Advanced Course on Tissue Remodeling
ACTR'04 – (pp.301–318) – Warsaw, 2004.

Cellular and Molecular Mechanisms of Bone Remodeling

Grzegorz Szczęsny [1,2], Wojciech Glinkowski [1,3,4], and Andrzej Górecki [1,3]

[1)] *Department of Orthopaedics and Traumatology of Locomotor System Medical University, Lindleya 4 02-005 Warsaw, Poland*

[2)] *Department of Surgical Research and Transplantology Medical Research Centre, Polish Academy of Sciences Pawińskiego 5 02-106 Warsaw, Poland*

[3)] *"TeleOrto" Center of Excellence*

[4)] *Department of Anatomy, Center of Biostructure Research Medical University, Chałubińskiego 5 02-005 Warsaw, Poland*

Bone remodeling is a continuous biological process leading to the replacement of the used bone by a newly formed one. The process restores function and continuity of the bone as whole skeleton and locally in particular bones, i.e. post fracture, immobilization, etc. During fracture healing phases remodeling covers the period of replacement of the woven bone within the fracture gap and callus by secondary osteonal bone. However, without previous fracture healing stages of the strict remodeling phase could not proceed. The callus remodeling leads to complete restoration of the injured bone, with no scar formation. It is possible to investigate and describe complicated mechanisms of bone remodeling based on the fracture healing process. The process of fracture repair is regulated by various molecular substances stimulating protein synthesis proliferation and chemotaxis of cells in the process of bone union. Many of those molecular substances BMPs, TGF-β, IGFs, PDGF and FGFs and interleukins evoke the most prominent influence on skeletal tissue. Selected topics of bone physiology, regulating molecules and mechanisms of remodeling and fracture healing are described in the paper.

1. Introduction

Bone remodeling is a continuous, biological process, supporting adaptation of the skeletal tissue to the local environment. Various cell populations take part in remodeling including mesenchymal progenitors being a reservoir of bone forming cells. Remodeling mechanisms are stimulated and regulated by molecular substances, namely growth factors, chemokines and cytokines. Molecular regulatory factors are controlled by local and/or systemic mechanical, chemical and molecular (including hormonal) environment. They are synthesized, released or activated due to several stimuli affecting the skeleton. The process of bone remodeling also enables ingrowths of porous coated implants and regeneration after fractures. Moreover, it leads to optimization of the internal structure of the skeletal tissue for its most effective weight-bearing and protective function. The phenomenon of bone remodeling proceeds due to cooperation of two main cellular components—osteoclasts, which degrade extracellular matrix dissolving minerals and digest its protein components, and osteoblasts forming new bone. In some specific way those two cellular populations cooperate; as soon as osteoclasts degrade the bone, osteoblasts fill the emptied spaces with newly formed bone matrix. The phenomenon of bone degradation and restoration is called "remodeling", and its intensity process is characterized by "bone turnover". The process consists of many remodeling episodes that proceed simultaneously in many places of the skeleton. Single remodeling phase starts as soon as osteclasts are being activated (Fig. 1). As osteoclasts transmigrate through the bone dissolving its mineral components and digesting extracellular proteins, vascular loops are created from the surrounding blood vessels in angiogenesis. Newly formed blood vessels originate from activated endothelial cells, which proliferate, migrate (chemotaxis in the gradient of molecular stimuli), and form three-dimensional tubulae. As soon as blood starts to circulate in newly formed vascular loops, osteoblastic progenitor cells originating from circulating stem cells repopulate spaces freed after osteoclastic digest of bone matrix, and differentiate into osteoblastic cells lineage. Osteoblasts synthesize and release extracellular matrix proteins and activators of calcification, forming a "new" bone filling erosions made by osteoclasts. The sequence of groups of osteoclasts followed by osteoblasts form the structure of cutting cone. Those cones have activated osteoclasts at the top, followed by vascular loops, and osteoblasts rebuilding extracellular matrix. The remodel-

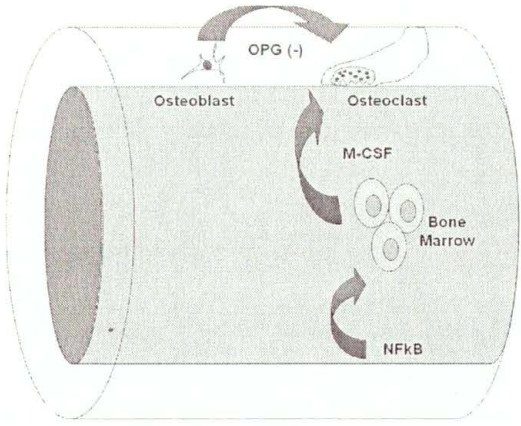

FIGURE 1. *Activation of osteoclasts.* Maturation of monocytes/macrophages into osteoclasts requires M-CSF, which is produced and secreted by bone marrow cells stimulated by the NF-kB. RANK (also known also as OPG) and its ligand (RANKL, OPGL) are members of the TNF-alpha superfamily. See text for description of the abbreviations.

ing is a permanent process throughout the whole life. Its intensity is highest at adolescence, achieves plateau in adults and gradually decreases after menopause/andropause. Remodeling proceeds in thousands of places at the same moment. Multilocular process recreates three dimensional structures of bone trabeculae affected by mechanical strains.

