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**REVIEW / SYNTHÈSE** 

# **PGE**<sub>2</sub> and **BMP-2** in bone and cartilage metabolism: 2 intertwining pathways

Marcel Haversath, Isabelle Catelas, Xinning Li, Tjark Tassemeier, and Marcus Jäger

**Abstract:** Osteoarthritis and lesions to cartilage tissue are diseases that frequently result in impaired joint function and patient disability. The treatment of osteoarthritis, along with local bone defects and systemic skeletal diseases, remains a significant clinical challenge for orthopaedic surgeons. Several bone morphogenetic proteins (BMPs) are known to have osteoinductive effects, whereof BMP-2 and BMP-7 are already approved for clinical applications. There is growing evidence that the metabolism of bone as well as the cartilage damage associated with the above disease processes are strongly inter-related with the interactions of the inflammation-related pathways (in particular prostaglandin  $E_2$  (PGE<sub>2</sub>)) and osteogenesis (in particular bone morphogenetic protein-2 (BMP-2)). There is strong evidence that the pathways of prostaglandins and bone morphogenetic proteins are intertwined, and they have recently come into focus in several experimental and clinical studies. This paper focuses on PGE<sub>2</sub> and BMP-2 intertwining pathways in bone and cartilage metabolism, and summarizes the recent experimental and clinical data.

Key words: osteoblasts, osteoclasts, prostaglandins, bone morphogenetic proteins, cellular signaling.

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Abbreviations: ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; ALP, alkaline phosphatase; BMP, bone morphogenetic proteins; BMP-2, bone morphogenetic protein-2; BMPR-Ia (ALK3), type Ia bone morphogenetic receptor; BMPR-Ib (ALK6), type Ib bone morphogenetic receptor; BMPR-II, type II bone morphogenetic receptor; cAMP, cyclic adenosine monophosphate; cFMS, colony-stimulating factor-1 receptor (c-fms proto-oncogene product); c-FOS, a transcription factor encoded by the FOS gene, a proto-oncogen; COX-2, cyclooxygenase-2; CSF-1, colony stimulating factor-1; Dlx5, a transcription factor encoded by the Dlx5 gene; EP1, prostaglandin E receptor 1 encoded by the PTGER1 gene; EP2, prostaglandin E receptor 2 encoded by the PTGER2 gene; EP3, prostaglandin E receptor 3 encoded by the PTGER3 gene; EP4, prostaglandin E receptor 4 encoded by the PTGER4 gene; ERK, extracellular-signal regulated kinase; ER- $\alpha$ , estrogen receptor  $\alpha$ ; FDA, US Food and Drug Administration; Fra-1, FOS-related antigen 1, a transcription factor belonging to the *c*-FOS gene family; Fra-2, FOS-related antigen 2, a transcription factor belonging to the *c-FOS* gene family; HO, heterotopic ossifications; IL-1α, interleukin-1α; IL-6, interleukin-6; IL-8, interleukin-8; IP3, inositol triphosphate; JNK, c-Jun N-terminal kinases, belonging to the mitogen-activated protein kinases family; MAPK, mitogen-activated protein kinases, responsive to extracellular stimuli; MMP-1, matrix metalloproteinase-1; MMP-13, matrix metalloproteinase-13; MMP-9, matrix metalloproteinase-9; Msx2, a transcription factor encoded by the msh homeobox 2 gene; NF-κB, nuclear factor κ-light-chain-enhancer of activated B cells; OA, osteoarthritis; OCN, osteocalcin; ONO-4891, a selective prostaglandin E receptor 4 activator; OPG, osteoprotegerin; OPN, osteopontin; Osx, osterix transcription factor, required for the expression of osteopontin; PG, prostaglandins: PGE2, prostaglandin E2: PGES, prostaglandin E synthase: PGHS-2, prostaglandin H synthase type 2; PKA, protein kinase A; PLA2, phospholipase A2; PLC, phospholipase C; PTH, parathormone; RANK, receptor activator of nuclear factor κB; RANKL, receptor activator of nuclear factor-κB ligand; rhBMP-2, recombinant human bone morphogenetic protein-2; R-SMAD, receptor regulated SMAD (a transcription factor family that transduce TGF-β signals); Runx2, runt-related transcription factor-2 (associated with osteoblastic differentiation); TGF- $\beta$ , transforming growth factor- $\beta$ ; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; TRAF6, TNF receptor associated factor-6 (a protein that transduces TNF signals); Vit. D, vitamin D.

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**Résumé :** L'ostéoarthrite et les lésions du cartilage sont des maladies qui résultent fréquemment en une réduction de la fonction articulaire et en une invalidité du patient. Le traitement de l'ostéoarthrite qui s'accompagne d'anomalies osseuses locales et de maladies squelettiques systémiques pose toujours un défi clinique significatif aux chirurgiens orthopédiques. Plusieurs protéines morphogènes de l'os, les BMP, sont connues pour exercer des effets ostéoinducteurs, parmi lesquelles BMP-2 et BMP-6 sont déjà approuvées aux fins d'applications cliniques. Il y a de plus en plus de preuves que le métabolisme de l'os et le dommage au cartilage associé aux maladies mentionnées plus haut sont fortement interreliés aux interactions des voies de l'inflammation (notamment la voie de la prostaglandine  $E_2$ , PGE<sub>2</sub>) et de l'ostéogenèse (notamment la voie de la *bone morphogenetic protein-2*, BMP-2). Il existe une preuve solide que les voies des prostaglandines et des BMP s'entrecroisent, et elles ont été l'objet d'une attention particulière dans plusieurs études expérimentales et cliniques. Cet article se concentre sur l'entrecroisement des voies de la PGE<sub>2</sub> et de la BMP-2 dans le métabolisme de l'os et du cartilage, et résume les dernières données expérimentales et cliniques.

