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Angiogenesis and bone repair

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The intimate connection, both physical and biochemical, between blood vessels and bone cells has long been recognized. Genetic, biochemical, and pharmacological studies have identified and characterized factors involved in the conversation between endothelial cells (EC) and osteoblasts (OB) during both bone formation and repair. The long-awaited FDA approval of two growth factors, BMP-2 and OP-1, with angiogenic and osteogenic activity confirms the importance of these two processes in human skeletal healing. In this review, the role of osteogenic factors in the adaptive response and interactive function of OB and EC during the multi-step process of bone repair will be discussed.

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▼ As early as 1763, the importance of blood vessels in bone formation was noted: 'the origin of bone is the artery carrying the blood and in it the mineral elements' [1]. Around the same time, Hunter suggested that blood vessels are key contributors to the process of osteogenesis, both in development and during repair [2]. During the following 150 years, studies of osteogenesis focused on the role of bone-forming osteoblasts, rather than on blood vessels. In 1963, Trueta revived interest in bone vasculature by publishing data that was based on years of bone studies. Building on Levander's proposal in 1938 that tissues produce substance(s) that initiate osteogenesis, Trueta suggested the existence of a 'vascular stimulating factor (VSF)' operating at sites of bone damage [3].

These early predictions have proven to be remarkably accurate. Inadequate or inappropriate bone vascularity is associated with decreased bone formation and bone mass [4,5]. Of the 6 million bone fractures reported annually in the United States, 5–10% have impaired healing, causing pain and disability, and, in some cases, deformity, with atrophic fracture non-unions representing the vast majority (up to 80%) of non-unions [6,7]. Accordingly, inhibition of angiogenesis during fracture repair in animals results in the formation of fibrous tissue, reminiscent of human atrophic non-unions [8]. Risk factors for impaired bone healing include: poor blood supply, poor apposition of fractured bone ends, interposition of soft tissues or necrotic bone between bone fragments, inadequate immobilization, infection, drug use (e.g. corticosteroid therapy or nicotine), advanced age, and systemic disorders, such as diabetes or poor nutrition [7]. Negative effects on the vascular system might be the mechanism whereby many other risk factors delay or impair bone healing [5].

Bone healing

The soft-tissue healing of a wound creates a fibrous scar but bone is unique in its scarless regenerative capacity [5]. Repair of fractures by callus production occurs in four overlapping phases [7,9–12] (Figure 1). Following damage to the musculoskeletal system, disruption of blood vessels leads to activation of the coagulation cascade and formation of a hematoma, which encloses the fracture area (Figure 1a). Removal of the hematoma significantly attenuates repair, and transplantation of the hematoma produces new bone [13,14], consistent with the angiogenic activity of the hematoma [15,16]. Inflammatory cells, fibroblasts, and stem cells are recruited to the site, and new blood vessels are formed from pre-existing ones (i.e. angiogenesis). The inflammatory response is associated with pain, heat, swelling, and the release of several growth factors and cytokines that have important roles in repair [7,10,12,17,18]. Initially, granulation tissue forms at the ends of bones, gradually being replaced by fibrocartilage, in a manner seemingly related to the vascular pattern [3]. Meanwhile, the periosteum undergoes direct bone formation, or intramembranous ossification, to create an external callus (Figure 1b). Subsequently, the internal callus becomes mineralized with calcium hydroxyapatite, to form a hard callus of woven bone (Figure 1c). In the final, remodeling phase of bone regeneration, the

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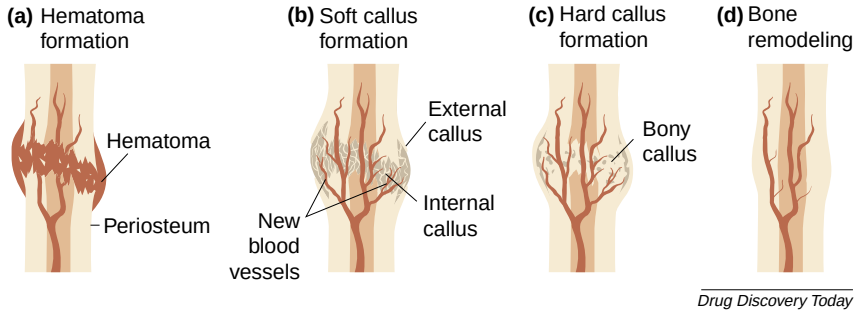

Figure 1. The stages of fracture repair. **(a)** Hematoma formation: following injury, disruption of blood vessels leads to formation of a hematoma; **(b)** Soft callus formation: this stage involves the formation of new blood vessels from pre-existing ones (angiogenesis), the external callus (intramembranous ossification) and the internal callus (fibrocartilage); **(c)** Hard callus formation: the callus becomes mineralized, forming a hard callus of woven bone; **(d)** Bone remodeling: the large fracture callus is replaced with secondary lamellar bone, and the vascular supply returns to normal.

large fracture callus is replaced by secondary lamellar bone; the size of the callus is reduced to that of pre-existing bone at the damage site, and the vascular supply reverts to a normal state (Figure 1d). This temporal progression of repair can been recapitulated in animal models, such as those of long bone fractures [19,20] (Figure 2).

The blood supply of mammalian long bones has been studied in many different bones and species [5,21,22]. Generally, long bones receive blood from several groups of arteries: proximal/distal metaphyseal arteries, proximal/distal epiphyseal arteries, diaphyseal nutrient arteries and periosteal arteries [5]. Bone fracture results in disruption of the marrow architecture and blood vessels within and around the fracture site [5,21,22]. During bone repair, the three components of the normal bone blood supply – medullary, periosteal, and osseous – can be enhanced according to physiological need [5,21]. The newly generated blood supply to the callus and cortical bone appears to persist until the medullary blood supply is fully regenerated [21]. The heterogeneity in vascularity after bone damage could help to explain local differences in bone formation in normal, delayed, and mal- unions [23].

Systemic and local responses

Many growth factors/cytokines, expressed during fetal skeletal development and induced in response to injury, are believed to have a significant role in the process of repair [7,10,12,17,18]. These include members of the fibroblast growth factor (FGF), transforming growth factor (TGF), bone morphogenetic protein (BMP), insulin-like growth factor (IGF) and platelet derived growth factor (PDGF) families [9,10,12,17], as well as vascular endothelial growth factor, VEGF [24,25] (Table 1). These factors are produced by and/or responded to by many cell types present at the fracture site [12,17,24,25]. Other angiogenic [12] and anti-angiogenic factors, such as those expressed in the growth plate of developing bones [26], might similarly be expressed and active in the fracture callus during endochondral ossification. Skeletal injury in humans is characterized by local (injury site) and systemic angiogenic responses [15,16,27]. Accordingly, systemic factors - such as parathyroid hormone (PTH), growth hormone, steroids, calcitonin and Vitamin D – can also modulate bone metabolism and vascularity [10] (Table 1).

Vascular endothelial growth factor

Previous studies have shown that endogenous VEGF is important for endochondral bone formation [28–31]. VEGF is expressed before blood vessels are detected in developing mouse bones, and this expression is tightly associated with cells involved in bone formation (osteoblasts) [28]. Inhibition of VEGF leads to expansion of the hypertrophic zone, loss of metaphyseal blood vessels and impaired trabecular bone formation in developing mice [29,30] and monkeys [31]. Thus, during development, VEGF is essential for blood vessel invasion of hyaline cartilage, growth plate morphogenesis, and cartilage remodeling.

