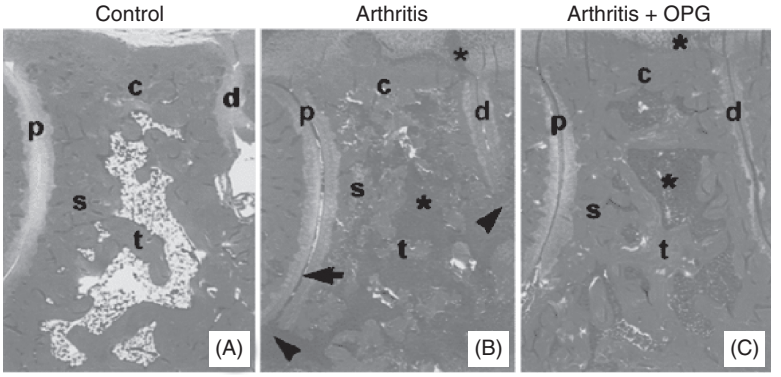
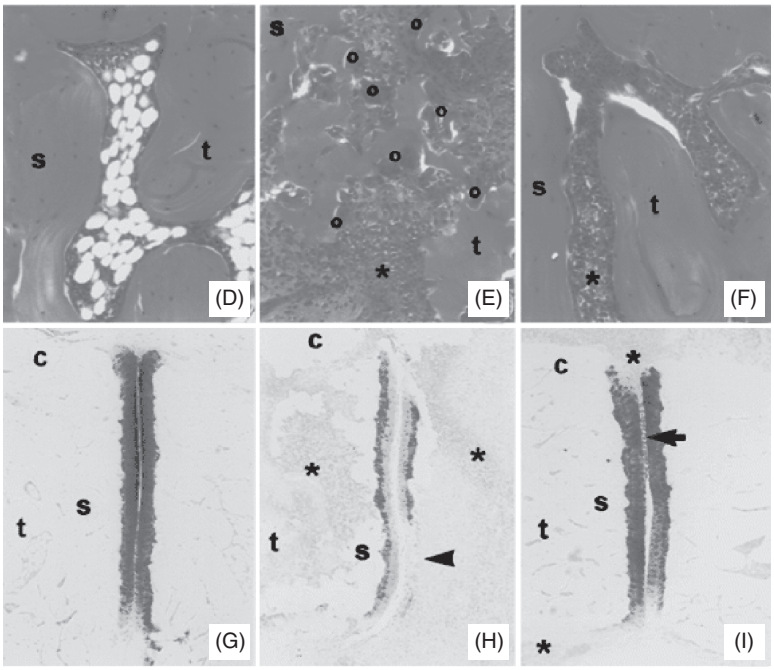
22

BONE AND CARTILAGE REGENERATIVE ENGINEERING



Osteoprotegerin (OPG, the decoy receptor for the tumor necrosis factor family molecule osteoprotegerin ligand or OPGL) prevents bone and cartilage destruction in the presence of severe inflammation. (A, D), Bone and joint structure in the normal hind paw showing dense bony plates, intact articular cartilages and marrow cavities containing scattered hematopoietic precursor cells. Proximal (p) distal (d) intertarsal joints. (B, E), Disrupted bone and joint structure in AdA rats (day 16) with severe mononuclear infiltration in the bone marrow (asterisk), and pannus (arrow); advanced destruction (arrowheads) of cortical (c), subchondral (s), and trabecular (t) bone; and erosion of the articular cartilages. The marrow cavity contains a marked mononuclear cell infiltration (asterisk) containing numerous osteoclasts (o). These rats show severe clinical crippling. (C, F), Preserved bone and joint structure of AdA rats (day 16) treated with OPG ($1\,mg\,kg^{-1}\,day^{-1}$) on days 9–15. OPG-treated rats exhibit extensive mononuclear cell infiltration of bone marrow (asterisk) and pannus formation (arrow), but cortical (c), subchondral (s), and trabecular (t) bone and articular cartilages are intact. Note the absence of osteoclasts as compared with non-OPG-treated rats (E). See color insert.

Bioregenerative Engineering: Principles and Applications, by Shu Q. Liu



These rats do not show any clinical signs of crippling. (G) Normal cartilage integrity in control rats as determined by toluidine blue staining. The matrix of normal articular cartilage is uniformly stained, and the underlying bony plates are dense. (H) Cartilage matrix degeneration in AdA rats (day 16). Uniform pallor (small arrow) in the upper half of articular cartilages denotes extensive loss of matrix proteoglycans. Subchondral (s), cortical (c), and trabecular (t) bone is extensively eroded, and the marrow cavity is filled with inflammatory cells (asterisks). (I) OPG treatment preserves matrix proteoglycans of arthritic rats (day 16) treated with OPG ($1\,mg\,kg^{-1}\,day^{-1}$) on days 9–15. Note modest reduction of toluidine blue staining in peripheral cartilage regions that are in direct contact with inflamed synovial tissue (asterisks) or pannus (arrows). Subchondral (s), cortical (c) and trabecular (t) bone is intact. Staining: H&E (A–F); toluidine blue (G–I). Magnifications: 50× (A–C); 250× (D–F); 75× (G–I). (Reprinted by permission from Macmillan Publishers Ltd.: Kong YY et al: Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand, *Nature* 402:304–9, copyright 1999.) See color insert.

ANATOMY AND PHYSIOLOGY OF THE BONE

Structure [22.1]

Bones are considered hard connective tissues and are major constituents of the skeletal system. A typical bone is composed of several structures, including the periosteum, compact bone, cancellous bone, and endosteum. The *periosteum* is a soft connective tissue structure, which covers the exterior of the bone. The periosteum is composed of several layers, including an external connective tissue layer containing blood vessels and nerve fibers and an internal cellular layer containing osteoblasts, osteoclasts, and osteochondral progenitor cells. The *compact bone* is a thick layer immediately beneath the periosteum. This layer consists of primarily hard bone matrix, a component that carries mechanical

loads. The compact bone consists of blood vessels, which enter the bone from the periosteum through perforating canals. The *cancellous bone* is located internal to the compact bone and is a spongy meshwork composed of trabeculae (about 100 μm in size) and small cavities between the trabeculae. The trabeculae are aligned in the direction of weight-bearing stress. The *endosteum* is the innermost layer of a bone, which is composed of cell types including osteoblasts, osteoclasts, and bone progenitor cells. In long bones, such as the femur and tibia, there is a central cavity known as the *medullary cavity*. Short and flat bones are not composed of a central cavity. The small cavities of the cancellous bones and the medullary cavity contain bone marrow, where hematopoietic stem cells and stromal cells are located.

At the micro-structural level, a bone is composed of two types of components: bone cells and matrix. There are four types of bone cells, including osteoblasts, osteocytes, osteoclasts, and osteochondral progenitor cells. *Osteoblasts* are cells that generate extracellular matrix components, including collagen and proteoglycans. These cells also play a critical role in osteogenesis or the formation of the hard bone matrix, known as hydroxyapatite crystals, by controlling the accumulation, release, and metabolism of calcium and phosphate ions. *Osteocytes* are mature osteoblasts that are located within the bone matrix. The function of the osteocytes is similar to that of the osteoblast. However, the activity of the osteocytes is reduced compared to the osteoblasts. *Osteoclasts* are cells that induce and regulate bone degeneration and resorption. These cells are located in the inner layer of the periosteum and endosteum. Osteoclasts can produce and release enzymes that break down the bone matrix. The matrix debris is endocytosed by osteoclasts. *Osteochondral progenitor cells* are cells that can give rise to osteoblasts and osteoclasts in the bone and chondroblasts in the cartilage. These cells are located in the internal layer of the periosteum and endosteum. Damaged osteoblasts and osteoclasts are replaced with newly generated cells from the osteochondral progenitor cells. A typical bone is composed of about 40% cellular and extracellular matrix components (primarily collage fibers) and about 60% hard mineral materials. The extracellular matrix and the mineral materials constitute the *bone matrix*. The mineral phase is composed of hydroxyapatite, a calcium phosphate crystal structure. The mineral materials provide mechanical strength, while the extracellular matrix provides flexibility to the bone.

Functions of the Bone [22.1]

Structural and Mechanical Support. Bones and cartilages provide support to tissues and organs, ensuring the structural integrity and stability of tissues and organs. There are a large number of bones in the body. These bones are connected by soft connective tissues known as *ligaments*. Bones participate in the movement of the body. Skeletal muscles attach to various bones by tendons. Forces generated by muscular contraction are transmitted to the bones, resulting in the movement of the involved body parts. Bones are the most important components for the protection of internal organs from mechanical injury. For instance, the skull protects the brain and the rib cage protects the heart and lungs.

Generation of Stem Cells. Bones contain bone marrow in the internal cavities. Bone marrow is composed of hematopoietic stem cells, which are capable of generating blood cells. In addition, the bone marrow contains marrow stromal cells, which support hematopoietic stem cells by providing necessary soluble factors for the development, survival,

and differentiation of the hematopoietic stem cells. The marrow stromal cells also contain stem cells for a number of systems, including the connective tissue, blood vessels, heart, brain, and liver. These aspects are discussed in detail on page in Chapter 9.