Mots-clés : ostéoblastes, ostéoclastes, prostaglandines, protéines morphogènes de l'os, signalisation cellulaire.

[Traduit par la Rédaction]

## Relevance of prostaglandins and bone morphogenetic proteins in orthopaedics

Prostaglandins (PG) play an important role in bone formation, inflammation processes, and perfusion. They are lipid, arachidonic acid-derived mediators that are produced by a variety of cells and act in an autocrine and paracrine fashion to maintain local homeostasis.  $PGE_2$  is one of the most investigated prostanoids and is also known to be crucially involved in regulating bone formation associated with fracture healing and heterotopic ossification on the one hand, and bone resorption associated with inflammation and osteolytic effects of metastatic cancer on the other (Blackwell et al. 2010).

Bone morphogenetic proteins (BMPs) are multi-functional growth factors belonging to the transforming growth factor  $\beta$  $(TGF\beta)$  gene superfamily. There are about 30 different molecules within this group of mediators. BMPs play an important role in mesoderm formation. Thus, besides their role in the formation of cartilage and bone, they are also involved in the development and maintenance of various other mesodermal tissues, including kidneys and blood vessels (Chen et al. 2004). In the post-natal skeleton, BMPs are produced by the periosteal cells and mesenchymal cells of the marrow stroma. Three of them, i.e., BMP-2, -4, and -7, have been reported to induce de novo bone formation in vitro and in vivo (Sampath et al. 1990). Recent research has revealed that other BMPs such as BMP-6 and BMP-9 are also osteoinductive, and might have potential for future clinical applications (Kamiya 2011; Luther et al. 2011).

This review focuses on  $PGE_2$  and BMP-2 as 2 crucial mediators involved in the pathogenesis and therapeutic treatment of orthopaedic diseases. In the first section, the main functions of both mediators in the pathogenesis of orthopaedic diseases are highlighted. In a second section, the biochemical intertwining pathways of  $PGE_2$  and BMP-2 are illustrated.

### PGE<sub>2</sub> and BMP-2: relevant mediators in bone formation and repair

 $PGE_2$ , as a subtype, is known to be one of the most important local regulators of bone metabolism. Within bone,  $PGE_2$ is primarily produced by osteoblasts. Enhanced production of  $PGE_2$  by local osteoblasts can be found adjacent to skeletal injury. In this inflammatory environment,  $PGE_2$  is essential for new bone formation and bone healing (the effects of  $PGE_2$  on osteoblasts in the presence of inflammation are illustrated in Fig. 1). For example, O'Keefe et al. (2006) showed that local delivery of PGE<sub>2</sub> enhanced bone formation at the cortical bone graft junction. Other studies have reported that mice lacking cyclooxygenase 2 (COX-2), and thus unable to produce  $PGE_2$ , as well as aged mice with reduced COX-2 expression showed significant delayed bone healing after injury (Zhang et al. 2002; Naik et al. 2009). PGE<sub>2</sub> activates osteoblasts directly and osteoclasts indirectly through interactions with osteoblasts via the RANK signaling pathway (Boyle et al. 2003). Four different G-protein-coupled PGE<sub>2</sub> receptors have been identified: EP1, EP2, EP3, and EP4. All of these were detected in osteoblasts of different animal species (Narumiya et al. 1999). However, in human osteoblasts, only EP3 and EP4 were observed. Activation of EP4 has been shown to rescue impaired bone fracture healing in  $COX-2^{-/-}$  mice (Xie et al. 2009). Similarly, Naik et al. (2009) have shown that COX-2/EP4 agonists may compensate for deficient molecular signals that result in the reduced fracture healing associated with aging.

Activation of EP2 and EP4 leads to a stimulation of adenylate cyclase and increased cAMP levels as a second messenger system. On the other hand, EP3 receptor activation results in lower cAMP levels. High cAMP levels are linked to anabolic bone remodelling (Hakeda et al. 1986). Therefore, EP2 and EP4 receptors seem to play a crucial role in bone remodelling. Furthermore, selective stimulation of EP2 receptors of osteoblasts leads to differentiation of osteoblasts (Choudhary et al. 2008), which in turn cause differentiation of osteoclasts via the RANK/RANKL signaling pathway. With regards to EP1 receptor, there is evidence that its activation inhibits osteoblast differentiation (Zhang et al. 2011). The EP1 receptor activates phospholipase C followed by induction of calcium mobilization.