Endogenous VEGF also plays a key role in bone repair [19,32,33]. VEGF is expressed in much the same temporal and spatial pattern in the fracture callus as that that occurs during long bone development [12,24,25]. Notably, the human fracture hematoma, present after injury but not during development, has potent angiogenic activity that appears to be predominantly due to VEGF [15,16]. Similarly, the angiogenic activity of injured patient plasma (systemic response) is primarily VEGF dependent, an effect particularly obvious in elderly patients, in whom the incidence of delayed and aberrant fracture repair is increased [15,16]. Inhibition of VEGF activity disrupts repair of femoral fractures and cortical bone defects in mice [19], and decreases blood flow and leads to non-unions in rabbit radial fractures [32]. Thus, VEGF activity is essential for normal angiogenesis and appropriate callus architecture and mineralization in response to bone injury. These findings support the hypothesis that blood vessel invasion has a key role in tissue repair, and suggest that VEGF production is the major mechanism by which angiogenesis and osteogenesis are tightly coupled during bone repair [19,32,33].

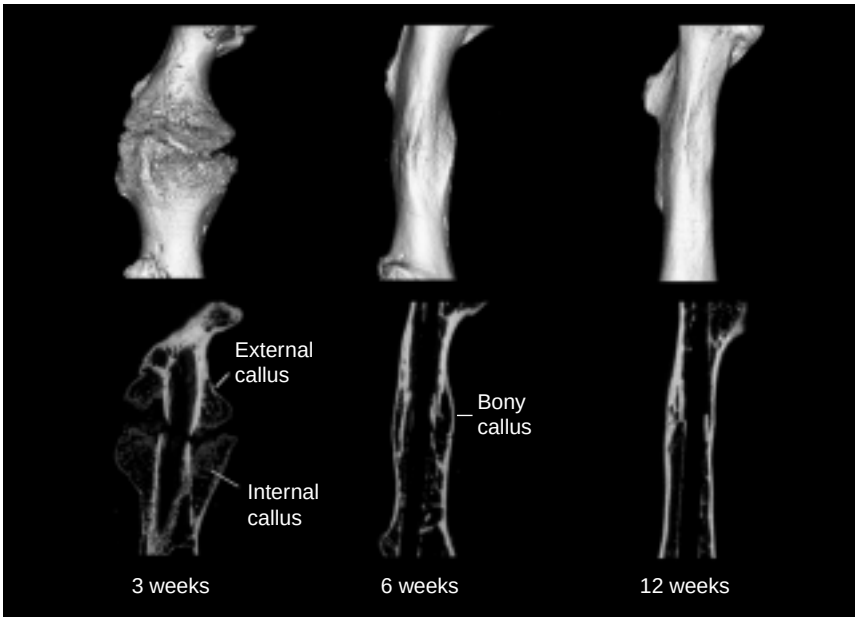

Figure 2. *Ex vivo* micro-computed tomography (μCT) of mouse femurs at different times (3, 6, or 12 weeks), after creation of challenged fractures [19]. 3D surface renderings (top row) and coronal μCT images through the center of each femur (bottom row). 3D renderings show calcified structures above a bone density threshold of 0.48 g-hydroxyapatite/cm^3. Soft tissues, such as fibrous tissue and cartilage, which bridge the gap at early timepoints (e.g. at 3 weeks), appear dark in μCT images. Images were acquired at a resolution of 16×16×16 microns.

VEGF expression is induced by most osteoinductive growth factors (Table 1) as well as prostaglandins [34] and ultrasound [35]. In fact, inhibition of VEGF blocks FGF-2- [36] or BMP-2-induced [37] angiogenesis, BMP-7 (OP-1) induction of primary osteoblast differentiation [38], and BMP-4-induced bone formation [33]. Thus, the ability of these factors to stimulate bone repair in animal models or clinical trials might be mediated, at least partly, by VEGF. VEGF, in turn, regulates recruitment, survival and activity of endothelial cells [39], osteoblasts [16,19,27,28,40–42] and osteoclasts [43–45].

Given the importance of VEGF in normal bone repair, treatment with exogenous VEGF might be expected to promote angiogenesis and bone formation after injury – a hypothesis that is supported by several studies. Local administration of exogenous VEGF, in the absence of a scaffold or progenitor cells, enhances bone formation in mouse femur fractures and rabbit radial critical-sized defects [19]. Studies using muscle-derived stem cells have shown that VEGF acts synergistically with BMP-4 to enhance bone formation through stimulation of cell recruitment, survival, and cartilage formation and resorption [33]. VEGF treatment increases bone blood flow in rabbit radial fractures [32] and in rabbit tibiae during distraction osteogenesis (i.e. gradual separation of bony fronts to stimulate new bone formation) [46]. Local adenoviral delivery of VEGF increases bone formation and decreases bone resorption in intact rabbit femurs [47], and stimulates angiogenesis and bone formation in rat femur defects [48]. In tibial fractures in matrix metalloproteinase 9 (MMP-9) knockout mice, VEGF treatment overcomes the skeletal repair defect that resembles human hypertrophic non-unions [49].

As might be expected with growth factor treatments, the timing, dose and cellular context can affect VEGF activity. In terms of timing, delivery of VEGF during the latency (time of rest after creation of bony defects) and distraction phase (period of separation of bone ends) of distraction osteogenesis does not increase blood flow in the regenerate [46]. Given the highly immature state of the early blood supply of the regenerate [50,51], and that bone formation is at a high level in the first week of the consolidation phase (period after completion of distraction), this phase might be an optimal time for treatments such as VEGF [46]. In terms of dose, the ratio of VEGF- to BMP-4- transduced muscle-derived stem cells was found to be important for enhanced bone formation in a critical size mouse skull defect [33]. These results - together with findings that VEGF-expressing muscle-derived stem cells do not promote repair of critical size skull defects in mice – might be due to increased differentiation of stem cells along the endothelial lineage, at the expense of cells becoming cartilage or bone cells [33]. Alternatively, such results might indicate that this timing and dose of VEGF (i.e. continuous exposure of cells to relatively high levels of VEGF during the 21-day repair process [33]), are not optimal to induce bone formation, because local levels of endogenous VEGF peak at 5 days and decrease towards normal levels after day 10 after fracture [52] or bony injury [53] in rodents. VEGF does not appear to drive progenitor cell differentiation into the chondro- or osteo-genic lineages. Therefore, combination therapies with BMPs [33], which have such activity, might be beneficial in specific types of defects, especially those in patients with a limited number of committed osteoblasts and/or those involving the use of stem cells.