Bone Formation and Resorption. The skeletal system conducts coordinated bone formation and resorption. The function of the bone is dependent on the maintenance of the dynamic balance of both processes. When bone resorption exceeds bone formation, the mass and strength of the bone reduces, resulting in a disorder known as *osteoporosis* (see page 918 of this chapter). Bone formation is a series of regulated processes, including the generation and release of collagen-dominant extracellular matrix (primarily type I collagen) by osteoblasts, the organization of collagen matrix into a framework, and the deposition of calcium and phosphorus minerals in the matrix framework, forming crystal structures of hydroxyapatite. Bone formation is influenced by a number of factors, including the activity of osteoblasts and the concentration of calcium and phosphorus. Osteoblasts control the production and release of collagen matrix, which nucleates mineral deposition and bone formation. Thus the rate of collagen production influences the rate of bone formation. A critical concentration of calcium and phosphorus is required for the formation of the mineral phase. The concentration of calcium and phosphorus is proportional to the rate of bone formation.

Osteoblasts are a major cell type that controls bone formation. These cells differentiate from the mesenchymal progenitor cells. The mesenchymal progenitor cells also give rise to myocytes, adipocytes, and chondrocytes. The specification of the mesenchymal progenitor cells to different cell types is controlled by distint proteins (Fig. 22.1). For instance, a protein known as runt-related transcription factor 2 (Runx2) promotes osteoblast formation. Another protein known as osterix (Osx) acts downstream of Runx2 to stimulate the maturation of osteoblasts. Two types of protein known as *myogenic regulatory factors* (MRFs) and *myocyte-enhancer factor 2* (MEF2) stimulate the mesenchymal progenitor cells to form myocytes. The proteins CCAAT-enhancer-binding protein (C/EBP) and peroxisome proliferator-activated receptor γ (PPAR γ) induce the differentiation of mesenchymal progenitor cells to adipocytes. The proteins Sox5, −6 and −9 and signal transducers and activators of transcription-1 (STAT1) stimulate the formation of chondrocytes from the mesenchymal progenitor cells.

A number of biochemical factors are known to regulate the activity of osteoblasts and bone development. A typical factor the Wnt protein. This protein promotes the survival, proliferation, and expansion of pre- and immature osteoblasts, thus enhancing bone formation. The Wnt-mediated signaling activities are counterregulated by several molecules, such as Dkks, Sfrps, and Wif-1. These molecules antagonize the Wnt signaling processes in osteoblasts to facilitate the death of immature cells, resulting in a reduction in bone formation (Fig. 22.2).

Bone resorption is a process by which the bone matrix is degenerated, collagen matrix is resorbed, and calcium and phosphorus ions are released from the bone to the blood. Osteoclasts are responsible for bone resorption. These cells can create a low pH environment and release proteinases. The acidic condition induces the degradation of the mineral phase of the bone. Proteinases can cleave extracellular matrix components, including collagen and proteoglycans. These activities result in bone resorption. The regulatory mechanisms of osteoclast activation are demonstrated in Fig. 22.3. The relative activities of bone formation and resorption vary during the different developmental stages. During the childhood, bone formation is dominant. During the adulthood, bone formation and resorption

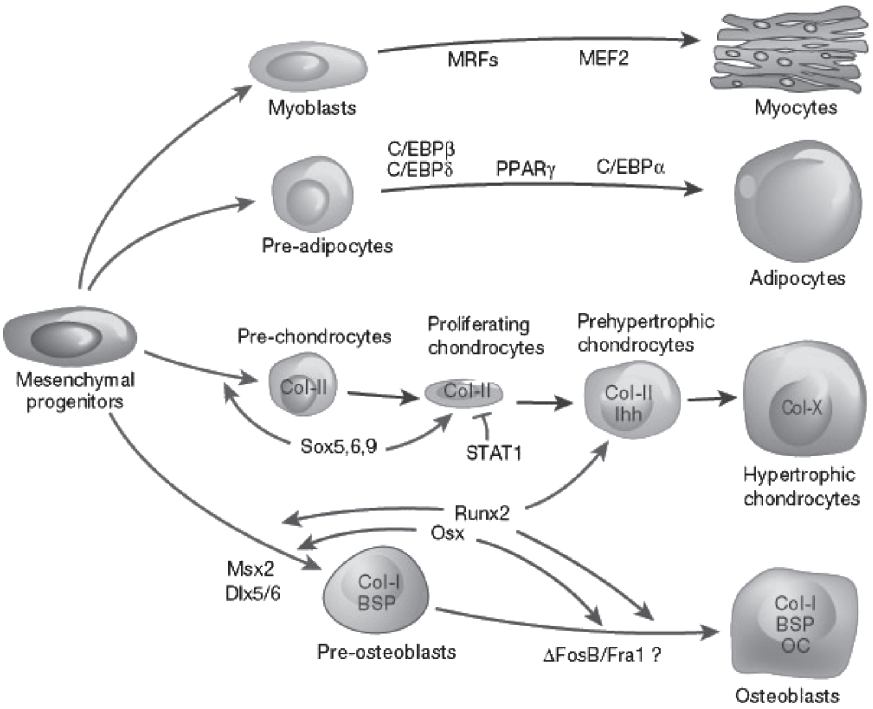


Figure 22.1. Transcriptional control of osteoblastic, chondrocytic, adipocytic and myocytic differentiation. Osteoblasts differentiate from mesenchymal progenitor cells that also give rise to myocytes, under the control of MRFs and MEF2[31], to adipocytes under the control of C/EBP α, β and δ and PPAR γ[30], and to chondrocytes under the control of Sox5, -6 and -9[33] and STAT1. Runx2 is essential for osteoblast differentiation and is also involved in chondrocyte maturation. Osterix (Osx) acts downstream of Runx2 to induce mature osteoblasts that express osteoblast markers, including osteocalcin. *Abbreviations*: MRFs, myogenic regulatory factors (including MyoD, myogenin, myogenic factor 5 and myogenic regulatory factor 4); MEF2, myocyte-enhancer factor 2; C/EBP, CCAAT-enhancer-binding protein; PPARgamma, peroxisome proliferator-activated receptor γ; STAT1, signal transducers and activators of transcription-1; Runx2, runt-related transcription factor 2; Col-I/II/X, type I/II/X collagen; Ihh, Indian hedgehog; BSP, bone sialoprotein; OC, osteocalcin. (Reprinted by permission from Macmillan Publishers Ltd.: Harada S Rodan GA: Control of osteoblast function and regulation of bone mass, *Nature* 423:349–55, copyright 2003.)

are dynamically balanced, maintaining a relatively constant density of bone matrix. During the late stage of the lifespan, the rate of bone resorption exceeds that of bone formation, often resulting in osteoporosis, a disorder with reduced bone matrix and strength (Fig. 22.4).

The process of bone resorption is controlled by the osteoclasts. These cells are differentiated from the haematopoietic precursor cells, which are present as bloodborne mononuclear cells. Osteoclastogenesis or the formation of osteoclasts is regulated by several biochemical fators, including M-CSF (CSF-1) and RANKL (Fig. 22.5). The activation of M-CSF (CSF-1) and RANKL can induce the recuitment of the haematopoietic precursor cells to the target bone. The recuited haematopoietic precursor cells can be transformed

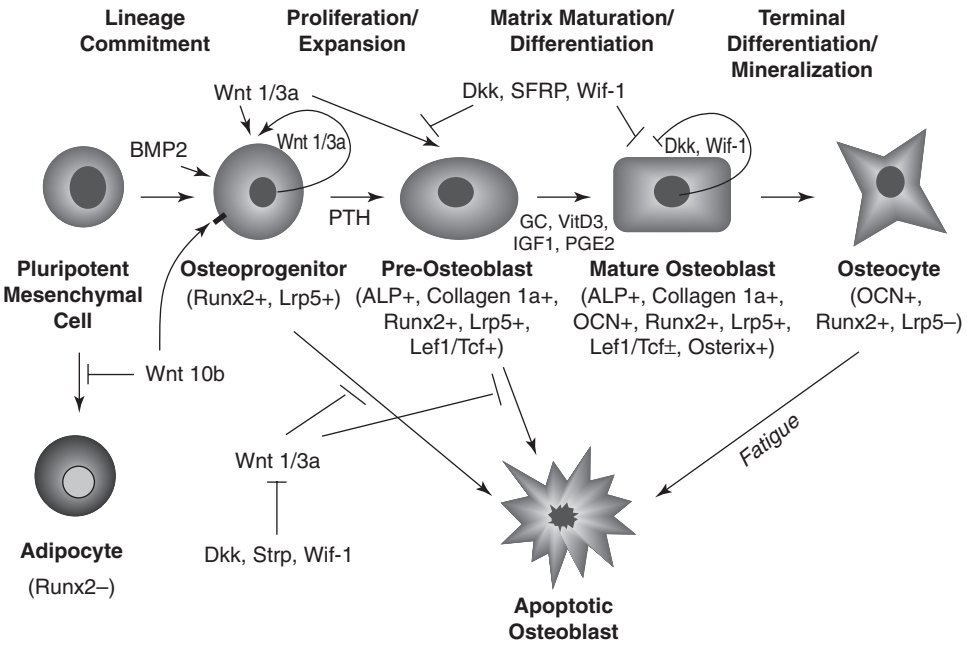


Figure 22.2. Effects of Wnt signaling on osseous cells. The canonical Wnt signaling pathway promotes the proliferation, expansion and survival of pre- and immature osteoblasts. Dkks, Sfrps, and Wif-1 antagonize Wnt signaling in osteoblasts to facilitate death of immature cells, but they may also downregulate the pathway in mature cells to induce terminal differentiation. (Reprinted from Westendorf JJ, Kahler RA, Schroeder TM: Wnt signaling in osteoblasts and bone diseases, *Gene* 341:19–39, copyright 2004, with permission from Elsevier.)

to osteoclasts. The molecular mechanisms of osteoclast formation and maturation are shown in Fig. 22.6. Another factor known as osteoprotegerin (OPG) serves as an antagonist for these osteoclast-stimulating factors. OPG can bind to and reduce the activity of RANKL and M-CSF, resulting in a reduction in osteoclastogenesis and the activity of osteoclasts.