During the development of the skeleton the process of modeling leads to the formation of proper three-dimensional structure of the bone. The modeling transforms bones from amorphous, calcifying cartilage. Repeating episodes of the modeling introduce vascular tree into the bone as a consequence of bone modeling. Newly formed blood vessels remain in the cortical bone after each "passage" of the cutting cone. Moreover, modeled bone after cutting cone passage is no longer amorphous. Three dimensional structure of osteon, with blood capillaries located at the centre, and bone lamellae are formed. Moreover, free spaces between trabeculae are settled by bone marrow cells. Osteons and bone marrow appear due to modeling of the bone. There is an obvious functional link between bone and bone marrow, as bone supports bone marrow cells as "scaffold". Apart from that, bone marrow provides molecular environment for skeletal development, regeneration and physiology. Disorders affecting one of them usually influence the other. There is a continuous adaptation process affecting the external shape of the skeleton and

its internal structure according to the mechanical forces loading the bones. Thus, the structure of the bone is functionally adapted to the local mechanical environment. However, the shape of the skeletal bones is determined by the genetic factors. The rise of mineral content is observed in the overloaded skeleton. Bone mineral content decreases in disuse (long-lasting limb immobilization with reduced weight-bearing or space flights in weightlessness).

The mechanical loads affect the external shape and internal structure of the bone. It allows optimizing the structure of the bone assuring its maximal strength. During skeletal development mechanical factors are considered as an important risk factor of neonatal anatomical deformations (flat foot, club foot or developmental hip dysplasia, etc.).

Bone remodeling phenomenon enables full restoration of the bone structure ("restitutio ad integrum") lost due to fracture. Repaired bone achieves the restoration of its continuity on macroscopic and microscopic level. The healed bone tissue regenerates completely with no connective tissue scar.

The changes in the net concentration of the mineral components of the skeleton are regulated by the differences between dissolve and deposition of bone minerals. Furthermore, plasma calcium ions released from its deposits in the extracellular matrix hydroxyapatites, participate in the calcium-dependent homeostasis. They are required for activation of peptides (coagulation factors, etc.) and are important for various intracellular processes. The remodeling mechanisms are able to accommodate circadian fluctuation in calcium balance and supply a temporary need for additional calcium lasting for a few months. The remodeling apparatus may slow elimination of surplus bone in response to the age-related decline in physical activity. Plasma calcium homeostasis requires rapid ion exchange, which is impeded by progression of secondary mineralization and loss of water from the surface mineral. Calcium homeostasis can be considered as one of metabolic expressions of bone remodeling, locally regulated by mechanical loads, and generally by the hormonal influence on the bone (growth hormone, estrogens, and parathormone).

2. Bone Physiology—Cells Responsible for Bone Remodeling

Bone physiology requires cooperation of various cells, including bone forming (osteoblasts, osteocytes) and bone resorbing cells (osteoclasts). Cells derived form other tissues as cartilage (chondroblasts, chondrocytes, chon-

droclasts), immune cells (macrophages, lymphocytes), and their progenitors of mesenchymal origin are involved also into the bone physiology.

Osteoclasts are bone resorbing cells of hematopoietic origin; all others originate from mesenchymal stem cells. Stem cells form a reservoir pool of cells for tissue regeneration. They are located in the inner layer of the endosteum, superficially to the hematopoietic cell lineages, near the cortical bone and distant to the venous sinusoids [1]. Stem cells activated by molecular stimuli, differentiate into a particular cell lineage in response to the local molecular and metabolic influences. Progenitor cells, transmigrating into the donor site, repopulate osteolysed bone and restore its structure (osteogenesis, regeneration) [2].

Several attempts have been made to characterize factors recruiting progenitors for tissue regeneration. They allowed to associate systemic inflammatory response to injury [3, 4] with tissue regeneration process (Fig. 2). Molecular mediators involved both in the systemic inflammatory response and in reparative process. It was suggested that stem cells are stimulated by various interleukins as IL-3 (Interleukin-3) and IL-11 [5], IL-6, soluble form of IL-6 (sIL-6R) [6, 7], IL-8 and also SCF-1 (stromal cell factor-1) as well through IL-6 receptor (IL-6R) [8].

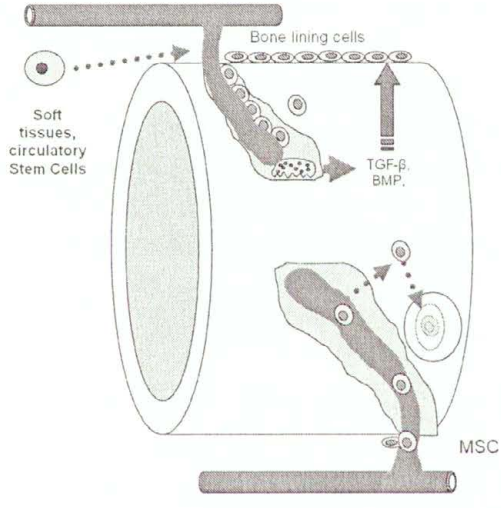

FIGURE 2. *Migration, extravasation of preosteoblasts and synthesis of bone matrix proteins.* Osteoblasts transmigration: activated from their precursors (mesenchymal stem cells) osteoblasts migrate through vascular loops. Once osteoblasts transmigrate through vascular wall they transform into osteocyte.