Table 1. Factors influencing bone repair

Treatment[a]	Osteoblastic[b]		Angiogenic[c]		Induction of VEGF[d]	
Activins	+	[134]	[f]	[f]	ND	
Bone Morphogenetic Proteins 2 and 4	+	[17]	+	[37]	+	[37]
Osteogenic Protein-1[e]	+	[17]	+	[128]	+	[38]
Fibroblast Growth Factor 1 and 2	+	[139]	+	[64,77]	+	[140]
Growth and Differentiation Factor-5	[g]		+	[129]	ND	ND
Growth Hormone	+	[141]	+	[142]	ND	ND
Insulin-like Growth Factor-1	+	[143]	+	[144]	+	[57,58]
Parathyroid hormone	+	[145]	+	[146]	+	[58]
Platelet-derived Growth Factors	+	[10]	+	[147]	+	[56]
Prostaglandins	+	[34]	+	[148]	+	[34]
Transforming Growth Factor βs	+	[113,114,120]	+	[149]	+	[59]
Vascular Endothelial Growth Factor	+	[19,40–42]	+	[19,39]		

[a]Factors that can enhance bone repair.
[b]'Osteoblastic' is defined as having direct effects on committed (pre)osteoblasts.
[c]'Angiogenic' is defined as the ability to form blood vessels *in vivo* and includes both direct and indirect mechanisms.
[d]Induction of VEGF *in vitro* and/or *in vivo*
[e]Also known as bone morphogenetic protein-7.
[f]Activin might be anti-angiogenic, at least in the context of cancer [150].
[g]Growth and Differentiation Factor 5 (GDF-5) induces ectopic cartilage and bone *in vivo* [92,131) and promotes spinal fusion [151,152]; *in vitro* studies indicate that GDF-5 stimulates formation of chondrogenic nodules by calvarial cells [92,131,151].
ND – not yet determined

Besides the paracrine effects of VEGF (i.e. stimulation of endothelial cell secretion of osteogenic molecules [54–56], VEGF can act directly on osteoblasts. Osteoblasts both synthesize VEGF in response to various stimuli [55–59] and respond to VEGF itself [19,28,40–42]. VEGF receptors (VEGFR-1, VEGFR-2, VEGFR-3, neuropilin-1 and -2) are expressed in cells of the osteoblast lineage [41,42,60] and during mouse fracture repair [49]. VEGF increases chemotaxis, proliferation, differentiation, and basal and PTH-induced cAMP production in osteoblasts [19,28,40–42]. VEGF also induces bone formation in organ cultures [28] and *in vivo*, due in part to direct recruitment and stimulation of osteoblasts [47]. Inhibition of VEGF decreases primary osteoblast differentiation *in vitro* [19]. Mice that express only the freely diffusible isoform of VEGF (VEGF120) exhibit reduced osteoblast activity; VEGF inhibition decreases growth of cultured mouse skull bones [28].

Bone-resorbing osteoclasts are also responsive to VEGF. VEGF is required for normal osteoclastic resorption during endochondral ossification in development and for the age-dependent recovery of osteoclasts in osteopetrotic (op/op) mice that lack functional macrophage colony-stimulating factor (M-CSF or CSF-1) [29,43,44]. VEGF stimulates osteoclast recruitment, survival and activity [43–45,61], and osteoclast differentiation *in vitro* in combination with osteoprotegerin ligand (OPGL)/receptor activator of NF-κB ligand (RANKL) [43]. The lack of lamellar bone remodeling and osteoclastic 'cutting cones' in fractured mouse femurs following VEGF inhibition suggests that VEGF is important for osteoclast activity during bone repair [19]. Surprisingly, adenovirus-mediated VEGF overexpression in intact rabbit femurs does not appear to increase osteoclast activity [47].

VEGF has also been implicated in cartilage maturation and resorption [28–30,33,49,62]. More specifically, mice that express only the freely diffusible VEGF isoform (VEGF120) [28,62] or have conditional inactivation of VEGF in areas of collagen 2α1 expression [30] have impaired endochondral bone formation. Inhibition of VEGF decreases cartilage formation and delays cartilage resorption during ectopic bone formation induced by BMP-4-transduced muscle-derived stem cells [33]. Overexpression of VEGF increases cartilage formation by BMP-4-transduced muscle-derived stem cells [33]. The effects of VEGF on cartilage cells might be direct. VEGF stimulation of hypertrophic chick chondrocytes *in vitro* leads to phosphorylation of VEGF-R2/KDR (kinase-insert domain receptor)/Flk-1 (fetal liver kinase 1) [63]. Inhibition of VEGF in developing mice appears to delay or inhibit chondrocyte death, without affecting chondrocyte growth or maturation [29]. Indirect effects of VEGF on cartilage during development

include changes in vascularity, and recruitment and/or differentiation of cells that resorb cartilage [28,29,33,44,49].

Fibroblast growth factors

Members of the fibroblast growth factor (FGF) family regulate cell proliferation, migration and differentiation as well as musculo-skeletal development [64,65]. Within this family, most bone studies have focused on FGF-2 or basic FGF (bFGF). The injection of FGF-2 into intact bone stimulates bone formation [66–68]. However, early bone repair studies provided conflicting results [69–71]. Treatment with a single dose of FGF-2 (2μg) at the time of injury increases callus formation and mechanical strength in a fibula fracture model in normal and diabetic rats [69]. By contrast, a single dose of FGF-2 (1μg) in a hydroxyapatite (HA) graft transiently reduces the mechanical strength of a rat segmental defect [71]. FGF-2 (3μg) applied four days after creation of a rabbit tibial defect does not affect the size or amount of cartilage or bone in the callus [70].

These initial differences in results with FGF-2 could be explained, at least partly, by findings that positive effects of FGF-2 on bone repair are dose- and time- dependent [69,72,73] and by the use of different species, carriers, and model systems. More specifically, in a rabbit tibial defect model, 100 μg of FGF-2 is required to increase the volume and density of new bone [73]. Use of a slow-release formulation allows for lower effective doses (as low as 1.4μg) of FGF-2 in a rabbit segmental gap six weeks after treatment, when a single injection of 2μg of FGF-2 is not sufficient to promote repair [72]. Studies in larger animals support the proposed bone repair function of FGF-2. In a canine tibial fracture model, 200μg of FGF-2 injected at the fracture site increases callus area, bone mineral content, and biomechanical strength [74]. FGF-2 can also accelerate repair in nonhuman primates [75,76]. FGF-2 (in hyaluronic acid) increases callus area, mechanical strength and vascularity in baboon fibula osteotomies [75].

The exact mechanism by which FGF-2 stimulates bone repair remains uncertain, but FGF-2 induces angiogenesis [77–79], and stimulates mitogenesis of mesenchymal cells and osteoblasts [80], which might be mediated and modulated by TGF-β [66,80]. The ability of FGF-2 to promote the formation of a larger callus at early stages of fracture repair [71,81], and thus, improve mechanical stability during this stage, could be one of the primary benefits of FGF-2 treatment [76]. FGF-2 increases osteoclastic bone remodeling [74,79,82–85] and down-regulates at least one BMP antagonist (noggin) [86]. Finally, FGF-2 might prove to be a useful systemic treatment for osteoporosis because it increases connections between neighboring trabeculae in ovariectomized rodents [67,68,87].

The transforming growth factor superfamily

In 1965, Marshall Urist demonstrated that demineralized bone matrix induces cartilage and bone formation when implanted at extra-skeletal sites [88]. Based on these studies, Urist proposed the existence of a substance(s) within bone matrix that induces differentiation of precursor cells into bone-forming cells. Subsequent protein purification and cloning [89,90] led to the identification of proteins within TGF-β superfamily [91,92].

This superfamily includes most factors known to induce cartilage and bone formation during development, including TGF-βs, BMPs, growth and differentiation factors (GDFs) and activins [92]. By virtue of shared receptors and structural homology, many of the members of this large family have overlapping activities, including stimulation of mesenchymal stem cell differentiation into chondrocytes or osteoblasts. However, their distinct expression pattern during bone repair [12,93], the developmental phenotypes observed when the genes are mutated or deleted [92] and their activities when added exogenously in animal models or humans [10, 94–112] suggest subtly distinct roles in the process of bone repair.