Bone Metabolism. The bone matrix is constituted with minerals including calcium and phosphorus. The bone is a tissue for the storage, transport, and metabolism of these elements. Calcium and phosphorus are transported from the blood to the bone for storage. When the blood level of these elements is reduced, the elements are released from the bone, ensuring an appropriate level for metabolic and regulatory processes. Calcium and phosphorus are fundamental elements that participate in the regulation of cellular processes such as cell division, migration, adhesion, and contraction.

There is about 1–2 kg calcium in an adult body. More than 95% of the total calcium is stored in the bone. Calcium can be mobilized and released from the bone matrix when the calcium concentration in the extracellular space is reduced below a critical level, whereas calcium is deposited to the bone matrix when the extracellular calcium concentration is increased. The concentration of extracellular calcium is maintained within a narrow range from 9 to 10 mg/dL. The maintenance of such a calcium concentration is essential

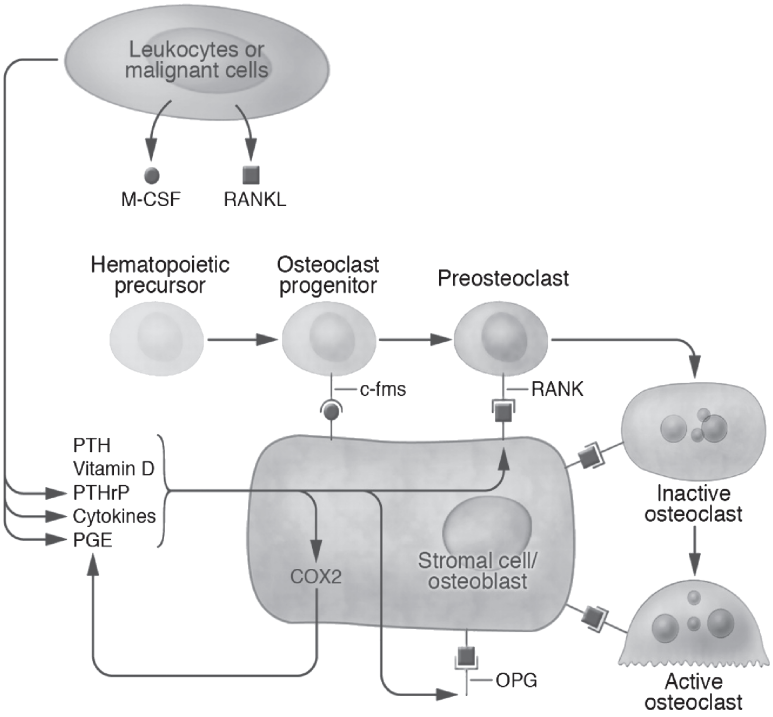


Figure 22.3. Regulation of osteoclast formation and activity. In physiologic remodeling, activation of bone resorption requires contact between cells of the osteoblast and osteoclast lineages. M-CSF, which may be either membrane bound or secreted, interacts with its receptor, c-fms, to stimulate differentiation and proliferation of hematopoietic progenitors, which then express RANK as pre-osteoclasts. Osteoclast differentiation and activity are stimulated by RANK/RANKL interaction, and this interaction can be blocked by soluble osteoprotegerin (OPG). Bone-resorbing factors can also stimulate COX2 activity, which may amplify responses to RANKL and OPG by producing prostaglandins. In pathologic conditions, inflammatory and malignant cells can increase osteoclastogenesis by producing soluble or membrane-bound M-CSF and RANKL as well as PTH-related protein (PTHrP), cytokines, and prostaglandins. (Reprinted with permission from Raisz LG, Pathogenesis of osteoporosis: concepts, conflicts, and prospects, *J Clin Invest* 115:3318–25, copyright 2005.)

to the regulation of cellular activities such as cell adhesion, migration, proliferation, and contraction.

The blood concentration of calcium is controlled by several factors, including calcium absorption from the intestinal tract, calcium mobilization from and deposition to the bone matrix, calcium excretion by the kidneys, and calcium loss via sweating. Calcium is absorbed from diets via active transport and diffusion in the small intestine. Vitamin D regulates the absorption of calcium (see section below). The urinary excretion of calcium is influenced by the concentration of blood calcium. A reduction in calcium intake and the level of blood calcium, or hypocalcemia, is associated with a decrease in urinary calcium excretion. Hypocalcemia may occur in the presence of vitamin D deficiency, intestinal disorders, and dietary calcium deprivation.

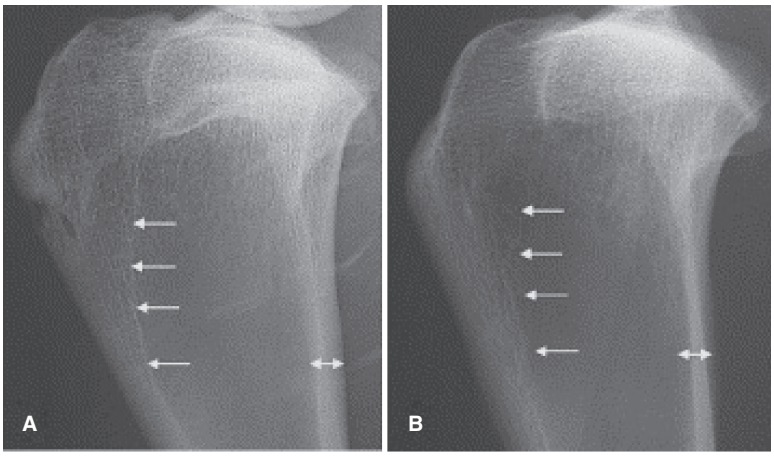


Figure 22.4. In vivo radiological survey of typical mineralization in young (18 months, panel A) and old (8 years, panel B) sheep tibia. Note in particular the overall reduced X-ray absorption in the old tibia due to lowered mineral density, the reduction in trabecular organization, the lack of a defined border towards the marrow cavity (arrows), and the thinning of the cortical bone (double arrowheads), all features pointing to significant osteoporosis in the old animal. (Reprinted from Sachse A et al: Osteointegration of hydroxyapatite-titanium implants coated with nonglycosylated recombinant human bone morphogenetic protein-2 (BMP-2) in aged sheep, *Bone* 37:699–710, copyright 2005 with permission of Elsevier.)

Phosphorus is not only a constituent of the bone matrix, but also an element participating in the regulation of molecular signaling processes, such as phosphorylation and dephosphorylation. There is about 1 kg phosphorus in an adult body. About 85% of the total phosphorus is stored in the bone matrix. When blood phosphorus is low, phosphorus can be mobilized from the bone matrix to form free phosphorus. Free phosphorus is present in the form of inorganic phosphate (HPO_4^{2-}, $H_2PO_4^-$, or $NaHPO_4^-$), which is found in the blood and extracellular space (3–4 mg/dL). Dietary phosphorus is absorbed in the small intestine. The rate of intestinal absorption controls the blood level of phosphorus. In addition to phosphorus mobilization from the bone and absorption from the intestine, the kidney also participates in the regulation of blood phosphorus. The renal tubules can efficiently reabsorb phosphorus from the glomerular filtrate. When the blood level of phosphorus is low, the renal tubules can reabsorb all phosphorus from the glomerular filtrate if necessary.