The mechanism activating cells to differentiate into a particular cell lineage is not fully understood. Nevertheless, it has been established that low tissue oxygenation is responsible for chondroblastic instead of osteoblastic differentiation, pointing out to the importance of adequate blood supply in the fracture repair [9]. The migration of the stem cells from bone marrow into destination site requires chemotactic stimuli, including SDF-1 (stromal cells derived factor-1), IL-8 and co-stimulation by SCF-1, involving activation of enzymatic break-down of the matrix proteins by matrix metalloproteinase's (e.g. MMP-9) [10]. Stem cells accumulate at the high SDF-1 concentration sites [11]. Increased SDF-1 expression is observed as a factor of bone marrow repopulation, tissue repair and heterotopic ossification [12].

Osteoblasts are formed from progenitors located inside the bone and proliferative layer of periosteum and endosteum. Their precursors are located in bone marrow, soft tissues adjacent to the bone, especially in striated, skeletal muscles, pericytes of blood vessels, and circulating blood as well [13, 14]. Osteoblasts present almost similar function and morphology to fibroblasts. All genes expressed by fibroblasts are also expressed by osteoblasts [15]. However, exceptional for osteoblasts is their ability to express genes for transcription factor Cbfa1 [16, 17], Osx [18] and osteocalcin coding gene (bone GLA protein) [19].

Osteoclasts are multinuclear cells formed from their precursors originating from monocyte/macrophage lineage in the bone marrow. Osteoclasts locate on the surface of the bone. They produce proteolytic enzymes (collagenases, hydrolases, and proteases) that lead to degradation of the mineral and organic components of its matrix at the low pH (below 4). They are responsible for the bone resorption. The differentiation of monocytes/macrophages cell lineage into osteoclasts requires activation by M-CSF (macrophage-colony stimulating factor), synthesized and secreted by bone marrow cells stimulated by the ligand of receptor for activation of the nuclear factor kappa B (NF-κB) [20, 21]. RANK (receptor for activator of NF-κB, located on osteoclastic cell membranes) and its ligand (RANKL, expressed by osteoblasts) are members of the TNF-α (tumor necrosis factor-α) superfamily [22, 23]. Osteoclasts are activated by RANKL through membrane receptors RANK for increasing their osteolytic capacity. Osteoprotegerine (OPG), a decoy receptor for RANKL, is secreted by osteoblasts. It inhibits osteoclasts differentiation and activation what leads to blocking their osteolytic capacities [24]. That mechanism allows osteoblasts to control osteoclastic activity. The spe-

cific equilibrium between osteolysis and osteogenesis balances the bone mass throughout life. Activated osteoblasts produce and release factors stimulating and controlling osteoclasts maturation [25, 26]. Osteoclastic degradation of the extracellular matrix releases growth factors (stimulating osteogenesis). Those factors are stored in a latent form in the extracellular matrix. This functional link between osteoblasts and osteoclasts clearly proceeds in early phases of bone formation. Once osteoblasts and osteoclasts are fully differentiated, this cooperation is not so clear.

Decrease of OPG-dependent down-regulation of osteoclastic cell lineages is assumed as the most obvious mechanism activating bone remodeling and repair.

Osteoblasts transmigrating into the excavated spaces after bone resorption inhibit osteoclast-dependent bone resorption through the OPG, and secrete and release extracellular matrix proteins. As soon as extracellular matrix begins to calcify, osteoblasts transform into osteocytes. Mononuclear cells, located in the lacunae filled with the non-calcified extracellular matrix, form a network of long cytoplasmic appendices making direct connections between bone cells [27].

Chondrocytes may participate in osteogenesis in two ways—as a scaffold for skeletal development (endochondral ossification) and fracture repair (endochondral fracture healing). Differentiating from their mesenchymal progenitors and accumulating in the fracture site in response to molecular stimuli released during the inflammatory phase of the healing process [28, 29]. Chondrocytes filing the fracture gap replace hematoma and form a scaffold for forthcoming angiogenesis and osteoblastic infiltration [30]. Moreover, chondrocytes actively participate in the process of ossification through synthesizing and releasing various enzymes and growth factors creating proper microenvironment for osteoblast immigration [31, 32].