Transforming growth factor-β

Of the five isoforms of TGF-β [92], TGF-β–1 and TGF-β–2 are the most studied in bone [93,94,113,114]. All isoforms will be referred to, here, as TGF-β. Early studies indicated that TGF-β stimulates bone formation when injected into rodent bones [94,100,115] or rabbit skull defects [105] and also increases callus volume and strength in tibial fractures [95,99]. Despite a few studies to the contrary [98,116,117], most reports indicate positive (if weak) effects of TGF-β on bone repair [113], [114]. TGF-β might also be useful for implant fixation [118,119].

Consistnet with its pleiotropic activities and pattern of expression during development and repair [93,94,113–115], TGF-β can affect the repair process in multiple ways. In fractured bones, TGF-β is released by degranulating platelets in the hematoma and by extracellular matrix at the site of damage. At this stage (Figure 1a), TGF-β could stimulate recruitment and proliferation of mesenchymal cells (stem cells, chondroblasts, and osteoprogenitors), and might affect inflammation and angiogenesis. At later stages of repair (Figure 1b and 1c), chondrocytes, osteoblasts, and osteoclasts synthesize and respond to TGF-β [93,94,115]. During these stages, TGF-β can regulate chondrocyte and osteoblast differentiation, extracellular matrix production and osteoblast/osteoclast coupling [120].

Bone morphogenetic proteins

Consistent with Urist's initial work, several BMPs have been found to induce ectopic bone, due, at least partly, to

stimulation of mesenchymal and osteoprogenitor cell proliferation and differentiation [121,17,112]. Many studies have shown that BMP-2 promotes bone repair [112,122]. Early pre-clinical studies indicated that BMP-2 stimulates bone formation in critical size defects [123], fractures [104,124] and spinal fusions [97,125]. In a rabbit ulna model, BMP-2 in a collagen sponge accelerates repair [126] and overcomes the inhibitory effects of chronic glucocorticoid therapy on bone repair [124]. In rabbit tibial fractures, BMP-2 accelerates repair only in non-stable fractures, not in stable fractures [104].

Based on positive data in pre-clinical models, BMP-2 was tested in humans [111,112,122]. Consistent with animal studies, BMP-2 is effective in human fractures and was recently approved for this indication (Wyeth Pharmaceuticals; http://www.wyeth.com/news/Pressed_and_Released/pr11_21_2002_15_57_08.asp). BMP-2 in a collagen sponge and implant cage device (InFUSE™ bone graft) promotes lumbar interbody spinal fusion in humans [127]. Patients treated with InFUSE™ exhibit higher rates of fusion, improved neurological status, and reduced back and leg pain in comparison with patients undergoing autogenous iliac crest bone graft [127]. The InFUSE™ bone graft device has been approved in America for lumbar interbody spinal fusion(Medtronic Sofamor Danek; http://sofamordanek.com/about-press-fda.html).

Like BMP-2, BMP-7 or osteogenic protein 1 (OP-1) induces ectopic bone formation *in vivo* [102] and enhances bone repair in preclinical models [96,101,106–108] and clinical studies [109,110]. OP-1 promotes bone repair of critical defects in the rabbit ulna [107], long bones of dogs [106] and calvarial defects in adult male baboons [101]. Clinically, OP-1, delivered with a type-1 collagen carrier, promotes bridging of a critical defect in the fibula of patients undergoing an upper tibial osteotomy [110]. Based on clinical and radiographic endpoints, OP-1-induced repair was found to be equivalent to the 'gold-standard', autogenous bone graft, in a clinical trial of patients with tibial nonunions [109]. Furthermore, OP-1 treatment avoids the potential problems that accompany autografts, namely, the need for an additional operative site and the associated morbidity at that location [109]. Although OP-1 did not initially receive FDA approval, OP-1 was subsequently granted a humanitarian device exemption, for the treatment of established non-unions (Stryker; http://www.op1.com).

The fracture repair benefits of BMP-2 and OP-1 have been attributed to their ability to stimulate proliferation and differentiation of mesenchymal and osteoprogenitor cells [17,112,122]. Interestingly, effects of OP-1 on osteoblasts might be mediated, at least in part, by VEGF [38]. In addition, both BMP-2 and OP-1 are angiogenic. OP-1 induces angiogenesis in a baboon calvarial defect model [101] and the chick chorioallantoic membrane (CAM) assay [128], and synergistically enhances the angiogenic activity of FGF or TGFβ [128]. Although BMP-2 does not induce angiogenesis in the CAM or rabbit cornea assay [129], BMP-2 can induce new vessel formation through stimulation of VEGF [37].

Summary

Most osteogenic factors stimulate angiogenesis, if not directly, then indirectly, through production of angiogenic molecules, such as VEGF (Table 1). Similarly, most factors that promote bone formation have direct, yet distinct, effects on osteoblasts. For example, both VEGF and BMP-2 stimulate osteoblast differentiation, yet VEGF inhibits [27] and BMP-2 stimulates [130] osteoblast apoptosis. VEGF stimulates osteoblast differentiation but only BMPs, such as BMP-2 or OP-1, convert mesenchymal cells to committed osteoblasts. Both FGF-2 and TGF-β are angiogenic but FGF-2 does not induce osteoblast differentiation, and TGF-β is not a potent inducer of fracture repair. Factors that can couple the activity of endothelial cells (angiogenesis) with that of osteoblasts (bone formation) are likely to have a clear advantage if used as single agents to enhance bone repair.

Many growth factors have been shown to promote bone repair in animal models [7,9,10,17,131]. However, activity in young, healthy animals might not be predictive of that in older animals nor translate to the more complex clinical situation. For example, TGF-β and BMP-2 are less active in older animals [132,133], perhaps due to changes in the number of responsive cells, and the stimulatory effect of activin on rat fibular fracture repair occurs only in older growing rats [134]. Despite the potential limitations of preclinical studies, at least two molecules, BMP-2 and OP-1, have given positive results in clinical trials.

The past 250 years of bone studies have attempted to address fundamental questions [1–3]. Intravital microscopy and angiographic analysis in bone chamber models indicate that angiogenesis temporally precedes osteogenesis [135], as predicted by Trueta's early work [3]. Molecular and cellular biology, together with improvements in imaging and histological techniques, have resulted in the identification of molecules with osteogenic and angiogenic activity (Table 1). The role of these molecules in the intimate conversation between endothelial cells and bone cells is still being elucidated, but their reciprocal relationship [54,55] and the potential synergism between potent pro-angiogenic factors (such as VEGF) and strong osteoinductive factors (such as BMP-4 [33]), suggest that combination therapies might produce optimum results, particularly for individuals at risk for delayed repair or non-unions.

The next 250 years of research on bone repair are likely to begin with studies aimed at understanding the responsiveness of different cell types (inflammatory cells, endothelial cells, chondrocytes, osteoblasts, osteoclasts) to various treatments and the relative contribution of these cell types to clinical indices of repair. Optimizing drug delivery – through improved carriers, stem cells, gene therapy and/or slow-release formulations – is likely to allow for lower effective doses [72] and fewer side effects. Ongoing and future molecular, cellular, chemical and clinical studies might uncover additional molecules, such as angiopoietins [12], small-molecule mimetics [136] or inhibitors, to treat the complications associated with skeletal injuries. As science and technology converge, superior methods, such as advanced non-invasive imaging techniques, transcription [11] and protein profiling, and identification of biomarkers for safety and efficacy, could enable clinicians to identify when and with which drug(s) to treat each patient. In addition, doctors might be able to monitor pharmacokinetic profiles and therapeutic outcomes during the course of treatment. The study of fossilized bones believed to be ~160 000-years-old has shown how our skeletons can provide information to help bridge evolutionary gaps [137,138]. In the future, we might determine how to use the protein messages that are embedded within our bones as seeds for bone regeneration.