 $PGE_2$  production is also increased through the upregulation of COX-2 expression via an autogenous stimulation mechanism (Suda et al. 1998). In addition, selective stimulation of EP4 receptors of osteoblasts leads to differentiation of osteoblasts and upregulation of COX-2 expression, but less than that of a single EP2 activation (Sakuma et al. 2004). Overall, PGE<sub>2</sub> has been shown to have anabolic effects on bone remodelling in vivo (Graham et al. 2009). However, severe side effects such as diarrhea, lethargy, and flushing preclude PGE<sub>2</sub> as a therapeutic agent in bone diseases. In recent animal experiments using selective EP2 and EP4 receptor agonists, minimal side effects were seen when compared with using PGE<sub>2</sub> alone (Paralkar et al. 2003). Therefore, selective agonists of EP2 or



**Fig. 2.** Computerized tomography (CT scan) and X-ray pictures of a 73-year-old patient with a severe and symptomatic acetabular osteolysis beyond the acetabular component of a hip arthroplasty, owing to wear particles (white arrow), and after up to 4 years of follow-up. At the time of revision, cancellous bone graft supplemented with BMP-2 was added after curettage of the defect and application of bone-marrow cells. (*a*) The critical size defect healed within 12 weeks, as demonstrated by the CT scans. (*b*) The images show that BMP-2 can lead to significant new bone formation within an inflammatory tissue. The latest follow-up showed solid implant integration over 4 years (white double-arrow). The patient is free of pain, has an unlimited walking distance, and can return to previous sport activities (such as bike riding).



EP4 may have a therapeutic potential for enhancing bone formation and bone healing.

BMPs are some of the most investigated cytokine molecules that are critically involved in osteoblast differentiation and bone formation. Specifically, BMP-2, -4, -6, -7, and -9 are known to be osteoinductive by inducing the differentiation of mesenchymal stem cells into osteoblast precursors, and promoting the maturation of osteoblasts through the increase of osteoblast differentiation gene expression. Recent research also suggests that BMP-2 in particular, directly promotes not only the differentiation of osteoblasts but also osteoclasts (Jensen et al. 2010).

BMP-2 activates osteoblasts directly via 2 types of transmembrane receptors: BMPR-I and BMPR-II, as illustrated in Fig. 1. BMPR-I is further subclassified into BMPR-IA (ALK3) and BMPR-IB (ALK6). All receptors possess intrinsic serine/ threonine kinase activity. Activation leads to phosphorylation of different specific intracellular signal molecules including so-called R-SMADs. Subsequent activation of transcriptional factors (i.e., Dlx5 and Runx2) leads to enhanced expression of osteoblast differentiation marker genes, resulting in new bone formation (Ryoo et al. 2006). The anabolic effect of BMP-2 for bone regeneration in vivo is used in clinical practice today: recombinant BMP-2 (rhBMP-2, Infuse®Bone Graft, Medtronic Sofamor Danek, Inc., Memphis, Tennessee; or dibotermin alfa as InductOs<sup>®</sup>, Wyeth Pharmaceuticals, Berkshire, UK) is locally applied in cases of impaired bone healing. Figure 2 illustrates clinical results obtained after treatment of a severe symptomatic acetabular osteolysis with cancellous bone graft supplemented with BMP-2. It has received approval by FDA for specific indications in open tibial fractures, anterior singlelevel lumbar spinal fusion, and certain oral maxillofacial and dental regeneration applications.

#### PGE<sub>2</sub> and BMP-2 in fracture repair

Bone fracture leads to local hypoxia due to disruption of the vasculature. Hypoxia is then followed by the release of PGE<sub>2</sub> from osteoblastic cells (Lee et al. 2010). Thus, a physiological increase of endogenous, local PGE2-production can be quantitatively measured after fracture. In a tibial diaphyseal fracture model, PGE<sub>2</sub> administration restored cartilage formation and significantly reduced the fracture gap in Fra-1 transgenic mice, which are normally incapable of initiating callus formation at the fracture site (Yamaguchi et al. 2009). PGE<sub>2</sub> has been reported to enhance fracture healing in murine experiments, mainly through activation of the EP4 receptor (Xie et al. 2009). Similarly, Marui et al. (2006) demonstrated accelerated bone healing and decreased incidence of sternal wound complications after median sternotomy in diabetic rats with local application of a PGE<sub>2</sub> EP4 receptor-selective agonist. By comparison with selective EP4 receptor activation, selective EP2 receptor activation leads to increased COX-2 expression (Sakuma et al. 2004). COX-2 is known to enhance bone healing in vivo (Xie et al. 2009). Xie et al. showed that administration of EP4 receptor-selective agonists can rescue impaired fracture healing in  $COX-2^{-/-}$  knockout mice, but not EP2-selective agonists (Xie et al. 2009). Although both activated receptors are followed by cAMP-elevation, they seem to play different roles during bone repair. Xie et al. also measured increased levels of matrix metalloproteinase-9 (MMP-9) after targeted stimulation of EP4-receptors but not EP2 receptors. MMP-9 is known to induce angiogenesis during endochondral ossification. Hence, these results demonstrate that EP2 and EP4 might in part operate via different signaling pathways.