3. Selected Mechanisms Related to Fracture Healing and Bone Remodeling

Bone fracture could be repaired in the process of endochondral ossification (mostly observed during long bones fractures healing) or intramembranous ossification. The process of endochondral ossification leads to the new bone formation "per analogiam" skeletogenesis. This process leads to mineralization of cartilaginous tissue in the consequence of action of proteases, phosphatases, and calcium ions exocytosis from chondrocytes [33]. Calcified

cartilage stimulates formation of microvascular loops sprouting from the vessels of the bone adjacent to fracture gap, and immigration of chondroclasts and osteoblasts through those newly formed vessels. Osteoblasts, transformed from activated precursors, transmigrate through microvasculature and colonize cartilaginous tissue. Once osteoblasts colonize cartilage, they start to produce and secrete specific proteins changing the composition of the extracellular matrix (replacing the cartilage collagen type II to osseous collagen type I [34], fibronectin, osteocalcin and osteopontin) [35]. Deposition of calcium phosphate around osteoblasts forms specific lamellar structures, and immobilizes osteoblasts in the extracellular matrix, where they transform into residual bone cells—osteocytes [36].

Intramembranous ossification proceeds due to proliferation and differentiation of activated mesenchymal progenitors directly into osteoblasts. It is suggested that TGF-β1 is responsible for this process [37]. TGF-β and BMP—Smad-dependent pathway was shown to be responsible for activation, proliferation and differentiation of osteoblastic progenitors [38], stimulation of osteoclasts for synthesis and release of growth factors, particularly BMPs, and extracellular matrix proteins, including type I collagen. [39].

Osteoblasts transmigrating into the fracture gap, restore bone continuity and structure. The crucial role in fracture repair is played by angiogenesis, i.e. the process of restoration of bone microvasculature. It starts immediately after fracture, and is mediated by cytokines and growth substances released from blood clot, traumatized soft tissues, bone cells and bone matrix. Activated osteoblasts transmigrate through the endothelium repopulating the fracture gap, as soon as blood starts to flow through newly formed vascular loops. Osteoblasts settle down around the blood vessels and then transform into osteocytes. They synthesize and release extracellular matrix proteins (type I collagen), biologically active substances (growth factors like Bone Morphogenetic Proteins, Transforming Growth Factor Beta, etc...). Since the process of intramembranous ossification is less complicated than endochondral one, it proceeds much faster. This type of fracture repair usually occurs without, or with only minimal external callus formation. Clinically, it is usually observed in anatomically reduced, firmly stabilized fractures without severe damage of bone fragments and surrounding soft tissues, with minimal blood volume extravasated into the fracture gap [40].

4. Molecular Regulators of Bone Physiology

Bone physiology is regulated by the molecular substances influencing cellular components of bone and surrounding tissues. Those substances are attracted to fracture gap from: the cellular components of blood extravasated into the fracture gap (platelets and immune cells), bone matrix and bone cells, surrounding soft tissues (i.e. skeletal muscle), and endings of the peripheral nervous system. Genetic control over bone remodeling is regulated through various gene expressions and also mechanisms of apoptosis [41]. Fracture healing is upregulated by various genes, expressed to affect all aspects of intracellular and extracellular processes, from transcription, intracellular signaling and extracellular protein synthesis and cell-matrix communication [42,43]. Up-regulation of signaling molecules, particularly growth factors and cytokines, lead to activation of cellular populations forming skeletal tissue from their undifferentiated precursors, their migration and homing at the site of injury [44]. They also regulate other processes, i.e. angiogenesis, providing vascularity needed for stem cells immigration and proper blood supply for newly formed bone [45].

Local ischemia and subsequent hypoxia and necrosis of osteocytes, result in the decrease of OPG expression [46]. Osteoclasts which are not sufficiently OPG-suppressed keep their activation, proliferation and migration, what finally increases degradation of the bone matrix.

Peptides from TGF-β family play an important role in skeletogenesis, chondrogenesis, bone remodeling and fracture repair [47, 48]. They possess capability to stimulate formation of the osteoblast progenitors from the non-differentiated mesenchymal stem cells [49]. Bone Morphogenetic Proteins belong to the most important factors of induction of the bone regeneration after injury [50]. The expression of BMP-2 and BMP-4 was observed in primary, non-differentiated mesenchyme during chondrogenesis [51], intramembranous bone healing [52] and morphogenesis as well [53]. The regulatory function of BMP-2 and BMP-3 on differentiation of mesenchymal cells was found during chondrogenesis [54]. But BMP-6 was detected as stimulator of chondrocyte maturation [55]. In opposite to other growth factors like PDGF, TGF-β, IGF, only BMPs have the ability to stimulate connective tissue pericytes to form the bone *de novo*.

BMPs transduce metabolic signals through two transmembrane receptors: type I and type II (BMP-RI and BMP-RII; both serine/threonine kinase receptors) [56]. The signal transduction into the nucleus proceeds through

the phosphorylation of Smad proteins, leading in consequence to activation of specific genes, or alternatively through Wnt signaling pathway [57]. A number of known BMPs antagonists (Noggin, Chordin, Gremlin, Dan, Sclerostatin and others) modulate their signaling, being regulated not only by local BMP synthesis and activation, but also by their antagonists [58].

Platelet Derived Growth Factor (PDGF) is a potent mitogen of cells of a mesenchymal origin that could also be found in bone cells. It was found that PDGF stimulate proliferation, chemotaxis and synthesis of matrix proteins by osteoblasts [59] as well as proliferation and differentiation of chondroblasts [60]. Increased concentration of PGDF protein and its mRNA was found at the sites of endochondral bone formation and bone remodeling [61].