Regulation of Bone Metabolism

Role of Parathyroid Hormone. Parathyroid hormone is a protein that stimulates bone resorption and mobilizes calcium and phosphorus from the bone, resulting in an increase in the blood concentration of calcium and phosphorus. The hormone is produced in the two parathyroid glands, which are located near the thyroid glands. The parathyroid glands are composed of several cell types, including the chief cells and oxyphil cells. The chief cells produce parathyroid hormone. The function of the oxyphil cells remains poorly understood. Parathyroid hormone is synthesized in the ribosomes in the form of a preprohormone polypeptide with about 110 amino acids. The preprohormone is modified in the

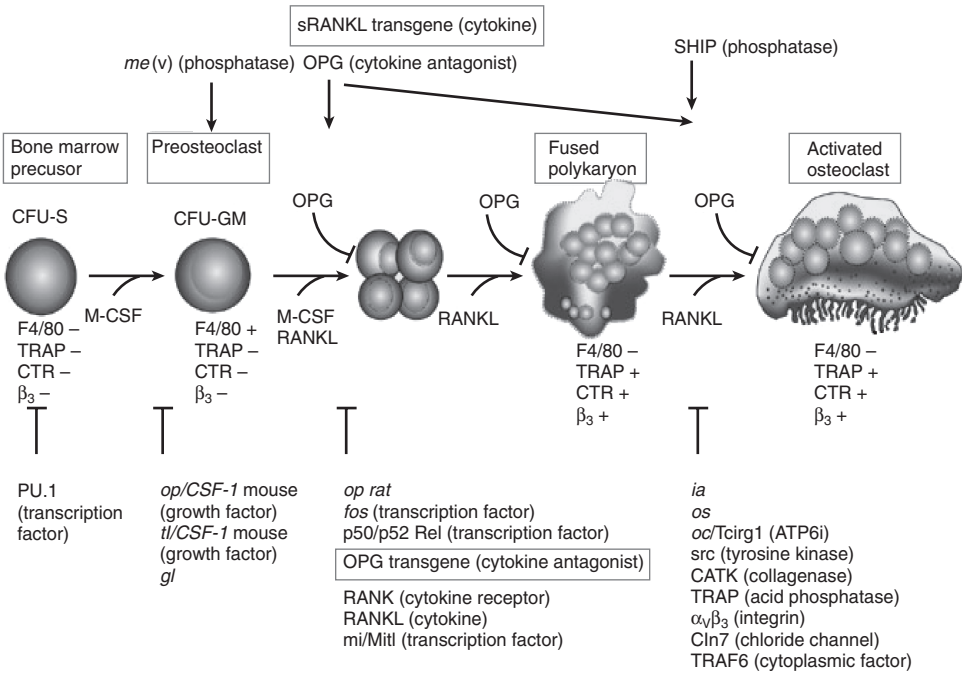


Figure 22.5. Osteoclastogenesis. Development schema of haematopoietic precursor cell differentiation into mature osteoclasts, which are fused polykaryons arising from multiple (10–20) individual cells. Maturation occurs on bone from peripheral bloodborne mononuclear cells with traits of the macrophage lineage shown below. M-CSF (CSF-1) and RANKL are essential for osteoclastogenesis, and their action during lineage allocation and maturation is shown. OPG can bind and neutralize RANKL, and can negatively regulate both osteoclastogenesis and activation of mature osteoclasts. Shown below are the single-gene mutations that block osteoclastogenesis and activation. Those indicated in italic font are naturally occurring mutations in rodents and humans, whereas the others are the result of targeted mutagenesis to generate null alleles. Shown above are the single-gene mutant alleles that increase osteoclastogenesis and/or activation and survival and result in osteoporosis. Note that all of these mutants represent null mutations with the exception of the OPG and sRANKL transgenic mouse overexpression models. (Reprinted by permission from Macmillan Publishers Ltd.: Boyle WJ, Simonet WS, Lacey DL: Osteoclast differentiation and activation, *Nature* 423:337–42, copyright 2003.)

endoplasmic reticulum and Golgi apparatus to form parathyroid hormone. The functional form of the hormone is stored in the secretory granules.

Parathyroid hormone can be released in response to a decrease in calcium concentration. The hormone can activate osteocytes and osteoblasts within a period as short as several minutes. These cells can release intermediate signals, which in turn activate osteoclasts, a type of bone-degrading cell. Note that, although the primary function of osteocytes and osteoblasts is to promote mineral deposition and bone formation, these cells mediate the activity of the bone-degrading osteoclasts in the presence of a high concentration of parathyroid hormone. Activated osteoclasts degrade bone matrix, releasing calcium and phosphorus from the bone matrix to the extracellular space and blood. In addition,

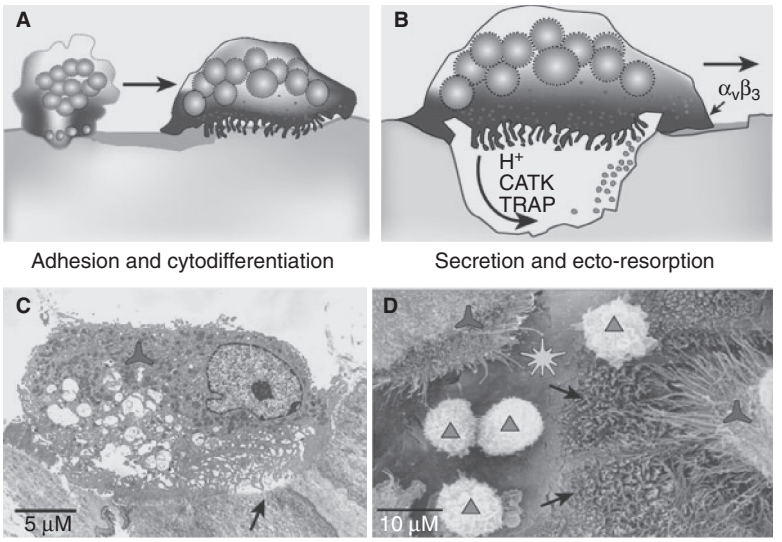


Figure 22.6. Activation of bone resorption. (A) Multinucleated polykaryons are recruited by the action of CSF1 and RANKL, which then adhere to bone and undergo cytodifferentiation into a mature osteoclast. (B) RANKL stimulates osteoclast activation by inducing secretion of protons and lytic enzymes into a sealed resorption vacuole formed between the basal surface of the osteoclast and the bone surface. Acidification of this compartment by secretion of protons leads to the activation of TRAP and CATK, which are the two main enzymes responsible for the degradation of bone mineral and collagen matrices. (C) Transmission electron micrograph of an activated mouse osteoclast with a visible ruffled border in a resorption lacunae on the periosteal femoral cortical bone surface. Red propeller, osteoclast; black arrow, a resorption pit. (D) Scanning electron micrograph of human osteoclasts generated in vitro on cortical bone slices from CSF1- and RANKL-treated peripheral blood mononuclear cells. Red propellers, osteoclasts; black arrows, a resorption pit where the normally smooth lamellar bone surface has been resorbed to expose collagen bundles; yellow star, nonresorbed bone surface; blue triangles, mononuclear cells (potential osteoclast precursors). (Reprinted by permission from Macmillan Publishers Ltd.: Boyle WJ, Simonet WS, Lacey DL: Osteoclast differentiation and activation, *Nature* 423:337–42, copyright 2003.)

parathyroid hormone can stimulate the proliferation of osteoclasts. Increased osteoclasts further enhance bone resorption and mineral release.

Parathyroid hormone can also act on the epithelial cells of the small intestine and the tubular cells of the kidney. In the intestine, parathyroid hormone enhances the absorption of calcium and phosphorus, resulting in an increase in the blood concentration of these ions. In the kidney, the effect of parathyroid hormone is more complicated than that in the intestine. The hormone stimulates the renal tubular epithelial cells to reabsorb calcium, while it reduces the reabsorption of phosphorus. Such activities result in an increase in the blood concentration of calcium and a decrease in the blood concentration of phosphorus. At the same time, the hormone also regulates the transport of magnesium, sodium, and potassium in the kidney. It enhances the reabsorption of magnesium, while it reduces the reabsorption of sodium and potassium.

Role of Vitamin D. Vitamin D is a hormone that is derived from 7-dehydrocholesterol, a precursor of cholesterol, and plays a critical role in the regulation of calcium metabolism

and homeostasis. Vitamin D is synthesized in the epidermal cells under the stimulation of ultraviolet radiation from the sunlight. Such a stimulation can induce a photobiochemical process, by which 7-dehydrocholesterol undergoes a conformational change, resulting in the formation of previtamin D_3. Previtamin D_3 can be spontaneously converted to vitamin D_3 under the stimulation of the body temperature. Vitamin D_3 is transported from the epidermis to the blood via the mediation of vitamin D-binding proteins. In the liver, vitamin D_3 is further converted to 25-hydroxyvitamin D [25(OH)D], a major form of vitamin D that exists in the blood. The blood concentration of 25-hydroxyvitamin D varies considerably under physiological conditions, ranging from 5 to 80 ng/mL. However, 25-hydroxyvitamin D is not active. This form of vitamin D is metabolized in the kidney and converted to an active form known as 1,25-hydroxyvitamin D [1,25$(OH)_2$D] under the action of 25(OH)D-1α hydroxylase. 1,25-hydroxyvitamin D is an active form of vitamin D.