Hypoxia not only induces PGE<sub>2</sub> release but also enhances BMP-2 expression in osteoblasts (Tseng et al. 2010). BMPs can be found along collagen fibers of human bone matrix. After fracture, BMPs diffuse from bone matrix and stimulate osteoprogenitor cells, further enhancing BMP production. There is evidence that BMP-2 is required for fracture repair. Indeed, mice lacking the ability to produce BMP-2 showed spontaneous fractures due to inferior bone strength, and these fractures do not heal with time. Interestingly, however, nearly normal skeletal development could be found in these animals (Tsuji et al. 2006). A prospective, randomized, controlled, single-blind study involving 450 patients with an open tibial fracture was published in 2002, showing that locally administered rhBMP-2 at the fracture site significantly reduces the frequency of secondary interventions, accelerates fracture and wound-healing, and reduces the infection rate (Govender et al. 2002). This study was the basis for pre-market approval by the FDA of rhBMP-2 for open tibial fractures. In a recently published, evidence-based Cochrane review, 11 randomized controlled trials and 4 economic evaluations were analyzed to assess the effectiveness of clinically used rhBMP (i.e., rhBMP-2 and rh-BMP-7) for fracture healing in adults (Garrison et al. 2010). The review ascertained the paucity of data on the use of BMP in fracture healing. Furthermore, the heavily industry-sponsored scientific involvement in currently available evidence-based studies was criticized. Garrison et al. (2010) stated that there is limited evidence for BMP being more effective than controls for acute tibial fracture healing. Economical assessment suggested that the use of BMP is only favourable in patients with the most severe fractures.

#### $PGE_2$ and BMP-2 in osteoporosis

Osteoporosis is a disease characterized by bone loss resulting in increased fracture risk. The disease is subdivided into 2 types: post-menopausal (type I), and senile (type II) osteoporosis. An imbalance of bone formation and bone resorption is considered to play the main role in pathophysiology of both types of osteoporosis.

Bone remodelling is a well-balanced, constant process of bone resorption by osteoclasts and bone formation by osteoblasts. In elderly post-menopausal women, the extent of resorption is higher than formation, leading to bone loss. The excess of resorption is linked to low levels of serum estrogen, such as  $17\beta$ -estradiol, known to induce osteoprotegerin in osteoblasts, and which inhibits RANKL-mediated osteoclast activation resulting in decreased bone resorption (Hofbauer and Heufelder 2001). Some studies have shown that bone loss can be suppressed by administration of  $PGE_2$  in vivo (Li et al. 1995; Ke et al. 1998). For example, Li et al. (1995) revealed that systemic PGE<sub>2</sub> treatment can prevent bone loss in orchidectomized rats. In an ovariectomized rat model with established osteopenia, Ke et al. (1998) demonstrated that systemic PGE<sub>2</sub> treatment completely restored maximum load and stiffness of bone after 30 days of treatment. In addition, a significant increase in maximum load and stiffness in both rapidly growing and adult male rats could be observed in nonorchidectomized animals. Yoshida et al. (2002) reported that restoration of bone mass was primarily mediated via the PGE<sub>2</sub> EP4 receptor. Indeed, using a murine model, the authors showed that only EP4-deficient mice (-/-) did not show de novo bone formation after PGE<sub>2</sub> treatment. In mice lacking the EP1, EP2, or EP3 receptor, massive formation of woven bone was histologically identified after treatment. However, animal studies using  $PGE_2$  in vivo have reported severe side effects that include severe diarrhea, hair loss, and decreased physical activity, precluding the use of  $PGE_2$  as a therapeutic agent for osteoporosis in humans (Graham et al. 2009). Interestingly, further investigations have demonstrated that ONO-4891, a selective EP4 agonist, prevented bone loss and restored bone mass and strength in ovariectomized rats (Yoshida et al. 2002). In contrast to PGE<sub>2</sub>, no severe side effects at the dose required for bone formation were observed when the EP4 agonist was utilized. Thus, selective EP4 agonists may represent promising therapeutic agents for osteoporosis (Yoshida et al. 2002).

BMPs play an important role in the pathophysiology of osteoporosis. In fact, Urist, who discovered BMPs in 1965, labelled osteoporosis as "a bone morphogenetic protein autoimmune disorder" in 1985. In a rat model, Bessho and Iizuka (1993) found an age-dependent decreasing activity of BMPs after implantation of purified BMP into the calf muscles. These results were supported by Fleet et al. (1996), who reported that aging impairs rhBMP-2-induced bone formation in rats, and explained their results by a reduced number of mesenchymal stem cells associated with aging or a change in the responsiveness of these target cells to rhBMP-2. Turgeman et al. (2002) showed that bone mass could be recovered by rhBMP-2 when administered systemically to osteopenic mice. This effect was coupled with an increased number of adult mesenchymal stem cells in bone. Recent research also demonstrated that  $17\beta$ -estradiol, which is known to be lowered in type I osteoporosis, promotes the induction of BMP-2 in mouse mesenchymal stem cells, mainly via the activation of estrogen receptor alpha (ER- $\alpha$ ) (Zhou et al. 2003). In opposition to 17β-estradiol, glucocorticoids are known to induce osteopenia, particularly as a result of long-term pharmacological intake. Luppen et al. (2008) revealed that expression of BMP-2 is inhibited in glucocorticoid-arrested osteoblasts, and that rhBMP-2 restores bone mass mainly via activation of R-SMAD signaling molecules.