The most potent influence on osteoblasts evokes the isomer PDGF-BB [62] which stimulates chemotaxis of various cells of mesenchymal origin, including osteoblasts [63]. It was found as a potent activator of fibroblast proliferation [64, 65]. PDGF-BB also activates fibroblasts to increase of DNA synthesis and proteins production. Nash and al. [66] injecting PDGF into a fracture gap showed its stimulatory effect on callus formation and acceleration of fracture healing. It was also shown that PDGF may lead to bone resorption by enhancement of prostaglandin (PG) synthesis and rise of intracellular PGE_2 concentration in bone cells [67] and $PGF_{2\alpha}$ in *in vitro* cultures of mesangial cells [68].

Osteoblasts synthesize and release IGF-I i IGF-II into extracellular matrix for further storage in latent form. Both forms evoke almost the same biological effects, but the effect of IGF-I is 4-7 times higher than IGF-II [69]. Production and release of bone IGFs depends on hormonal regulation of the parathormone (PTH) and the growth hormone (GH). It seems to be possible that biological effect of GH on bone is mediated by IGF's. The most important influence of IGFs on bone is their potent activation of growth plates. IGFs, present in serum in nanomolar concentrations are mostly synthesized in liver. They activate chondroblasts to release proteoglycans and osteoblasts to produce collagen [70, 71]. Their biological half time is about few minutes, increases up to 15 hours when bound with one of the seven known IGFs binding proteins (IGF-BPs). Among them IGF-BP3 binds 80-95% of whole circulating IGF-I and IGF-II. Together with ALS (acid labile subunit) it forms a storage form of IGF - stable protein complex [72] and increases the resistance of its degradation, prolongs their half-time, modulates the biological effect of IGFs, and regulates IGFs transport [73].

The FGF-1 and FGF-2 (known previously as acidic a-FGF and basic b-FGF) are the best known from the seven already distinguished FGFs. Both proteins possess 53% homology and express the capability to bind heparin. Both forms have the molecular weight of 17-18kD, although the forms with higher molecular weight (21-25 kD) occur as a consequence of development of N-terminal peptide chain. FGF binds to membraneous receptor, which results in intracellular activation of protein kinase C [74]. It was shown that during fracture repair the activated fibroblasts express FGF-2. When injected into fracture gap, FGF increases callus formation and synthesis of collagen and proteoglycans [75]. It was also shown that FGF increases expression of TGF-β in osteoblasts [76]. An application of autologous cells containing high amount of regulatory substances, i.e. blood platelets, is a permissible alternative. Clinical results with the platelet-rich plasma require further investigations. They could be used as a natural, autogenic source of growth factors regulating the regenerative process [77, 78].

5. Conclusion

Bone remodeling appears in later stage of fracture repair. It is possible to investigate and describe complicated mechanisms of bone remodeling based on fracture healing process.

The process of fracture repair is regulated by molecular substances stimulating protein synthesis proliferation and chemotaxis of cells orchestrating in the process of union. From those molecular substances BMPs, TGF-β, IGFs, PDGF and FGFs and interleukins evoke the most prominent influence on skeletal tissue.

References

1. S.K. Nilsson, H.M. Johnston, and J.A. Coverdale, *Spatial localization of transplanted hemopoietic stem cells: inferences for the localization of stem cell niches*, Blood, **97** : 2293–2299, 2001.

2. M.F. Pittenger, A.M. Mackay, S.C. Beck et al., *Multilineage potential of adult human mesenchymal stem cells*, Science, **284** : 143–147, 1999.

3. P.V. Giannoudis, *Current concepts of the inflammatory response after major trauma: an update*, Injury, **34** : 397–404, 2003.

4. J. KIVIOJA, V. OZENCI, L. RINALDI ET AL., *Systemic immune response in whiplash injury and ankle sprain: elevated IL-6 and IL-10*, Clin. Immunol., **101**: 106–112, 2001.

5. G.P. VEER REDDY, C.I. MCAULIFFE, L. PANG ET AL., *Cytokine receptor repertoire and cytokine responsiveness of Hodull/Rhdull stem cells with differing potentials for G/S phase progression*, Exp. Hematol., **30**: 792–800, 2002.

6. S. ROSE-JOHN, *GP130 stimulation and the maintenance of stem cells*, TRENDS in Biotech, **20**: 417–419, 2002.

7. B. KLARLUND PEDERSEN, A. STEENSBERG, and P. SCHJERLING, *Muscle-derived interleukin-6: possible biological effects*, J. Physiol., **536**: 329–337, 2001.

8. I. CORRE, D. PINEAU, and S. HERMOUET, *Interleukin-8: an autocrine/paracrine growth factor for human hematopoietic progenitors acting in synergy with colony stimulating factor-1 to promote monocyte-macrophage growth and differentiation*, Exp. Hematol., **27**: 28–36, 1999.

9. J.E. SCOTT, *Oxygen and the connective tissues*, Trends Biochem Sci., **17**: 340–343, 1992.

10. T. LAPIDOT and I. PETIT, *Current understanding of stem cell mobilization: The roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells*, Exp. Hematol. **30**: 973–981, 2002.