The level of 1,25-hydroxyvitamin D is controlled by a number of factors, including the level of sunlight exposure, the intensity of ultraviolet light, aging, the calcium concentration, and the level of parathyroid hormone. An increase in exposure to sunlight enhances vitamin D_3 synthesis and promotes the formation of 1,25-hydroxyvitamin D. Aging is associated with a progressive reduction in the rate of vitamin D_3 synthesis. A decrease in the blood concentration of calcium stimulates the conversion of 25-hydroxyvitamin D to 1,25-hydroxyvitamin D. Such a calcium change also stimulates the secretion of parathyroid hormone, which acts on renal tubule cells and enhances the formation of 1,25-hydroxyvitamin D. There exist many forms of vitamin D metabolites in the blood and extracellular matrix. These forms are mostly products of vitamin D degradation and possess vitamin D activity. However, the vitamin D metabolites are not as active as 1,25-hydroxyvitamin D.

There are a number of functions for 1,25-hydroxyvitamin D. These include the regulation of calcium and phosphorus absorption in the epithelial cells of the small intestine, the regulation of bone resorption, and the control of cell proliferation and differentiation. It is well known that 1,25-hydroxyvitamin D stimulates the absorption of calcium and phosphorus in the epithelial cells of the small intestine, resulting in an elevation in the blood concentration of these ions. The influence of vitamin D on bone resorption is dependent on the concentration of vitamin D. At a high concentration in the extracellular space, 1,25-hydroxyvitamin D enhances bone resorption. This is possibly due to the stimulatory effect of vitamin D on the bone-resorption activity of the parathyroid hormone. Such an effect results in an increase in the concentration of calcium and phosphorus in the extracellular space and blood. However, at a low level, 1,25-hydroxyvitamin D enhances bone mineralization and matrix formation. A possible mechanism for this phenomenon is that a low level of vitamin D may reduce the activity of the parathyroid hormone. 1,25-hydroxyvitamin D has also been hsown to inhibit the development and activation of osteoclasts (Fig. 22.7), thus reducing bone resorption.

Experimental investigations have demonstrated that 1,25-hydroxyvitamin D exerts inhibitory effects on the proliferation of normal and cancer cells. For instance, 1,25-hydroxyvitamin D inhibits the generation and secretion of renin in the renal arteries, resulting in a decrease in the level and activity of angiotensin II. Since angiotensin II enhances the proliferation of vascular smooth muscle cells, 1,25-hydroxyvitamin D suppresses vascular mitogenic activities via mediating the function of angiotensin II. Vitamin D is also known to promote cell differentiation A treatment with 1,25-hydroxyvitamin D can induce the differentiation of monocytes to osteoclast-like cells.

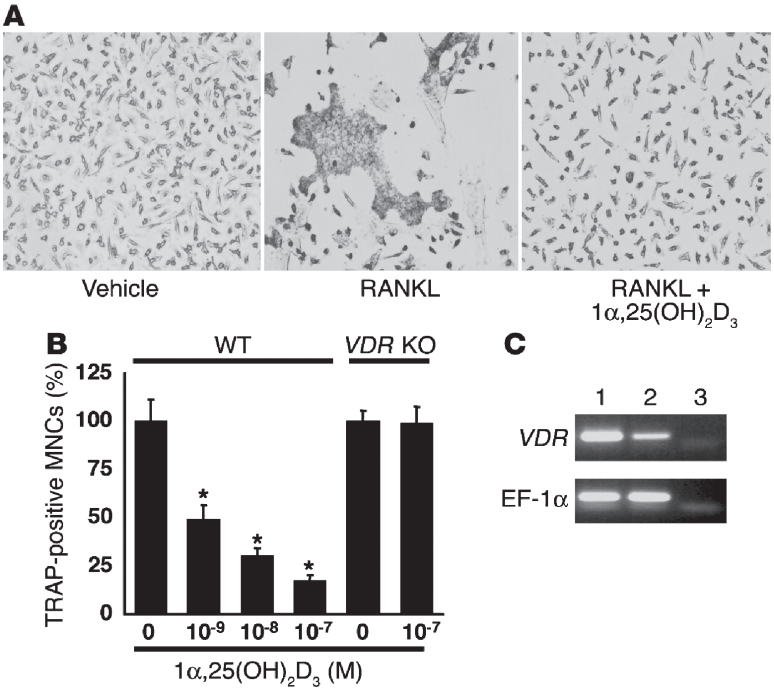


Figure 22.7. 1α-25$(OH)_2D_3$ inhibits osteoclast development through *VDR* by acting directly on osteoclast precursor cells in bone marrow. (A) Osteoclast precursor cells were isolated from the bone marrow of wildtype C57BL/6J and *VDR* Knockout mice as M-CSF–dependent adherent cells, and were further treated with RANKL (40 ng/mL) in the absence or presence of 10^{-7} M 1α-25$(OH)_2D_3$ for 3 days. Note that the development of TRAP-positive multinucleate osteoclasts induced by RANKL (receptor activator of NFκB ligand) was markedly inhibited by cotreatment with 1α-25$(OH)_2D_3$. (B) The inhibitory effect of 1α-25$(OH)_2D_3$ on the formation of TRAP-positive multinucleate cells (MNCs) was dose-dependent and was not seen in marrow cultures derived from *VDR* knockout mice, even at the highest dose of 10^{-7}M. Data are expressed as a percentage of vehicle-treated cultures. *$P < 0.05$ versus vehicle group, $n = 6$. (C) Expression of *VDR*s in the intestine (lane 1) and osteoclast precursor cells (lane 2) as detected by RT-PCR. EF-1α mRNA served as control for PCR. Lane 3 contained water as a negative control. (Reprinted with permission from Takasu H et al: c-Fos protein as a target of antiosteoclastogenic action of vitamin D, and synthesis of new analogs, *J Clin Invest* 116:528–35, copyright 2006.)

Mammalian cells express a nuclear receptor for 1,25-hydroxyvitamin D. The interaction of 1,25-hydroxyvitamin D with this receptor induces the phosphorylation and activation of the receptor. The complex of 1,25-hydroxyvitamin D and activated nuclear receptor serves as a transcription factor and can translocate to the cell nucleus, initiating the transcription of specific target genes. The gene products in turn regulate related physiological activities.

ANATOMY AND PHYSIOLOGY OF THE CARTILAGE [22.1]

Cartilage is a connective tissue that is mostly associated with the bone except for several organs such as the large airways and ears. Cartilage is composed of two structures: peri-

chondrium and internal cartilage. The perichondrium contains an external and internal connective tissue layer. The external layer is composed of fibroblasts, extracellular matrix (collagen fibers, elastic fibers, and proteoglycans), blood vessels, and nerves. The internal layer is composed of cells known as *chondroblasts*. These cells can generate cartilage matrix components, including collagen and proteoglycans. Chondroblasts can self-reproduce and are responsible for cartilage growth. The internal cartilage is composed of chondrocytes and cartilage matrix. *Chondrocytes* are developed from chondroblasts and are surrounded by cartilage matrix. The cartilage matrix is an amorphous structure, which contains scattered chondrocytes. It should be noted that a cartilage layer can be found on the terminal surface of the bone at a joint. This type of cartilage, called articular cartilage, does not contain perichondrium.

There are several functions for the cartilage. It provides support to adjacent bones, serves as a structural framework for several organs, including the trachea, nose, and ear, and constitutes the surface structure of the joint, ensuring smooth interaction between bone surfaces. In addition, cartilage plays a critical role in mediating bone growth. In long bones such as the femur and tibia, there exists a cartilage plate between the end and the body of the bone. This plate is responsible for the growth of the bone, which determines the height of the entire body. When bone growth is ceased, the cartilage growth plate is ossified into a thin bone matrix structure known as the epiphyseal line.

BONE AND CARTILAGE DISORDERS

Osteoporosis

Pathogenesis, Pathology, and Clinical Features [22.2]. *Osteoporosis* is a bone disorder characterized by increased degeneration or resorption of the bone matrix in the entire skeletal system, resulting in a progressive reduction in the mass of the bone, the thickness of the compact bone, and the size and number of the trabeculae of the cancellous bone. Osteoporosis often results in bone deterioration and fracture. Osteoporosis is a consequence of imbalance between bone formation and resorption. In the healthy population, the rate of bone formation is dynamically balanced with the rate of bone resorption. When the rate of bone resorption exceeds that of bone formation, the bone mass reduces and osteoporosis occurs. In such a case, the skeletal system is no longer able to resist the physiological level of mechanical loads. A slight increase in the mechanical load may result in bone fracture. In the normal population, bone resorption starts to exceed bone formation at the age of 40–50. The rate of bone resorption increases with aging. Osteoporosis is a common disorder in the population over the age of 60.

Based on etiological factors, osteoporosis is classified into several types, including idiopathic, postmenopausal, glucocorticoid, thyrotoxicotic, and inherited osteoporosis. *Idiopathic osteoporosis* is defined as osteoporosis without identified etiological factors. This disorder is often found in young people, especially premenopausal women. The disorder is associated with a decrease in blood calcium and phosphorus. Idiopathic osteoporosis can be self-cured spontaneously within several years. *Postmenopausal osteoporosis* is found in women with reduced production and release of estrogen, a hormone that suppresses bone resorption. A decrease in the level of estrogen in postmenopausal women induces increased bone resorption and osteoporosis.