#### PGE<sub>2</sub> and BMP-2 in local osteolysis

Periprosthetic osteolysis is a common cause of aseptic loosening in total joint arthroplasty. Wear particles in periprosthetic tissues stimulate the production of inflammatory cytokines such as TNF- $\alpha$ , PGE<sub>2</sub>, and IL-6 (Bukata et al. 2004). In particular, titanium wear particles were found to activate PGE<sub>2</sub> production in fibroblasts through a COX-2 dependent pathway (Bukata et al. 2004). Other investigations also demonstrated a pro-inflammatory response of human osteoblasts to cobalt ions, leading to increased secretion of chemokines including  $PGE_2$  (Queally et al. 2009). A decrease in the secretion of alkaline phosphatase and in calcium deposition, markers of osteoblastic differentiation, was measured. Other studies demonstrated that PGE<sub>2</sub> could lead to the activation of periprosthetic fibroblasts through EP4, followed by an elevated expression of RANKL, which is known as the final effector of osteoclastogenesis and bone resorption (Tsutsumi

et al. 2009). Aseptic loosening due to osteolysis can therefore, at least in part, be initiated by local PGE<sub>2</sub> production resulting in a subsequent indirect activation of osteoclasts predominantly and short-handed periprosthetic osteoblasts. Taken together, one must consider that besides the role of PGE<sub>2</sub> in indirect activation of osteoclasts via osteoblasts or fibroblasts, other cytokines such as TNF- $\alpha$ , IL-1 $\alpha$ , IL-6, and IL-8, which are produced by a variety of cells, can also lead to osteoclast activation. Hence, periprosthetic osteolysis is a complex, inflammatory process mediated by different cytokines, including PGE<sub>2</sub>, which collectively result in an excess of osteoclastic activity.

rhBMP-2 has been reported to lead not only to the differentiation of osteoblasts, but also of osteoclasts (Majid et al. 2010). In recent studies using rhBMP-2 for spinal lumbar interbody fusions, several adverse effects were discovered, including vertebral osteolysis, which is known to be a selflimiting event (Rihn et al. 2009). Rihn et al. discovered vertebral osteolysis in 5 out of 86 patients who underwent singlelevel transforaminal interbody fusion (TLIF) in combination with rhBMP-2. However, 2 cases of ectopic bone formation were also described (Rihn et al. 2009). Although the precise pathophysiology of rhBMP-2-induced vertebral osteolysis remains unclear, there are hints that this complication may be a dose-dependent phenomenon. The current literature suggests that the carrier used to apply rhBMP-2 may play an important role in the development of vertebral osteolysis. Different studies have proposed that scaffolds providing more sustained and localized delivery would be advantageous for decreasing the rate of systemic and local complications such as vertebral osteolysis (Xu et al. 2009). The dose-response relationship between locally released rhBMP-2 and the extent of osteoclastogenesis remains controversial (Kaneko et al. 2000; Toth et al. 2009). In a sheep model, Toth et al. reported that increasing local rhBMP-2 levels lead to enhanced osteoclastic resorption of peri-implant bone. However, this osteoclastic effect was transient, and was followed by progressive bone healing and bone formation (Toth et al. 2009). BMPR-I and BMPR-II receptors were detected in osteoblasts as well as osteoclasts, and current literature suggests that BMP-2 activates osteoclasts directly (Kaneko et al. 2000; Jensen et al. 2010) and indirectly through osteoclastic-promoting factors such as RANKL produced by osteoblasts or stromal cells (Abe et al. 2000). Recent in vitro studies have revealed that BMP-2 leads to direct enhanced activation of osteoclasts via SMAD signaling (P-SMAD1/5/8) (Jensen et al. 2010). However, enhanced expression of osteoclast differentiation genes by BMP-2 was RANKLdependent. In the absence of RANKL, BMP-2 was not able to induce differentiation (Jensen et al. 2010). These findings were supported by Itoh et al. (2001), suggesting that BMP-2induced enhancement of osteoclast differentiation was caused by cross-communication between BMP receptor-mediated signals and RANK-mediated signals. However, they assumed that the MAPK pathway rather than the SMAD pathway was involved in osteoclast differentiation by BMP-2.

### $PGE_2$ and BMP-2 in ectopic bone formation (heterotopic ossifications)

Injury to soft tissue near bone can induce heterotopic ossifications (HO) that are a common problem after orthopaedic surgery. Using a rabbit model, Bartlett et al. (2006) found that