References

1 Haller, A. (1763) Experimentorum de ossiem formatione. In *Opera minora* (Vol. 2), pp. 400, Francisci Grasset
2 Hunter, J. (1794) *Treatise on the Blood, Inflammation and Gunshot Wounds.*, Thomas Bradford
3 Trueta, J. (1963) The role of the vessels in osteogenesis. *J. Bone Jt Surg. Ser A.* 45B, 402–418
4 Burkhardt, R. *et al.* (1987) Changes in trabecular bone, hematopoiesis and bone marrow vessels in aplastic anemia, primary osteoporosis, and old age: a comparative histomorphometric study. *Bone* 8, 157–164
5 Glowacki, J. (1998) Angiogenesis in fracture repair. *Clin. Orthop.* (Suppl 355), S82–S89
6 Praemer, A. *et al.* (1992) In *Musculoskeletal Conditions in the United States*, pp. 85–124, The American Academy of Orthopaedic Surgeons
7 Einhorn, T.A. (1995) Enhancement of fracture-healing. *J. Bone Joint Surg. Am.* 77, 940–956
8 Hausman, M.R. *et al.* (2001) Prevention of fracture healing in rats by an inhibitor of angiogenesis. *Bone* 29, 560–564
9 Einhorn, T.A. (1998) The cell and molecular biology of fracture healing. *Clin. Orthop.* (Suppl 355), S7–21
10 Mandracchia, V.J. *et al.* (2001) Current concepts of bone healing. *Clin. Podiatr. Med. Surg.* 18, 55–77
11 Hadjiargyrou, M. *et al.* (2002) Transcriptional profiling of bone regeneration. Insight into the molecular complexity of wound repair. *J. Biol. Chem.* 277, 30177–30182
12 Gerstenfeld, L.C. *et al.* (2003) Fracture healing as a post-natal developmental process: molecular, spatial, and temporal aspects of its regulation. *J. Cell. Biochem.* 88, 873–884
13 Grundnes, O. and Reikeras, O. (1993) The importance of the hematoma for fracture healing in rats. *Acta Orthop. Scand.* 64, 340–342
14 Mizuno, K. *et al.* (1990) The osteogenetic potential of fracture haematoma. Subperiosteal and intramuscular transplantation of the haematoma. *J. Bone Joint Surg. Br.* 72, 822–829
15 Street, J. *et al.* (2000) Is human fracture hematoma inherently angiogenic? *Clin. Orthop.* 378, 224–237
16 Street, J.T. *et al.* (2001) The angiogenic response to skeletal injury is preserved in the elderly. *J. Orthop. Res.* 19, 1057–1066
17 Barnes, G.L. *et al.* (1999) Growth factor regulation of fracture repair. *J. Bone Miner. Res.* 14, 1805–1815
18 Hauser, C.J. *et al.* (1997) The immune microenvironment of human fracture/soft-tissue hematomas and its relationship to systemic immunity. *J. Trauma* 42, 895–903
19 Street, J. *et al.* (2002) Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proc. Natl. Acad. Sci. U. S. A.* 99, 9656–9661
20 Le, A.X. *et al.* (2001) Molecular aspects of healing in stabilized and non-stabilized fractures. *J. Orthop. Res.* 19, 78–84
21 Rhinelander, F.W. (1974) Tibial blood supply in relation to fracture healing. *Clin. Orthop.* 105, 34–81
22 Brighton, C.T. and Hunt, R.M. (1991) Early histological and ultrastructural changes in medullary fracture callus. *J. Bone Joint Surg. Am.* 73, 832–847
23 Marsh, D. (1998) Concepts of fracture union, delayed union, and nonunion. *Clin. Orthop.* (Suppl 355), S22–S30
24 Ferguson, C. *et al.* (1999) Does adult fracture repair recapitulate embryonic skeletal formation? *Mech. Dev.* 87, 57–66
25 Tatsuyama, K. *et al.* (2000) Expression of various growth factors for cell proliferation and cytodifferentiation during fracture repair of bone. *Eur. J. Histochem.* 44, 269–278
26 Gerber, H.P. and Ferrara, N. (2000) Angiogenesis and bone growth. *Trends Cardiovasc. Med.* 10, 223–228
27 Street, J. *et al.* (2000) Multiple angiogenic cytokines protect against osteoblast apoptosis. *Surg. Forum* L1, 465–467
28 Zelzer, E. *et al.* (2002) Skeletal defects in VEGF(120/120) mice reveal multiple roles for VEGF in skeletogenesis. *Development* 129, 1893–1904
29 Gerber, H.P. *et al.* (1999) VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. *Nat. Med.* 5, 623–628
30 Haigh, J.J. *et al.* (2000) Conditional inactivation of VEGF-A in areas of collagen2a1 expression results in embryonic lethality in the heterozygous state. *Development* 127, 1445–1453
31 Ryan, A.M. *et al.* (1999) Preclinical safety evaluation of rhuMAbVEGF, an antiangiogenic humanized monoclonal antibody. *Toxicol. Pathol.* 27, 78–86
32 Chu, T.W. *et al.* (2002) [Effect of endothelial growth fracture in fracture healing]. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi* 16, 75–78
33 Peng, H. *et al.* (2002) Synergistic enhancement of bone formation and healing by stem cell-expressed VEGF and bone morphogenetic protein-4. *J. Clin. Invest.* 110, 751–759
34 Harada, S. *et al.* (1994) Induction of vascular endothelial growth factor expression by prostaglandin E2 and E1 in osteoblasts. *J. Clin. Invest.* 93, 2490–2496
35 Doan, N. *et al.* (1999) *In vitro* effects of therapeutic ultrasound on cell proliferation, protein synthesis, and cytokine production by human fibroblasts, osteoblasts, and monocytes. *J. Oral Maxillofac. Surg.* 57, 409–419
36 Claffey, K.P. *et al.* (2001) Fibroblast growth factor 2 activation of stromal cell vascular endothelial growth factor expression and angiogenesis. *Lab. Invest.* 81, 61–75
37 Deckers, M.M. *et al.* (2002) Bone morphogenetic proteins stimulate angiogenesis through osteoblast-derived vascular endothelial growth factor A. *Endocrinology* 143, 1545–1553
38 Yeh, L.C. and Lee, J.C. (1999) Osteogenic protein-1 increases gene expression of vascular endothelial growth factor in primary cultures of fetal rat calvaria cells. *Mol. Cell. Endocrinol.* 153, 113–124
39 Ferrara, N. *et al.* (2003) The biology of VEGF and its receptors. *Nat. Med.* 9, 669–676

40 Midy, V. and Plouet, J. (1994) Vasculotropin/vascular endothelial growth factor induces differentiation in cultured osteoblasts. *Biochem. Biophys. Res. Commun.* 199, 380–386

41 Deckers, M.M. *et al.* (2000) Expression of vascular endothelial growth factors and their receptors during osteoblast differentiation. *Endocrinology* 141, 1667–1674