An excessive increase in glucocorticoids, as seen in Cushing's syndrome, can reduce bone formation and simultaneously enhance bone resorption, often resulting in osteoporosis. Glucocorticoids have been shown to enhance the effect of parathyroid hormone, which activates the osteoclasts and mobilizes calcium from the bone matrix. Furthermore, glucocorticoids inhibit calcium absorption in the intestine. All these effects contribute to the development of osteoporosis.

Thyrotoxicity is a condition with increased secretion of the thyroid hormone, which is also referred to as hyperthyroidism. Increased thyroid hormone mobilizes calcium and phosphorus from the bone, promotes bone resorption, and enhances the excretion of calcium and phosphorus via urine and feces. These changes can lead to osteoporosis if hyperthyroidism prolongs. In postmenopausal women, hyperthyroidism may induce more severe osteoporosis than the general population.

Osteogenesis imperfecta is an inherited form of osteoporosis and is characterized by heterogeneous reduction in the mass of bone matrix. There are two types of disorder: autosomal dominant and autosomal recessive osteogenesis imperfecta. The autosomal dominant type of imperfecta is associated with relatively mild bone resorption and functional defects. In contrast, the autosomal recessive subtype is often found within a short period after birth and is associated with a severe reduction in the bone mass. Patients may experience recurrent fracture of long bones such as femurs and tibias. Pathological examinations usually demonstrate reduced synthesis of type I collagen and altered organization of collagen matrix.

Conventional Treatment [22.2]. Osteoporosis is a group of disorders induced by different etiological factors. One of the strategies for the treatment of osteoporosis is to eliminate or alleviate the primary etiological factors, if known. For instance, postmenopausal osteoporosis is caused by a reduction in the level of estrogen. Administration of estrogen is the primary choice of method for the treatment of postmenopausal osteoporosis. In osteoporosis induced by increased level of glucocorticoids and thyroid hormone, a primary approach is to treat the original diseases that enhance the production and secretion of these hormones. Since calcium is a major component of the bone matrix, calcium administration is often necessary. An increase in the level of extracellular calcium reduces bone resorption. In addition, fluorides can be incorporated into the bone matrix, enhancing the crystal formation and strength of the bone. Thus fluorides have been used to treat osteoporosis.

Molecular Engineering Therapy. Osteoporosis is induced by progressive degeneration of the bone matrix. Molecular regenerative approaches can be used to protect the bone from degeneration and promote bone matrix formation. There are a number of genes encoding proteins that are known to regulate bone matrix formation and mineralization, including vitamin D receptor, estrogen receptor alpha, type I collagen, transforming growth factor, and interleukin-6. The mutation of these genes and/or disorders in regulating the expression of these genes may predispose to bone degeneration, leading to osteoporosis. Thus, the genetic manipulation of these genes may potentially enhance bone formation and prevent bone degeneration. Here the application of these genes to osteoporosis is briefly discussed.

Vitamin D Receptor (VDR) [22.3]. The vitamin D receptor (Table 22.1) interacts with vitamin D and participates in the regulation of calcium metabolism and bone formation.

TABLE 22.1. Characteristics of Vitamin D Receptor*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Vitamin D receptor	VDR, 1,25-dihydroxyvitamin D3 receptor	427	48	Ubiquitous	Serving as a *trans*-acting transcriptional regulatory factor, which is similar to steroid and thyroid hormone receptors in structure, regulating genes involved in mineral (calcium) metabolism

*Based on bibliograhpy 22.3.

TABLE 22.2. Characteristics of Selected Collagen Molecules*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Collagen type I α1	COL1A1, collagen α1(I) chain	1464	139	Bone, skin, tendon	Constituting collagen type 1 together with collagen type I α2
Collagen type I α2	COL1A2, collagen I α2 polypeptide	1366	129	Bone, skin, tendon	Constituting collagen type 1 together with collagen type I α1

*Based on bibliograhpy 22.4.

At a relatively low level, vitamin D can activate the vitamin D receptor, enhancing calcium deposition and bone mineralization. Furthermore, vitamin D inhibits the activity of osteoclasts, suppressing bone resorption. The mutation of the vitamin D receptor gene induces disorder of calcium metabolism, influencing the mineralization process of the bone matrix. When the mutation of the vitamin D receptor is an identified cause for osteoporosis, the transfer of the wildtype vitamin D receptor gene may be considered for the treatment of the disorder.

Type I Collagen Gene [22.4]. Type I collagen is a major type of matrix molecule in the bone. This collagen is composed of collagen type I α1 and collagen type I α2 chains (both are listed in Table 22.2). The genes encoding these chains are COLIA1 and COLIA2,

respectively. The mutation of these genes may influence the formation of the collagen matrix. For instance, there exists a polymorphism in the introns of the COLIA1 gene. This polymorphism induces alterations in the regulation of gene transcription, resulting in a shift in the ratio of the alpha1 to alpha2 collagen chains. These abnormalities affect the formation of type I collagen as well as the collagen matrix in the bone. Since the integrity of the collagen matrix is critical to the mineralization of the bone matrix, any disorder in the formation of collagen matrix may contribute to bone degeneration and the development of osteoporosis. When disordered formation of collagen matrix is the cause of osteoporosis, the transfer of the wildtype type I collagen gene should be considered for the treatment of the disorder. Furthermore, when gene mutation is the cause of bone disorders, targeted correction of mutant genes by transfecting cells with viral vectors may be an effective approach for the treatment of bone disorders.

Estrogen Receptor [22.5]. This receptor interacts with the hormone estrogen and enhances bone formation. Postmenopausal women are often associated with osteoporosis because of estrogen reduction or deficiency. Estrogen is known to suppress the activity of cytokines, including tumor necrosis factor α, interleukin (IL)1, IL6, IL11, macrophage-colony stimulating factor, and prostaglandin E, via interaction with the estrogen receptor. The loss of estrogen and/or estrogen receptor is often associated with activation of these cytokines, which stimulate the proliferation of osteoclasts. Activated osteoclasts induce bone resorption. Mutation of the estrogen receptor gene may also lead to enhanced bone resorption. In the case of osteoporosis due to the malfunction of the estrogen receptor gene, the transfer of a functional gene into the skeletal system may help to enhance bone formation.

Characteristics of several estrogen receptors are listed in Table 22.3.

Calcitonin [22.6]. Calcitonin (Table 22.4) is a peptide hormone that is synthesized by the parafollicular cells of the thyroid. Its function is to inhibit the activity of osteoclasts, resulting in the suppression of bone resorption and a reduction in serum calcium. Calcitonin exerts an effect opposite to that of the parathyroid hormone. The protein form of calcitonin can be delivered to disordered bones for the treatment of osteoporosis. Clinical studies have provided promising results for the use of calcitonin. Alternatively, the gene of calcitonin can be cloned, amplified, and used for therapeutic purposes. As for other therapeutic genes, the delivery of the calcitonin gene can prolong the effectiveness of the hormone compared with direct delivery of the calcitonin protein.

Osteoprotegerin (OPG) and Osteoprotegerin Ligand (OPGL) [22.7]. Osteoprotegerin is a secreted soluble receptor protein that belongs to the tumor necrosis factor receptor superfamily and exists in the extracellular matrix. Osteoprotegerin is produced by the osteoblasts and is capable of suppressing the activity of osteoclasts and thus inhibiting bone resorption. Osteoprotegerin can bind to a ligand known as osteoprotegerin ligand (OPGL), which is a member of the tumor necrosis factor cytokine family and is also known as tumor necrosis factor ligand superfamily member 11 (TNFSF11). Osteoprotegerin ligand is a protein that can interact with a cell membrane receptor in hematopoietic cells to induce the differentiation of hematopoietic stem cells to osteoclasts. The binding of osteoprotegerin, which serves as a decoy receptor in the extracellular matrix, to osteoprotegerin ligand inhibits the interaction of the osteoprotegerin ligand to cell membrane receptor and thus suppresses the formation of osteoclasts from hematopoietic stem cells.

TABLE 22.3. Characteristics of Selected Estrogen Receptors*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Estrogen receptor α	Estrogen receptor 1 (ESR1), estrogen receptor (ER)	595	66	Uterus, ovary, breast, bones, skeletal muscle, blood vessels, lung, skin	Serving as a nuclear receptor and transcription factor, regulating the development of the sex organs, and regulating cell proliferation
Estrogen receptor β	ER β, estrogen receptor 2 (ESR2), ESRB, ESR β	530	59	Uterus, ovary, breast, prostate gland, bones, skeletal muscle, blood vessels, brain, lung, skin	Similar to functions of estrogen receptor α

*Based on bibliograhpy 22.5.

TABLE 22.4. Characteristics of Calcitonin*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Calcitonin	CT, α calcitonin, katacalcin	141	15	Nervous system, bone, thyroid gland	Suppressing bone resorption and reducing the level of serum calcium

*Based on bibliograhpy 22.6.