 $PGE_2$  was required for the development of ectopic bone. Non-steroidal anti-inflammatory drugs (NSAID) have been given prophylactically to patients, resulting in a lower incidence of HO. For example, Rapuano et al. (2008) found that COX-2 inhibitors significantly reduced PGE<sub>2</sub> levels at the site of the injury. Bartlett et al. (2006) reported on locally elevated levels of different prostaglandins, including PGE<sub>2</sub>, prior to and during the development of HO after muscle trauma in a rabbit model. Within only 24 h after injury, they measured enhanced  $PGE_2$  levels at the site of the injury, and suggested that HO prophylaxis with NSAIDs or prostaglandin receptor antagonists should be started immediately in patients undergoing orthopaedic surgery. Furthermore, they demonstrated that the induction of HO was mainly mediated via the PGE<sub>2</sub> EP2 receptor, and that elevated cAMP levels were critical in this process. After administration of the EP1 and EP2 receptor antagonist AH 6809, no development of HO could be found in the presence of PGE<sub>2</sub>. These findings were partly supported by Nakagawa et al. (2007) showing that ONO-4819 alone, a selective agonist for the EP4 prostanoid receptor, was not capable of inducing alkaline phosphatase, which is an early marker of osteoblast differentiation. However, in the presence of rhBMP-2, some studies demonstrated that systemic and local administration of ONO-4819 enhanced ectopic bone formation both in vivo and in vitro (Toyoda et al. 2005; Nakagawa et al. 2007). Toyoda et al. assumed that ONO-4819 and BMP-2 had cooperative anabolic effects on bone metabolism, and that administration of ONO-4819 together with rhBMP-2 could have clinical relevance in the future for reducing the dose of BMP needed for new bone formation (Toyoda et al. 2005). Similarly, in a very recent study, Kamolratanakul et al. (2011) showed that the combined delivery of ONO-AE1-437 (EP4 receptor agonist) and low-dose BMP-2, via a nanogel-based hydrogel, efficiently activated bone cells to regenerate calvarial bone and could therefore provide a new system for bone repair.

Ectopic bone formation was fundamental to the discovery of BMPs in 1965 (Urist 1965). After successful production of recombinant BMPs, it was shown that injection of recombinant human BMP-2 alone into muscle tissue induced ectopic bone formation (Wang et al. 1990). This was the basis for further experimental investigations that led to the current clinical utilization of recombinant BMP-2 and BMP-7 to enhance bone regeneration and formation.

### $\mathrm{PGE}_2$ and BMP-2 in osteoarthritis and cartilage metabolism

The role of the inflammatory mediator  $PGE_2$  in osteoarthritis (OA) was recently investigated. Some studies have shown a correlation between the extent of cartilage damage and the production of IL-1 $\beta$ , which induces the expression of COX-2 followed by elevated PGE<sub>2</sub> levels in OA joints (Shimpo et al. 2009).

The effect of  $PGE_2$  on chondrocytes depends on the predominant type of EP receptor. Thus, because of the different patterns of EP receptor expression in chondrocytes, different effects for  $PGE_2$  stimulation have been reported in the literature (Aoyama et al. 2005). For example, some authors have speculated that  $PGE_2$  had a protective effect on articular cartilage. Aoyama et al. (2005) found significant amounts of EP2 receptor and minor amounts of EP3 receptor, and saw no significant expression of EP1 and EP4 in the normal chondrocytes of human and mouse articular cartilage. Furthermore, Aoyama et al. (2005) demonstrated that PGE<sub>2</sub> signal through EP2 promoted the growth of articular cartilage cells in an animal model with chondral defects. Stimulation of these chondrocytes led to the suppression of osteopontin (OPN), which, when increased in chondrocytes in osteoarthrotic joints, has been linked to cartilage destruction (Yumoto et al. 2002). Nishitani et al. (2010) reported that PGE<sub>2</sub> inhibits IL-1-induced extracellular matrix metalloproteinase (i.e., MMP-1 and MMP-13) production via EP4, suggesting a potentially beneficial effect from PGE<sub>2</sub> on articular chondrocytes in OA. However, numerous studies have identified catabolic, degrading effects of PGE<sub>2</sub> on articular cartilage (Attur et al. 2008; Li et al. 2009). Attur et al. found the expression of all 4 EP receptor subtypes in both normal and OA chondrocytes, with a predominance of EP4 receptors in OA chondrocytes. Overall, they discovered catabolic effects of PGE<sub>2</sub> in OA cartilage that may have been mediated via EP4 signaling leading to increased expression of extracellular matrix degrading enzymes, such as metalloproteinases (MMP) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) (Attur et al. 2008). The catabolic effect of  $PGE_2$ in OA cartilage was also supported by Li et al. (2009), who suggested that PGE<sub>2</sub> does not modulate the expression of cartilage-degrading enzymes, but exerts its effects primarily by inhibiting aggrecan biosynthesis in human chondrocytes via activation of EP2. PGE<sub>2</sub> production, induced by mechanical shear stress on OA cartilage via COX-2, led to a significantly increased production of IL-6, a pro-inflammatory cytokine that is suspected to participate in the catabolic processes associated OA (Goekoop et al. 2010).