42 Mayr-Wohlfart, U. *et al.* (2002) Vascular endothelial growth factor stimulates chemotactic migration of primary human osteoblasts. *Bone* 30, 472–477

43 Niida, S. *et al.* (1999) Vascular endothelial growth factor can substitute for macrophage colony-stimulating factor in the support of osteoclastic bone resorption. *J. Exp. Med.* 190, 293–298

44 Engsig, M.T. *et al.* (2000) Matrix metalloproteinase 9 and vascular endothelial growth factor are essential for osteoclast recruitment into developing long bones. *J. Cell Biol.* 151, 879–889

45 Nakagawa, M. *et al.* (2000) Vascular endothelial growth factor (VEGF) directly enhances osteoclastic bone resorption and survival of mature osteoclasts. *FEBS Lett.* 473, 161–164

46 Eckardt, H. *et al.* (2003) Effects of locally applied vascular endothelial growth factor (VEGF) and VEGF-inhibitor to the rabbit tibia during distraction osteogenesis. *J. Orthop. Res.* 21, 335–340

47 Hiltunen, M.O. *et al.* (2003) Adenovirus-mediated VEGF-A gene transfer induces bone formation *in vivo*. *FASEB J.* 17, 1147–1149

48 Tarkka, T. *et al.* (2003) Adenoviral VEGF-A gene transfer induces angiogenesis and promotes bone formation in healing osseous tissues. *J. Gene Med.* 5, 560–566

49 Colnot, C. *et al.* (2003) Altered fracture repair in the absence of MMP9. *Development* 130, 4123–4133

50 Ilizarov, G.A. *et al.* (1980) Reconstruction of large defects of blood vessels on extremities by means of a gradual distraction. (An experimental study). *Acta Chir. Plast.* 22, 156–165

51 Aronson, J. (1994) Experimental and clinical experience with distraction osteogenesis. *Cleft Palate Craniofac. J.* 31, 473–481

52 Pufe, T. *et al.* (2002) Quantitative measurement of the splice variants 120 and 164 of the angiogenic peptide vascular endothelial growth factor in the time flow of fracture healing: a study in the rat. *Cell Tissue Res.* 309, 387–392

53 Uchida, S. *et al.* (2003) Vascular endothelial growth factor is expressed along with its receptors during the healing process of bone and bone marrow after drill-hole injury in rats. *Bone* 32, 491–501

54 Wang, D.S. *et al.* (1997) Anabolic effects of 1,25-dihydroxyvitamin D3 on osteoblasts are enhanced by vascular endothelial growth factor produced by osteoblasts and by growth factors produced by endothelial cells. *Endocrinology* 138, 2953–2962

55 Bouletreau, P.J. *et al.* (2002) Hypoxia and VEGF up-regulate BMP-2 mRNA and protein expression in microvascular endothelial cells: implications for fracture healing. *Plast. Reconstr. Surg.* 109, 2384–2397

56 Bouletreau, P.J. *et al.* (2002) Factors in the fracture microenvironment induce primary osteoblast angiogenic cytokine production. *Plast. Reconstr. Surg.* 110, 139–148

57 Goad, D.L. *et al.* (1996) Enhanced expression of vascular endothelial growth factor in human SaOS-2 osteoblast-like cells and murine osteoblasts induced by insulin-like growth factor I. *Endocrinology* 137, 2262–2268

58 Wang, D.S. *et al.* (1996) Increase of vascular endothelial growth factor mRNA expression by 1,25-dihydroxyvitamin D3 in human osteoblast-like cells. *J. Bone Miner. Res.* 11, 472–479

59 Saadeh, P.B. *et al.* (1999) Transforming growth factor-beta1 modulates the expression of vascular endothelial growth factor by osteoblasts. *Am. J. Physiol.* 277, C628–C637

60 Harper, J. *et al.* (2001) Neuropilin-1 expression in osteogenic cells: down-regulation during differentiation of osteoblasts into osteocytes. *J. Cell. Biochem.* 81, 82–92

61 Kohno, S. *et al.* (2003) Expression of vascular endothelial growth factor and the effects on bone remodeling during experimental tooth movement. *J. Dent. Res.* 82, 177–182

62 Maes, C. *et al.* (2002) Impaired angiogenesis and endochondral bone formation in mice lacking the vascular endothelial growth factor isoforms VEGF164 and VEGF188. *Mech. Dev.* 111, 61–73

63 Carlevaro, M.F. *et al.* (2000) Vascular endothelial growth factor (VEGF) in cartilage neovascularization and chondrocyte differentiation: auto-paracrine role during endochondral bone formation. *J. Cell Sci.* 113, 59–69

64 Klagsbrun, M. (1989) The fibroblast growth factor family: structural and biological properties. *Prog. Growth Factor Res.* 1, 207–235

65 Gonzalez, A.M. *et al.* (1996) Distribution of fibroblast growth factor (FGF)-2 and FGF receptor-1 messenger RNA expression and protein presence in the mid-trimester human fetus. *Pediatr. Res.* 39, 375–385

66 Nakamura, T. *et al.* (1995) Stimulation of endosteal bone formation by systemic injections of recombinant basic fibroblast growth factor in rats. *Endocrinology* 136, 1276–1284

67 Nakamura, K. *et al.* (1998) Stimulation of bone formation by intraosseous injection of basic fibroblast growth factor in ovariectomised rats. *Int. Orthop.* 22, 49–54

68 Liang, H. *et al.* (1999) Bone anabolic effects of basic fibroblast growth factor in ovariectomized rats. *Endocrinology* 140, 5780–5788

69 Kawaguchi, H. *et al.* (1994) Stimulation of fracture repair by recombinant human basic fibroblast growth factor in normal and streptozotocin-diabetic rats. *Endocrinology* 135, 774–781

70 Bland, Y.S. *et al.* (1995) Exogenous fibroblast growth factors-1 and -2 do not accelerate fracture healing in the rabbit. *Acta Orthop. Scand.* 66, 543–548

71 Andreshak, J.L. *et al.* (1997) Tibial segmental defect repair: chondrogenesis and biomechanical strength modulated by basic fibroblast growth factor. *Anat. Rec.* 248, 198–204

72 Inui, K. *et al.* (1998) Local application of basic fibroblast growth factor minipellet induces the healing of segmental bony defects in rabbits. *Calcif. Tissue Int.* 63, 490–495

73 Kato, T. *et al.* (1998) Single local injection of recombinant fibroblast growth factor-2 stimulates healing of segmental bone defects in rabbits. *J. Orthop. Res.* 16, 654–659

74 Nakamura, T. *et al.* (1998) Recombinant human basic fibroblast growth factor accelerates fracture healing by enhancing callus remodeling in experimental dog tibial fracture. *J. Bone Miner. Res.* 13, 942–949

75 Radomsky, M.L. *et al.* (1999) Novel formulation of fibroblast growth factor-2 in a hyaluronan gel accelerates fracture healing in nonhuman primates. *J. Orthop. Res.* 17, 607–614

76 Kawaguchi, H. *et al.* (2001) Acceleration of fracture healing in nonhuman primates by fibroblast growth factor-2. *J. Clin. Endocrinol. Metab.* 86, 875–880

77 Montesano, R. *et al.* (1986) Basic fibroblast growth factor induces angiogenesis *in vitro*. *Proc. Natl. Acad. Sci. U. S. A.* 83, 7297–7301