Since osteoclasts are responsible for bone resorption, the activation of osteoprotegerin leads to a reduction in bone resorption. Animals without the osteoprotegerin gene usually develop osteoporosis-like disorders. The application of osteoprotegerin to osteoporotic bones has been shown to prevent bone degeneration. In animal models with oophorectomy (surgical removal of the ovary), the administration of osteoprotegerin reduces estrogen deficiency-induced bone degeneration. The transfer of the osteoprotegerin gene into osteoporotic bones is a potential approach for the treatment of osteoporosis.

Properties of osteoprotegerin and its ligand are listed in Table 22.5.

Integrin-Binding Proteins [22.8]. The integrin complex αvβ3 is expressed in osteoclasts, plays a role in regulating the adhesion and proliferation of osteoclasts, and thus enhances osteoclast-mediated bone resorption. A family of integrin-binding proteins, known as disintegrins, can bind to the αvβ3 integrin and inhibit the activity of the integrin, resulting in the suppression of osteoclast activation and bone resorption. Disintegrins are small proteins found in the venom of snakes. The disintegrin family includes several members, which are echistatin, kistrin, albolabrin, bitistatin, elegantin, flavoridin, halysin, and triflavin. These proteins can specifically bind to β1 and β3 integrins and block the interaction of integrins with extracellular matrix components. Disintegrins also inhibit the activity of parathyroid hormone-induced bone resorption. Thus, the proteins or genes of disintegrins can be potentially used for the treatment of osteoporosis.

Growth Factors [22.9]. Several growth factors, including insulin-like growth factor (IGFβ1), fibroblast growth factor (FGF), the bone morphogenetic proteins (BMPs), and transforming growth factor-beta (TGFβ), are known to stimulate osteoblast proliferation and promote bone formation. Mutation of the genes encoding these growth factors is associated with an increased incidence of osteoporosis. A typical example is the association of the mutation of the transforming growth factor β gene (e.g., C509→T polymorphism) with osteoporosis. Local delivery of these growth factors enhances bone regeneration and recovery from osteoporosis. Alternatively, the genes of these growth factors can be used and transferred into disordered bones. As shown in experimental investigations, the overexpression of the bone morphogenetic protein gene in osteoblasts by gene transfer enhances bone formation. The construction of the bone morphogenetic protein gene vector is shown in Fig. 22.8. The effectiveness of the bone morphogenetic protein gene in bone formation is shown in Fig. 22.9.

Cell Therapy for Bone Regeneration [22.10]. Osteoporosis is a disorder induced by reduced osteogenesis due to impaired function of the osteoblasts, which regulate calcium deposition and bone formation. A potential approach for improving the function of osteoblasts is to transplant stem or progenitor cells to target bone tissue and replace malfunctioned osteoblasts. Candidate stem and progenitor cell types include embryonic, fetal, and adult stem and progenitor cells. It is important to point out the osteoporosis is a disorder that involves the entire skeletal system. Thus a systematic approach, such as intravenous delivery of osteogenic stem or progenitor cells, is required for the treatment of the disorder. To achieve a therapeutic goal, it is necessary to carry out several steps: (1) identify and collect a stem or progenitor cell type; (2) expand the cells in vitro; (3) genetically manipulate the cells (e.g., transfection of the cells with desired genes to enhance selected functions), if necessary; and (4) deliver expanded cells to the venous system.

TABLE 22.5. Characteristics of Osteoprotegerin and Osteoprotegerin Ligand*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Osteoprotegerin	OPG, osteoclastogenesis inhibitory factor (OCIF), tumor necrosis factor receptor superfamily member 11B (TNFRSF11B)	401	46	Thyroid gland, kidney	Inhibiting the formation and activation of osteoclasts, suppressing bone resorption, and regulating lymph node organogenesis and vascular calcification
Osteoprotegerin ligand	OPGL, tumor necrosis factor ligand superfamily, member 11 (TNFSF11), receptor activator of NKκB ligand (RANKL), TNF-related activation-induced cytokine, osteoclast differentiation factor, ODF	317	35	T-cell, dendritic cell, thymus, lymph node	Stimulating osteoclast differentiation and activation, inducing bone resorption, serving as a dentritic cell survival factor, and regulating T-cell-dependent immune responses

*Based on bibliograhpy 22.7.

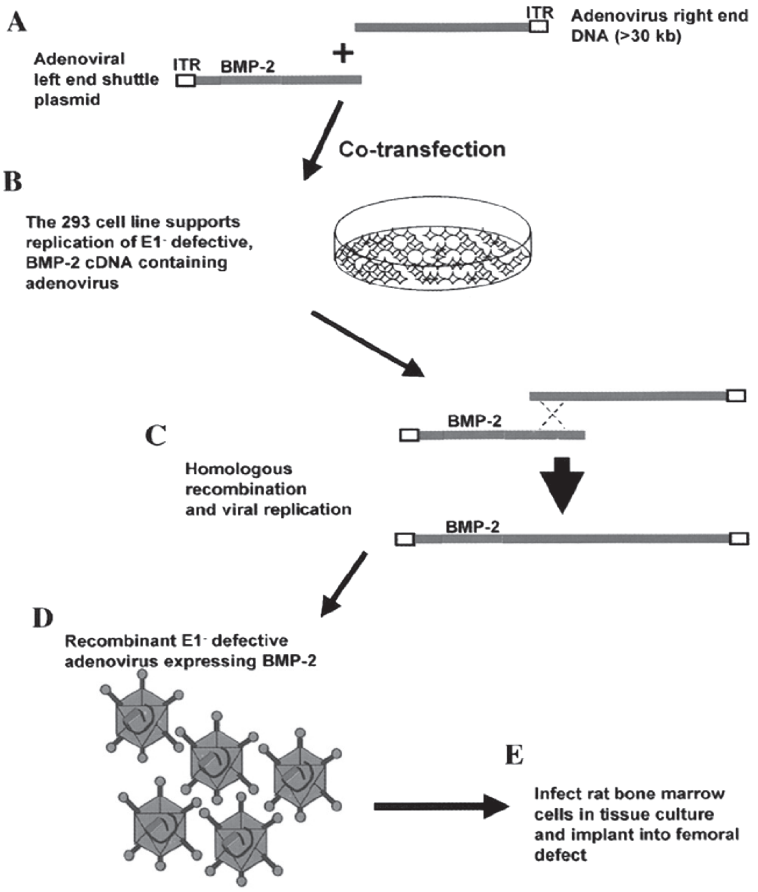


Figure 22.8. Diagram showing the construction of recombinant adenovirus containing rhBMP-2 cDNA. (A) Adenovirus E1 genes were deleted and replaced by BMP-2 cDNA on a plasmid (shuttle plasmid) containing the left inverted terminal repeat (ITR) required for viral replication. This BMP-2 shuttle plasmid and the large adenoviral right genome (~30 kb) were transfected into a human embryonic kidney fibroblast cell line, referred to as 293 cells. (B) The 293 cells contain integrated adenoviral E1 genes and express E1 proteins (key growth-regulatory proteins of the adenovirus) constitutively. Thus, the E1-defective adenovirus (the E1 genes have been deleted) can be propagated only in the 293 cells. (C) The cotransfected BMP-2 shuttle plasmid DNA and the adenoviral right end DNA can undergo recombination through the shared homologous viral sequence in vivo in the 293 cells. The resultant BMP-2-expressing E1-defective adenovirus will be able to replicate and form plaques on the 293 cells. (D) BMP-2 recombinant adenoviral clones are further purified and expanded from individual plaques, and their DNA structure is confirmed. (E) The purified BMP-2 recombinant adenovirus then can be used to infect the rat-bone-marrow cells that have been grown in tissue culture. (Reprinted with permission from Lieberman JR et al: The effect of regional gene therapy with bone morphogenetic protein-2-producing bone-marrow cells on the repair of segmental femoral defects in rats, *J Bone Joint Surg* 81:905–17, copyright 1999.)

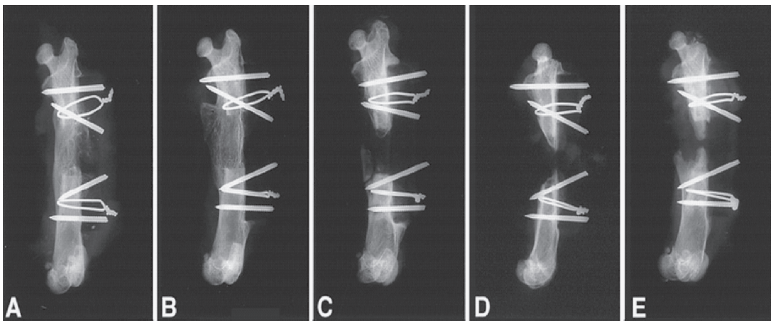


Figure 22.9. Radiographs of the specimens, made 2 months after the operation; 20 mg of guanidine hydrochloride-extracted demineralized bone matrix was used as a substrate in all defects. (A) Group I—local delivery of BMP-2-producing bone marrow cells (5×10^6), established by transferring a BMP2 gene-containing adenoviral vector. Dense, coarse trabecular bone, which was remodeling to form a new cortex, was present in these defects. (B) Group II—local delivery of rhBMP-2 (recombinant human BMP2, 20 μg). The healed defect is filled with lace-like trabecular bone. (C–E) Group III—β-galactosidase-producing rat bone marrow cells; group IV—uninfected rat bone marrow cells; group V—demineralized bone matrix alone. Minimum bone formation was noted in these three groups. (Reprinted with permission from Lieberman JR et al: The effect of regional gene therapy with bone morphogenetic protein-2-producing bone-marrow cells on the repair of segmental femoral defects in rats, *J Bone Joint Surg* 81:905–17, copyright 1999.)