BMPs can have opposing effects on cartilage from development and protection, to degradation, and they are crucial for the homeostasis of cartilage tissue. In healthy articular cartilage, BMP-2 can hardly be detected, whereas in OA cartilage, BMP-2 is highly expressed (Blaney Davidson et al. 2007). BMP-2 has been proposed to have a regenerative effect on chondrocytes by promoting differentiation and enhancing the expression of extracellular matrix such as type II collagen and proteoglycan (Schmitt et al. 2003). However, initially, BMP-2 also stimulates the expression of matrix degrading enzymes such as MMPs and ADAMTS (Blaney Davidson et al. 2007). Blaney Davidson et al. (2007) interpreted this expression as a short-term impulse to create space for newly produced extracellular matrix. Overall, the anabolic effects of BMP-2 in cartilage could be observed as matrix production exceeds degradation. However, one must consider that BMP activity alone is not sufficient to adequately protect cartilage against destruction. Several studies have indicated that the balance of TGF- $\beta$ /BMP signaling is of particular importance for cartilage maintenance (Li et al. 2006). Loss of TGF-B signaling promotes enhanced BMP signaling, which leads to terminal differentiation of chondrocytes (hypertrophy) and results in development of osteoarthritis (Li et al. 2006). Li et al. demonstrated that BMP-2 induces maturation of chondrocytes via R-SMAD signaling molecules (i.e., SMAD1, SMAD5, SMAD8), whereas TGF-β signaling via SMAD3 inhibits maturation and downregulates expression of BMP-2 (Li et al. 2006). Couchourel et al. (2009) investigated normal human and OA osteoblasts from tibial plateaus to analyze the mechanisms that lead to undermineralized bone tissue in OA. Elevated levels of TGF<sup>β1</sup> were found in OA osteoblasts. TGF<sup>β1</sup> is known to have an inhibitory effect on bone formation and it exerts its effects, at least in part, through suppression of BMP-2 production in OA osteoblasts (Li et al. 2006). TGFB1 also induces enhanced, abnormal type I collagen expression in OA osteoblasts (Couchourel et al. 2009). Thus, undermineralized bone tissue in OA osteoblasts may be the result of the suppression of BMP-2 production as well as abnormal osteoid production mediated by TGF $\beta$ 1. The dual role of BMPs in articular cartilage formation and repair and in OA development and progression was recently described in a published review (van der Kraan et al. 2010). BMP-2 would induce cartilage formation and repair with the expression of extracellular matrix which can ultimately progress to chondrocyte hypertrophy, production of matrix degrading enzymes (i.e., MMP-13) and development of OA (van der Kraan et al. 2010).

### The intertwining pathways: intracellular signaling of PGE<sub>2</sub> and BMP-2 in bone

The pathways of PGE<sub>2</sub> and BMP-2 are closely related and influence each other on different intra- and extra-cellular levels. Understanding these intertwining pathways may be relevant for the development of new therapeutic substances to enhance bone formation and (or) cartilage metabolism. It was shown that selective EP2 and EP4 agonists activate both the PKA pathway and MAPK pathways (predominantly p38 MAPK and ERK) in rat calvaria cell cultures. BMP-2 effects are mediated via R-SMADs and in part via PKA and MAPK pathways, and this is where the signaling pathways of both PGE<sub>2</sub> and BMP-2 are intertwined. Consequently, application of EP2 and EP4 selective agonists together with BMP-2 showed an additive effect on mineralized bone nodule formation (Minamizaki et al. 2009). Promising results for future clinical application were recently published by Kamolratanakul et al. (2011) who, as previously described, used nanogel-based scaffolds with a selective EP4 agonist (ONO-AE1-437) in combination with a low dose of BMP-2 to heal critical-size bone defects in a murine calvaria model.

#### Intertwining pathways in osteoblasts

The main intracellular signaling cascades in osteoblasts are displayed in Fig. 1. Overall, both PGE<sub>2</sub> and BMP-2, have cooperative anabolic effects on bone metabolism. Inflammation, along with injury to bone or soft tissue, leads to increased production of local PGE<sub>2</sub> and release of BMP-2 from bone matrix. Hypoxic conditions during injury or inflammation stimulate PGE<sub>2</sub> and BMP-2 production (Tseng et al. 2010). PGE<sub>2</sub> exerts its anabolic effects mainly via osteoblastic EP2 and EP4 receptor activation and increase in cAMP-levels with activation of protein kinase A (Graham et al. 2009). The activation results in the induction of BMP-2 and COX-2. There is growing evidence that PGE<sub>2</sub>-mediated BMP-2 production is mainly stimulated via the EP4 receptor, while COX-2 production is stimulated via the EP2 receptor (Sakuma et al. 2004; Graham et al. 2009). In turn, BMP-2 induces COX-2 expression, which leads to increased PGE<sub>2</sub> production (Chikazu et al. 2002).

To summarize, the production of  $PGE_2$  and BMP-2 in osteoblasts is reciprocally promoted. Additionally,  $PGE_2$  induces its own production not only via EP2 and EP4 receptors, but also EP1 through an autogenous stimulation mechanism (Suda et al. 1998; **Fig. 3.** Intertwining pathways in osteoblast and osteoclast interactions. In the presence of PGE<sub>2</sub>, BMP-2 activates osteoblasts to produce osteoclast-stimulating proteins such as Receptor Activator of NF-κB Ligand (RANKL). BMP-2 also promotes osteoclastogenesis directly via SMAD signaling and possibly via MAPK signaling, but only in the presence of RANKL. On the other hand, PGE<sub>2</sub> can directly inhibit osteoclastogenesis in the absence of osteoblasts via activation of protein kinase A. During osteoclastogenesis in an inflammatory environment, EP2 and EP4 receptors are down-regulated to maintain bone resorption.