78 Hayek, A. *et al.* (1987) An *in vivo* model for study of the angiogenic effects of basic fibroblast growth factor. *Biochem. Biophys. Res. Commun.* 147, 876–880

79 Collin-Osdoby, P. *et al.* (2002) Basic fibroblast growth factor stimulates osteoclast recruitment, development, and bone pit resorption in association with angiogenesis *in vivo* on the chick chorioallantoic membrane and activates isolated avian osteoclast resorption *in vitro*. *J. Bone Miner. Res.* 17, 1859–1871

80 Globus, R.K. *et al.* (1988) Regulation of bovine bone cell proliferation by fibroblast growth factor and transforming growth factor beta. *Endocrinology* 123, 98–105

81 Nakajima, F. *et al.* (2001) Spatial and temporal gene expression in chondrogenesis during fracture healing and the effects of basic fibroblast growth factor. *J. Orthop. Res.* 19, 935–944

82 Simmons, H.A. and Raisz, L.G. (1991) Effects of acid and basic fibroblast growth factor and heparin on resorption of cultured fetal rat long bones. *J. Bone Miner. Res.* 6, 1301–1305

83 Hurley, M.M. *et al.* (1998) Basic fibroblast growth factor induces osteoclast formation in murine bone marrow cultures. *Bone* 22, 309–316

84 Nakagawa, N. *et al.* (1999) Basic fibroblast growth factor induces osteoclast formation by reciprocally regulating the production of osteoclast differentiation factor and osteoclastogenesis inhibitory factor in mouse osteoblastic cells. *Biochem. Biophys. Res. Commun.* 265, 158–163

85 Kawaguchi, H. *et al.* (2000) Direct and indirect actions of fibroblast growth factor 2 on osteoclastic bone resorption in cultures. *J. Bone Miner. Res.* 15, 466–473

86 Warren, S.M. *et al.* (2003) The BMP antagonist noggin regulates cranial suture fusion. *Nature* 422, 625–629

87 Lane, N.E. *et al.* (2003) Basic fibroblast growth factor forms new trabeculae that physically connect with pre-existing trabeculae, and this new bone is maintained with an anti-resorptive agent and enhanced with an anabolic agent in an osteopenic rat model. *Osteoporos. Int.* 14, 374–382

88 Urist, M.R. (1965) Bone: formation by autoinduction. *Science* 150, 893–899

89 Wozney, J.M. *et al.* (1988) Novel regulators of bone formation: molecular clones and activities. *Science* 242, 1528–1534

90 Celeste, A.J. *et al.* (1990) Identification of transforming growth factor beta family members present in bone-inductive protein purified from bovine bone. *Proc. Natl. Acad. Sci. U. S. A.* 87, 9843–9847

91 Wozney, J.M. and Rosen, V. (1998) Bone morphogenetic protein and bone morphogenetic protein gene family in bone formation and repair. *Clin. Orthop.* 346, 26–37

92 Chang, H. *et al.* (2002) Genetic analysis of the mammalian transforming growth factor-beta superfamily. *Endocr. Rev.* 23, 787–823

93 Cho, T.J. *et al.* (2002) Differential temporal expression of members of the transforming growth factor beta superfamily during murine fracture healing. *J. Bone Miner. Res.* 17, 513–520

94 Joyce, M.E. *et al.* (1990) Transforming growth factor-beta in the regulation of fracture repair. *Orthop. Clin. North Am.* 21, 199–209

95 Lind, M. *et al.* (1993) Transforming growth factor-beta enhances fracture healing in rabbit tibiae. *Acta Orthop. Scand.* 64, 553–556

96 Margolin, M.D. *et al.* (1998) Maxillary sinus augmentation in the non-human primate: a comparative radiographic and histologic study between recombinant human osteogenic protein-1 and natural bone mineral. *J. Periodontol.* 69, 911–919

97 Muschler, G.F. *et al.* (1994) Evaluation of human bone morphogenetic protein 2 in a canine spinal fusion model. *Clin. Orthop.* 308, 229–240

98 Heckman, J.D. *et al.* (1999) Bone morphogenetic protein but not transforming growth factor-beta enhances bone formation in canine diaphyseal nonunions implanted with a biodegradable composite polymer. *J. Bone Joint Surg. Am.* 81, 1717–1729

99 Nielsen, H.M. *et al.* (1994) Local injection of TGF-beta increases the strength of tibial fractures in the rat. *Acta Orthop. Scand.* 65, 37–41

100 Noda, M. and Camilliere, J.J. (1989) *In vivo* stimulation of bone formation by transforming growth factor-beta. *Endocrinology* 124, 2991–2994

101 Ripamonti, U. *et al.* (1996) Complete regeneration of bone in the baboon by recombinant human osteogenic protein-1 (hOP-1, bone morphogenetic protein-7). *Growth Factors* 13, 273–289

102 Sampath, T.K. *et al.* (1992) Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation *in vivo* with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation *in vitro*. *J. Biol. Chem.* 267, 20352–20362

103 Sakai, R. *et al.* (2000) Activin increases bone mass and mechanical strength of lumbar vertebrae in aged ovariectomized rats. *Bone* 27, 91–96

104 Bax, B.E. *et al.* (1999) Bone morphogenetic protein-2 increases the rate of callus formation after fracture of the rabbit tibia. *Calcif. Tissue Int.* 65, 83–89

105 Beck, L.S. *et al.* (1991) Rapid publication. TGF-beta 1 induces bone closure of skull defects. *J. Bone Miner. Res.* 6, 1257–1265

106 Cook, S.D. *et al.* (1994) Recombinant human bone morphogenetic protein-7 induces healing in a canine long-bone segmental defect model. *Clin. Orthop.* 301, 302–312

107 Cook, S.D. *et al.* (1994) The effect of recombinant human osteogenic protein-1 on healing of large segmental bone defects. *J. Bone Joint Surg. Am.* 76, 827–838

108 Cook, S.D. *et al.* (1994) *In vivo* evaluation of recombinant human osteogenic protein (rhOP-1) implants as a bone graft substitute for spinal fusions. *Spine* 19, 1655–1663

109 Friedlaender, G.E. *et al.* (2001) Osteogenic protein-1 (bone morphogenetic protein-7) in the treatment of tibial nonunions. *J. Bone Joint Surg. Am.* 83A (Suppl 1), S151–S158

110 Geesink, R.G. *et al.* (1999) Osteogenic activity of OP-1 bone morphogenetic protein (BMP-7) in a human fibular defect. *J. Bone Joint Surg. Br.* 81, 710–718

111 Valentin-Opran, A. *et al.* (2002) Clinical evaluation of recombinant human bone morphogenetic protein-2. *Clin. Orthop.* 395, 110–120

112 Reddi, A.H. (2001) Bone morphogenetic proteins: from basic science to clinical applications. *J. Bone Joint Surg. Am.* 83A(Suppl 1), S1–S6

113 Rosier, R.N. *et al.* (1998) The potential role of transforming growth factor beta in fracture healing. *Clin. Orthop.* (Suppl 355), S294–S300

114 Bostrom, M.P. and Asnis, P. (1998) Transforming growth factor beta in fracture repair. *Clin. Orthop.* (Suppl 355), S124–S131

115 Joyce, M.E. *et al.* (1990) Transforming growth factor-beta and the initiation of chondrogenesis and osteogenesis in the rat femur. *J. Cell Biol.* 110, 2195–2207