11. J.P. LEVESCUE, J. HENDY, Y. TAKAMATSU ET AL., *Disruption of the CXCR4/CXCL12 chemotactic interaction during hematopoietic stem cell mobilization induced by GCSF or cyclophosphamide*, J. Clin. Invest., **111**: 187–196, 2003.

12. M. KUCIA, J. RATAJCZAK, R. RECA ET AL., *Tissue - specific muscle, neural and liver stem/progenitor cells reside in the bone marrow, respond to an SDF-1 gradient and are mobilized into peripheral blood during stress and tissue injury*, Blood Cells, Molecules and Disease, **32**: 52–57, 2004.

13. L. DIAZ-FLORES, R. GUITERREZ, A. LOPEZ-ALONSO ET AL., *Pericytes as a supplementary source of osteoblasts in periosteal osteogenesis*, Clin. Orthop., **275**: 280–286, 1992.

14. S. HARADA and G.A. RODAN, *Control of osteoblast function and regulation of bone mass*, Nature, **423**: 349–355, 2003.

15. J.M. LANE and J.R. WERNTZ, *Biology of fracture healing*, In: J.M. Lane (ed.), *Fracture Healing*, Churchill Livingstone 1987.

16. H. ENOMOTO, M. ENOMOTO-IWAMOTO, M. IWAMOTO ET AL., *Cbfa1 is a positive regulatory factor in chondrocyte maturation*, J. Biol. Chem., **275**: 8695–8702, 2000.

17. P. DUCY, T. SCHINKE, and G. KARSENTY, *The osteoblast: a sophisticated fibroblast under central surveillance*, Science, **289**: 1501–1504, 2000.

18. X. YANG and G. KARSENTY, *Transcription factors in bone: developmental and pathological aspects*, Trends in Mol. Med., **8**: 340–345, 2002.

19. P. DUCY, C. DESBOIS, B. BOYCE ET AL., *Increased bone formation in osteocalcin-deficient mice*, Nature, **382**: 448–452, 1996.

20. W.S. SIMONET, D.L. LACEY, C.R. DUNSTAN ET AL., *Osteoprotegerin: a novel secreted protein involved in the regulation of bone density*, Cell, **89**: 309–319, 1997.

21. E. FILVAROFF and R. DERYNCK, *Bone remodelling: a signalling system for osteoclast regulation*, Curr. Biol., **8**: R679–682, 1998.

22. S.L. TEITELBAUM, *Bone resorption by osteoclasts*, Science, **289**: 1504–1508, 2000.

23. G.A. RODAN and T.J. MARTIN, *Therapeutic approaches to bone diseases*, Science, **289**: 1508–1514, 2000.

24. W. WITTRANT, S. COUILLAUD, S. THEOLEYRE ET AL., *Osteoprotegerin differentially regulates protease expression in osteoclast cultures*, Bioch. Biophys. Res. Comm., **293**: 38–44, 2002.

25. Q. QU, P.L. HARKONEN, J. MONKKONEN, and H.K. VAANANEN, *Conditioned medium of estrogen-treated osteoblasts inhibits osteoclast maturation and function in vitro*, Bone, **25**: 211–215, 1999.

26. W.J. BOYLE, W.S. SIMONET, and D.L. LACEY, *Osteoblast differentiation and activation*, Nature, **423**: 337–342, 2003.

27. P.A. REVELL, *Pathology of bone*, Springer Verlag, New York, 1986.

28. B. MCKIBBIN, *The biology of fracture healing in long bones*, J. Bone Joint Surg. (Br), **60**: 150–162, 1978.

29. T.A. EINHORN, R.J. MAJESKA, E.B. RUSH ET AL., *The expression of cytokine activity by fracture callus*, J. Bone Min. Res., **10**: 1272–1281, 1995.

30. T.A. EINHORN, *The cell and molecular biology of fracture healing*, Clin. Orthop. Rel. Res. **355S**: S7–S21, 1999.

31. B.M. CLANCY, J.D. JOHNSON, and A.J. LAMBERT ET AL., *A gene expression profile for endochondral bone formation: oligonucleotide microarrays establish novel connections between known genes and BMP-2-induced bone formation in mouse quadriceps*, Bone, **33**: 46–63, 2003.

32. R.K. AARON, S. WANG, and MCK. D. CIOMBOR, *Upregulation of basal TGFSS1 levels by EMF coincident with chondrogenesis - implications for skeletal repair and tissue engineering*, J. Orthop. Res., **20**: 233–240, 2002.

33. M. FERRETTI, C. PALUMBO, M. CONTRI, and G. MAROTTI, *Static and dynamic osteogenesis: two different types of bone formation*, Anat. Embryol. (Berl.), **206**(1-2): 21–29, 2002.

34. D.M. LAWTON, J.G. ANDREW, and D.R. MARSH ET AL., *Expression of the gene encoding the matrix gla protein by mature osteoblasts in human fracture non-unions*, Mol. Pathol., 52(2): 92–96, 1999.