Sakuma et al. 2004). Furthermore, cAMP-signaling induces the activation of Runx2, which is known to be an important transcription factor promoting osteoblastic differentiation (Yoshida et al. 2002). Recent research has suggested that EP2 and EP4 enhance Runx2 expression through cAMPdependent MAPK-pathways: EP2 mainly via p38 MAPK, EP4 via ERK, both possibly promoted by protein kinase C (PKC) (Minamizaki et al. 2009). JNK, as the third MAPKpathway, seems equally activated by both EP2 and EP4, which is also involved in Runx2 activation. Furthermore, BMP-2 mediated SMAD (SMAD 1/5/8) and MAPK-signaling (p38) are also pathways linked to the activation of Runx2 in osteoblasts (Ryoo et al. 2006). Additionally, SMAD signaling pathways also activate other transcriptional factors like Dlx5 and Osx in both a direct and indirect manner (Ryoo et al. 2006). Although the intertwining relations of different transcription factors are not fully understood yet, it is recognized that the main effect of cAMP-signaling via PGE<sub>2</sub>, as well as SMAD signaling via BMP-2, is the induction of osteoblastic differentiation as indicated by elevated levels of osteoblastic marker proteins such as OPN, alkaline phosphatase (ALP), or osteocalcin (OCN).

The use of a selective  $PGE_2$  EP4 agonist (ONO-4819) in combination with BMP-2 showed a significant augmentation of bone mass in different in vivo and in vitro studies (Nakagawa et al. 2007). Since selective  $PGE_2$  EP4 agonists

were found to have less of an adverse effect in animal models compared with  $PGE_2$  alone (Graham et al. 2009), together with rhBMP-2 they may prove to be promising therapeutic agents for the future enhancement of bone formation and bone healing in humans.

### Intertwining pathways in osteoblast and osteoclast interactions

Bone formation is always coupled with the bone resorbing process. Although the main effect of PGE<sub>2</sub> and BMP-2 on bone is anabolic, osteoclastic activity can also be stimulated by these mediators in a direct and, even more importantly, indirect manner (Fig. 3). PGE<sub>2</sub> administration and elevated cAMP levels have been shown to induce the expression of RANKL and inhibition of osteoprotegerin (OPG) in osteoblasts (Jurado et al. 2010). BMP-2 signaling via R-SMADs also stimulates RANKL production in osteoblasts, but this effect is only observed in the presence of  $PGE_2$  (Blackwell et al. 2009). However, BMP-2 alone can induce the production of colony stimulating factor-1 (CSF-1) in osteoblasts, which is known to promote osteoclastogenesis by activating the cFMS receptor of osteoclasts (Mandal et al. 2009). One must consider that it is not only PGE<sub>2</sub> and BMP-2 that are involved in the regulation of RANKL-production by osteoblasts, but also other mediators such as parathormone (PTH), glucocorticoids, and others (Fig. 3). PGE<sub>2</sub>, EP2, and EP4 receptors, as well as BMP-2,

BMPR-I, and BMPR-II receptors, were detected in osteoclasts (Kaneko et al. 2000). Direct activation of EP2 and EP4 receptors in osteoclasts has been shown to inhibit osteoclastogenesis in the absence of osteoblasts (Mano et al. 2000). This effect can be seen as a protective mechanism to avoid excessive bone resorption during inflammatory conditions in the absence of osteoblasts. On the other hand, RANKL-mediated osteoclastogenesis leads to down-regulation of EP2 and EP4 receptors in osteoclasts (Kobayashi et al. 2005). This mechanism provides the maintenance of RANKL-induced osteoclastogenesis by preventing direct inhibition through PGE<sub>2</sub>. RANKLstimulated differentiation of osteoclasts can further be augmented by BMP-2-mediated SMAD signaling and possibly MAPKsignaling (Itoh et al. 2001; Jensen et al. 2010). However, without RANKL, BMP-2 does not induce osteoclastogenesis.

To summarize, PGE<sub>2</sub> can induce the expression of RANKL in osteoblasts, leading to osteoclastic differentiation further enhanced by the co-presence of RANKL and BMP-2. On the other hand, PGE<sub>2</sub> can also directly inhibit osteoclastogenesis through the activation of EP2 and EP4 receptors on osteoclasts. However, this inhibition is counter-balanced by the down-regulation of these receptors by RANKL-mediated osteoclastogenesis.

In the absence of PGE<sub>2</sub> and RANKL, BMP-2-induced stimulation of osteoclasts can be mediated indirectly via CSF-1 production in osteoblasts. However, BMP-2 alone does not affect the production of RANKL in osteoblasts (Blackwell et al. 2009).

#### Conclusions

Inflammation processes and bone remodelling are strongly interconnected. The overall effect of PGE<sub>2</sub> and BMP-2 on bone is anabolic. Recent research has demonstrated that the intracellular signaling pathways of both mediators are intertwined and that their production in osteoblasts is reciprocally promoted. Contrary to PGE<sub>2</sub>, selective PGE<sub>2</sub> EP4 receptor agonists caused no severe side effects while providing similar anabolic effects. Hence, EP4 agonists together with rhBMPs, and in particular rhBMP-2, may be promising therapeutic agents for enhancing bone formation and bone healing in human skeletal disorders.

#### **Competing interests**

The authors declare that there is no conflict of interest associated with this work.

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