116 Critchlow, M.A. *et al.* (1995) The effect of exogenous transforming growth factor-beta 2 on healing fractures in the rabbit. *Bone* 16, 521–527

117 Tielinen, L. *et al.* (2001) Inability of transforming growth factor-beta 1, combined with a bioabsorbable polymer paste, to promote healing of bone defects in the rat distal femur. *Arch. Orthop. Trauma Surg.* 121, 191–196

118 Sumner, D.R. *et al.* (1995) Enhancement of bone ingrowth by transforming growth factor-beta. *J. Bone Joint Surg. Am.* 77, 1135–1147

119 Lind, M. *et al.* (1996) Transforming growth factor-beta 1 stimulates bone ongrowth to weight-loaded tricalcium phosphate coated implants: an experimental study in dogs. *J. Bone Joint Surg. Br.* 78, 377–382

120 Erlebacher, A. *et al.* (1998) Osteoblastic responses to TGF-beta during bone remodeling. *Mol. Biol. Cell* 9, 1903–1918

121 Wang, E.A. *et al.* (1990) Recombinant human bone morphogenetic protein induces bone formation. *Proc. Natl. Acad. Sci. U. S. A.* 87, 2220–2224

122 Croteau, S. *et al.* (1999) Bone morphogenetic proteins in orthopedics: from basic science to clinical practice. *Orthopedics* 22, 686–695

123 Yasko, A.W. *et al.* (1992) The healing of segmental bone defects, induced by recombinant human bone morphogenetic protein (rhBMP-2). A radiographic, histological, and biomechanical study in rats. *J. Bone Joint Surg. Am.* 74, 659–670

124 Luppen, C.A. *et al.* (2002) Recombinant human bone morphogenetic protein-2 enhances osteotomy healing in glucocorticoid-treated rabbits. *J. Bone Miner. Res.* 17, 301–310

125 Sandhu, H.S. *et al.* (1995) Evaluation of rhBMP-2 with an OPLA carrier in a canine posterolateral (transverse process) spinal fusion model. *Spine* 20, 2669–2682

126 Bouxsein, M.L. *et al.* (2001) Recombinant human bone morphogenetic protein-2 accelerates healing in a rabbit ulnar osteotomy model. *J. Bone Joint Surg. Am.* 83A, 1219–1230

127 Burkus, J.K. *et al.* (2002) Clinical and radiographic outcomes of anterior lumbar interbody fusion using recombinant human bone morphogenetic protein-2. *Spine* 27, 2396–2408

128 Ramoshebi, L.N. and Ripamonti, U. (2000) Osteogenic protein-1, a bone morphogenetic protein, induces angiogenesis in the chick chorioallantoic membrane and synergizes with basic fibroblast growth factor and transforming growth factor-beta1. *Anat. Rec.* 259, 97–107

129 Yamashita, H. *et al.* (1997) Growth/differentiation factor-5 induces angiogenesis *in vivo*. *Exp. Cell Res.* 235, 218–226
130 Hay, E. *et al.* (2001) Bone morphogenetic protein-2 promotes osteoblast apoptosis through a Smad-independent, protein kinase C-dependent signaling pathway. *J. Biol. Chem*. 276, 29028–29036
131 Yoon, S.T. and Boden, S.D. (2002) Osteoinductive molecules in orthopaedics: basic science and preclinical studies. *Clin. Orthop.* 395, 33–43
132 Critchlow, M.A. *et al.* (1994) The effects of age on the response of rabbit periosteal osteoprogenitor cells to exogenous transforming growth factor-beta 2. *J. Cell Sci.* 107, 499–516
133 Fleet, J.C. *et al.* (1996) The effects of aging on the bone inductive activity of recombinant human bone morphogenetic protein-2. *Endocrinology* 137, 4605–4610
134 Sakai, R. *et al.* (1999) Local administration of activin promotes fracture healing in the rat fibula fracture model. *Bone* 25, 191–196
135 Winet, H. *et al.* (1990) A control model for tibial cortex neovascularization in the bone chamber. *J. Bone Miner. Res.* 5, 19–30
136 Paralkar, V.M. *et al.* (2003) An EP2 receptor-selective prostaglandin E2 agonist induces bone healing. *Proc. Natl. Acad. Sci. U. S. A.* 100, 6736–6740
137 Clark, J.D. *et al.* (2003) Stratigraphic, chronological and behavioural contexts of Pleistocene Homo sapiens from Middle Awash, Ethiopia. *Nature* 423, 747–752
138 White, T.D. *et al.* (2003) Pleistocene Homo sapiens from Middle Awash, Ethiopia. *Nature* 423, 742–747
139 Rodan, S.B. *et al.* (1989) Effects of acidic and basic fibroblast growth factors on osteoblastic cells. *Connect. Tissue Res.* 20, 283–288
140 Saadeh, P.B. *et al.* (2000) Mechanisms of fibroblast growth factor-2 modulation of vascular endothelial growth factor expression by osteoblastic cells. *Endocrinology* 141, 2075–2083
141 Slootweg, M.C. *et al.* (1988) Growth hormone is mitogenic for fetal mouse osteoblasts but not for undifferentiated bone cells. *J. Endocrinol.* 116, R11–R13
142 Gould, J. *et al.* (1995) Angiogenic activity of anterior pituitary tissue and growth hormone on the chick embryo chorio-allantoic membrane: a novel action of GH. *Life Sci.* 56, 587–594
143 Ernst, M. and Froesch, E.R. (1987) Osteoblastlike cells in a serum-free methylcellulose medium form colonies: effects of insulin and insulinlike growth factor I. *Calcif. Tissue Int.* 40, 27–34
144 Hansson, H.A. *et al.* (1989) Transient expression of insulin-like growth factor I immunoreactivity by vascular cells during angiogenesis. *Exp. Mol. Pathol.* 50, 125–138
145 Zerega, B. *et al.* (1999) Parathyroid hormone [PTH(1-34)] and parathyroid hormone-related protein [PTHrP(1-34)] promote reversion of hypertrophic chondrocytes to a prehypertrophic proliferating phenotype and prevent terminal differentiation of osteoblast-like cells. *J. Bone Miner. Res.* 14, 1281–1289
146 Carter, W.B. *et al.* (2000) Parathyroid-induced angiogenesis is VEGF-dependent. *Surgery* 128, 458–464
147 Risau, W. *et al.* (1992) Platelet-derived growth factor is angiogenic *in vivo*. *Growth Factors* 7, 261–266
148 Ziche, M. *et al.* (1982) Role of prostaglandin E1 and copper in angiogenesis. *J. Natl. Cancer Inst.* 69, 475–482
149 Yang, E.Y. and Moses, H.L. (1990) Transforming growth factor beta 1-induced changes in cell migration, proliferation, and angiogenesis in the chicken chorioallantoic membrane. *J. Cell Biol.* 111, 731–741
150 Breit, S. *et al.* (2000) The N-myc oncogene in human neuroblastoma cells: down-regulation of an angiogenesis inhibitor identified as activin A. *Cancer Res.* 60, 4596–4601
151 Spiro, R.C. *et al.* (2000) Inductive activity of recombinant human growth and differentiation factor-5. *Biochem. Soc. Trans.* 28, 362–368
152 Spiro, R.C. *et al.* (2001) Spinal fusion with recombinant human growth and differentiation factor-5 combined with a mineralized collagen matrix. *Anat. Rec.* 263, 388–395

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