35. G. DACULSI, P.PILET, M. COTTREL, and G. GUICHEUX, *Role of fibronectin during biological apatite crystal nucleation: ultrastructural characterization*, J. Biomed. Mater. Res., **47**: 228–233, 1999.

36. J.A. BUCKWALTER, M.J. GLIMCHER, R.R. COOPER, and R. RECKER, *Bone biology. Part II: formation, form, remodeling and regulation of cell function*, J Bone Joint Surg., **77-A**: 1276–1289, 1995.

37. D.S. STEINRECH, B.J. MEHRARA, and N.M. ROWE ET AL., *Gene expression of TGF-β, TGF-β receptor, and extracellular matrix proteins during membranous bone healing in rats*, Plast. Reconstr. Surg., **105**: 2025–2038, 2000.

38. H. QI, D.J. AGUIAR, S.M. WILLIAMS, A. LA PEAN ET AL., *Identification of genes responsible for osteoblast differentiation from human mesodermal progenitor cells*, PNAS., **100**:3305–3310, 2003.

39. J.A. SPECTOR, J.S. LUCHS, B.J. MEHRARA ET AL., *Expression of bone morphogenetic proteins during membranous bone healing*, Plast. Reconstr. Surg., **107**:124–134, 1999.

40. J. HOLLINGER and M.E.K. WONG, *The integrated processes of hard tissue regeneration with special emphasis on fracture healing*, Oral Surg. Oral Med. Oral Pathol., **82**:594–606, 1996.

41. J.M. HOCK, V. KRISHNAN, J.E. ONYIA, J.P. BIDWELL, J. MILAS, and D. STANISLAUS, *Osteoblast apoptosis and bone turnover*, J. Bone Miner. Res., **16**:975–984, 2001.

42. M. HADJIARGYROU, F. LOMBARDO, S. ZHAO ET AL., *Transcriptional Profiling of Bone Regeneration. Insight into the molecular complexity of wound repair*, J. Biol. Chem., **277**(33):30177–30182, 2002.

43. G. WIECZOREK, C. STEINHOFF, R. SCHULZ ET AL., *Gene expression profile of mouse bone marrow stromal cells determined by cDNA microarray analysis*, Cell Tissue Res., **311**:227–237, 2003.

44. K. YAGAMI, Y. UYAMA, Y. YOSHIZAWA ET AL., *A human chondrogenic cell line retains multi-potency that differentiates into osteoblasts and adipocytes*, Bone, **34**:648–655, 2004.

45. M. HADJIARGYROU, W. AHRENS, and C.T. RUBIN, *Temporal expression of th chondrogenic and angiogenic growth factor CYR61 during fracture repair*, J. Bone Min. Res., **15**(6):1014–1023, 2000.

46. S. KHOSLA, *Minireview the OPG/RANKL/RANK system*, Endocrinology, **142**(12):5050–5055, 2001.

47. M.W.LONG, J.A. ROBINSON, E.A. ASHCRAFT, and K.G. MANN, *Regulation of human bone-derived osteoprogenitor cells by osteogenic growth factors*, J. Clin. Invest., **95**:881–887, 1995.

48. M. IWASAKI, H. NAKAHARA, K. NAKATA ET AL., *Regulation of proliferation and osteochondrogenic differentiation of periosteum-derived cells*

by transforming growth factor-beta and basic fibroblast growth factor, J. Bone Joint Surg. **77-A** : 543–554, 1995.

49. S.E. GITELMAN, M.S. KOBRIN, J.Q. YE ET AL., *Recombinant Vgr-1/BMP-6-expressing tumors induce fibrosis and endochondral bone formation in vivo*, J. Cell Biol., **126** : 1595–1609, 1994.

50. A. HULTH, O. JOHNELL, and A. HENRICSON, *The implantation of demineralized fracture matrix yields more bone formation than does intact matrix*, Clin. Orthop. Rel. Res., **234** : 235–239, 1988.

51. K. ELIMA, *Osteoinductive proteins*, Ann. Med., **25** : 395–402, 1993.

52. P.H. FRANCIS, M.K. RICHARDSON, P.M. BRICKNELL, and C. TICKLE, *Bone morphogenetic proteins and a signaling pathway that controls patterning in the developing chick limb*, Development, **120** : 209–218, 1994.

53. J.S. LUCHS, P.J. MEHRARA, M.F. PACCIONE ET AL., *The expression of bone morphogenetic proteins 2/4 and 7 during membranous fracture healing*, Surg. Forum, **50** : 530–531, 1999.

54. J.L. CARRINGTON, P. CHEN, M. YANANGISHITA, and A.H. REDDI, *Osteogenin (BMP-3) stimulates cartilage formation by chick limb bud cells in vitro*, Dev. Biol., **146** : 406–415, 1991.

55. C.D. GRIMSRUD, P.R. ROMANO, M. D'SOUZA ET AL., *BMP-6 is an autocrine stimulator of chondrocyte differentiation*, J. Bone Min. Res., **14** : 475–482, 1999.

56. C.H. HELDIN, K. MIYAZONO, and P. TEN DIJKE, *TGF-b signalling from cell membrane to nucleus through SMAD proteins*, Nature **390** : 465–471, 1997.

57. A. NOHE, E. KEATING, P. KNAUS, and N.O. PETERSEN, *Signal transduction of bone morphogenetic protein receptors*, Cell Signalling **16** : 291–299, 2004.

58. W. BALEMANS and W. VAN HUL, *Extracellular regulation of BMP signaling in vertebrates: a cocktail of modulators*, Dev. Biol., **250** : 231–250, 2002.

59. J. PFEIFSHIFTER, M. OECHSNER, A. NAUMANN ET AL., *Stimulation of bone matrix apposition in vitro by local growth factors: a comparison between insulin-like growth factor I, platelet derived growth factor, and transforming growth factor β*, Endocrinology, **127**: 69–75, 1999.

60. P.A. GUERNE, F. BLANCO, A. KAELIN ET AL., *Growth factor responsiveness of human articular chondrocytes in aging and development*, Arthritis Rheum., **38**: 960–968, 1995.

61. A. HORNER, S. BORD, P. KEMP ET AL., *Distribution of platelet-derived growth factor (PDGF) A chain mRNA, protein, and PDGF-α receptor in rapidly forming human bone*, Bone, **19**: 353–362, 1996.

62. L. ZHANG, E. LEEMAN, D.C. CARNES, and D.T. GRAVES, *Human osteoblasts synthesize and respond to platelet-derived growth factor*, Am. J. Physiol., **261**: C348–354, 1991.

63. C.H. HELDIN and B. WESTERMARK, *Mechanism of action and in vivo role of platelet-derived growth factor*, Physiol. Rev., **79**: 1283–1316, 1999.

64. C.H. HELDIN and B. WESTERMARK, *Platelet-derived growth factor: Three isoforms and two receptor types*, Trends Genet., **5**: 108–111, 1989.

65. R. ROSS, E.W. RAINES, and D.F. BOWEN-POPE, *The biology of platelet-derived growth factor*, Cell, **46**: 155–169, 1986.

66. T.J. NASH, C.R. HOWLETT, C. MARTIN ET AL., *Effect of platelet-derived growth factor on tibial osteotomies in rabbits*, Bone, **15**: 203–208, 1994.

67. A.H. TASHJIAN, E.L. HOHMANN, H.N. ANTONIADES, and L. LEVINE, *Platelet-derived growth factor stimulates bone resorption via prostaglandin-mediated mechanism*, Endocrinology, **111**: 118–124, 1982.

68. J. FLOEGE, N. TOPLEY, K. WESSEL ET AL., *Monokines and platelet-derived growth factor modulate prostanoid production in growth arrested, human mesangial cells*, Kidney Intern., **37**: 859–869, 1990.

69. R.D. FINKELMAN, S. MOHAN, J.C. JENNINGS ET AL., *Quantitation of growth factors IGF-I, SGF/IGF-II, and TGF-beta in human dentin*, J. Bone Min. Res., **5**: 717–723, 1990.

70. A. STAAL, W.M. GEERTSMA-KLEINEKOORT, G.J. VAN DEN BEMD ET AL., *Regulation of osteocalcin production and bone resorption by 1,25-dihydroxyvitamin D3 in mouse long bones: interaction with the bone-derived growth factors TGF-beta and IGF-I*, J. Bone Min. Res., **13**:36–43, 1998.

71. K. BERNEIS, M. OEHRI, M. KRAENZLIN, and U. KELLER, *Effects of IGF-I combined with GH on glucocorticoid-induced changes of bone and connective tissue turnover in man*, J. Endocrinol., **162**:259–264, 1999.

72. R. BAXTER, *Circulating binding proteins for the insulin - like growth factors*, Trends Endocrinol. Metab., **4**:91–96, 1993.

73. Y. KOSTECKA and J. BLAHOVEC, *Insulin-like growth factor binding proteins and their functions (minireview)*, Endocrine Regulations, **33**:90–94, 1999.

74. K.C. KENT, S. MII, E.O. HARRINGTON, J.D. CHANG ET AL., *Requirement for protein-kinase C activation in Basic Fibroblast Growth Factor-induced human endothelial cell proliferation*, Circul. Res., **77**:231–238, 1995.

75. S. JINGSHI, A. HEYDEMANN, S.K. KANA ET AL., *Acidic fibroblast growth factor (aFGF) injection stimulates cartilage enlargement and inhibits cartilage gene expression in rat fracture healing*, J. Orthop. Res., **8**:364–370, 1990.

76. M. NODA and R. VOGEL, *Fibroblast growth factor enhances type b1 transforming growth factor gene expression in osteoblast-like cells*, J. Cell Biol., **109**:2529–2535, 1989.

77. S. FONTANA, D.G. OLMEDO, J.A. LINARES ET AL., *Effect of Platelet-Rich Plasma on the Peri-implant Bone Response: An Experimental Study*, Implant Dent., 13:73–78, 2004.

78. J.C. ROLDAN, S. JEPSEN, J. MILLER ET AL., *Bone formation in the presence of platelet-rich plasma vs. bone morphogenetic protein-7*, Bone, **34**:80–90, 2004.