Among the stem and progenitor cell types, including embryonic, fetal, and adult stem and progenitor cells, the adult bone marrow stem cells have been studied extensively, since these cells can directly differentiate to osteoblasts and autogenous cells can be used to prevent immune rejection reactions (Fig. 22.10). As discussed Chapter 9, the bone marrow contains adult stem cells, which can self-replicate and differentiate into various types of specialized cells, depending on the presence of environmental cues. Bone marrow stem cells are composed of hematopoietic and mesenchymal stem and progenitor cells. The hematopoietic stem and progenitor cells can give rise to all blood cells, including the erythrocytes, leukocytes, and platelets, whereas the mesenchymal stem and progenitor cells can differentiate into mesenchymal lineages of connective tissues, such as osteoblasts, chondroblasts, adipocytes, and fibroblasts. The bone marrow mesenchymal stem cells have been considered potential candidate cells for treatment of osteoporosis.

There are different types of mesenchymal stem cells in the bone marrow. While not all these cell types are characterized, the bone marrow stromal cells have been shown to contain mesenchymal stem cells that can transform to cell lineages of soft and hard connective tissues (Fig. 22.11). These cells show several unique features, including the capability of adhering to a substrate and forming colonies. These features can be used to isolate bone marrow stromal cells, since other types of bone marrow cells, including the hematopoietic stem cells, do not show these features. The bone marrow stromal cells have been studied and used extensively for the repair and reconstruction of impaired bones.

When expanded autogenous bone marrow stromal cells are delivered into the circulatory system, these cells can engraft and integrate into various mesenchymal tissues, including the bone, cartilage, and soft connective tissue. In the bone, engrafted osteogenic progenitor cells can transform into osteoblasts, which produce collagen matrix, promote

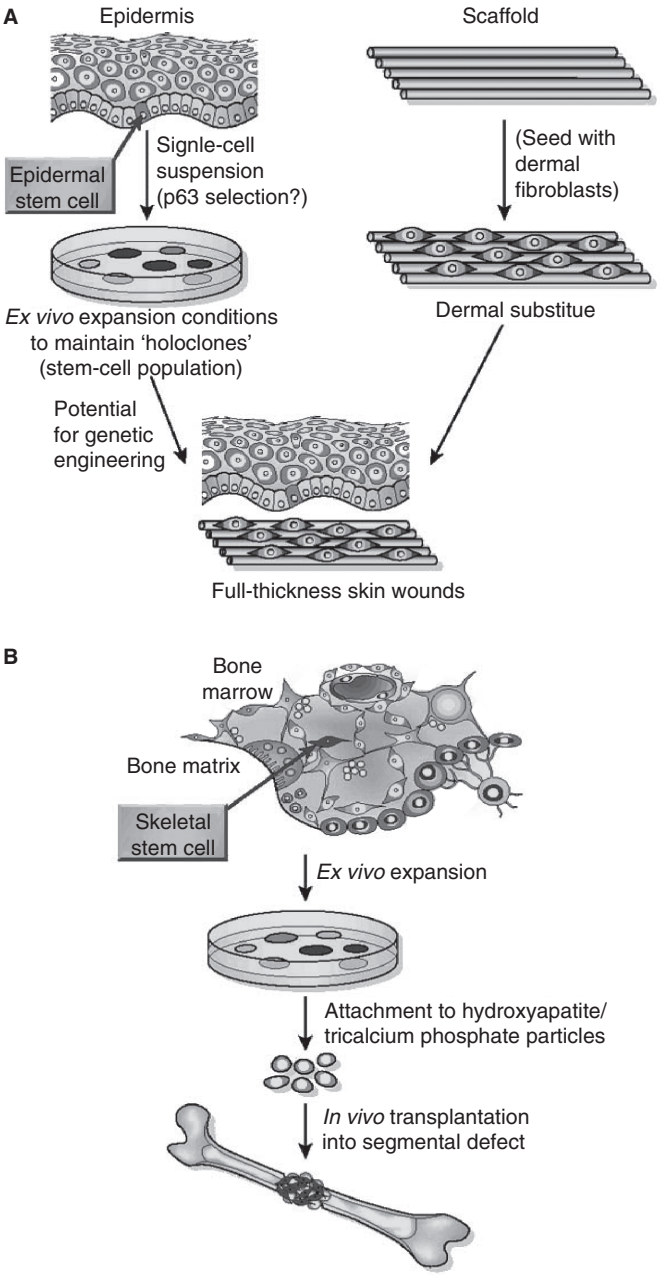


Figure 22.10. Regeneration of two-dimensional (skin) and three-dimensional (bone) tissues using stem cells. (A) Skin autografts are produced by culturing keratinocytes (which may be sorted for p63, the more recently described, epidermal stem cell marker) under appropriate conditions not only to generate an epidermal sheet but also to maintain the stem cell population (holoclones). The epidermal sheet is then placed on top of a dermal substitute comprising devitalized dermis or bio-engineered dermal substitutes seeded with dermal fibroblasts. Such two-dimensional composites, generated ex vivo, completely regenerate full-thickness wounds. (B) Bone regeneration requires ex vivo expansion of bone marrow-derived skeletal stem cells and their attachment to three-dimensional scaffolds, such as particles of a hydroxyapatite/tricalcium phosphate ceramic. This composite can be transplanted into segmental defects and will subsequently regenerate an appropriate three-dimensional structure in vivo. (Reprinted by permission from Macmillan Publishers Ltd.: Bianco P, Robey PG: Stem cells in tissue engineering, *Nature* 414:118–21, copyright 2001.)

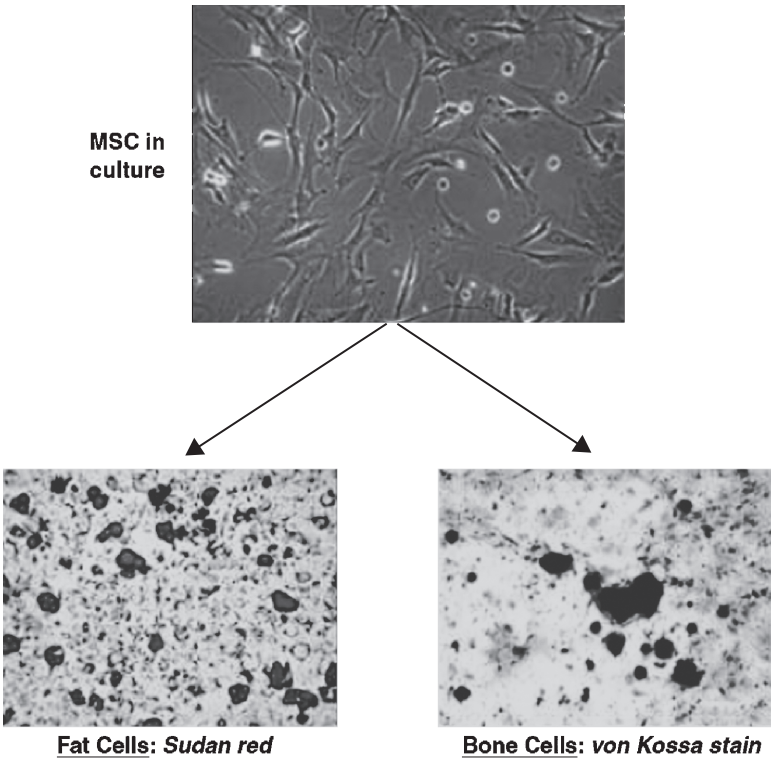


Figure 22.11. Mesenchymal stem cells (MSC) were characterized by their ability to differentiate into adipocytes and osteocytes under culture conditions. (Reprinted with permission from Togel F et al: Administered mesenchymal stem cells protect against ischemic acute renal failure through differentiation-independent mechanisms, *Am J Physiol Renal Physiol* 289:F31–42, copyright 2005.)

bone mineralization, and stimulate bone formation. Extensive investigations have demonstrated promising results for the treatment of bone osteoporosis and other disorders with cell transplantation. Allogenic osteogenic stem and progenitor cells can also be used for such a purpose. However, immune rejection is a serious problem. Immunosuppressive drugs must be administrated when allogenic cells are used.

Osteoporosis is associated with a progressive reduction in the bone strength due to the loss of bone mass and mineralization, often resulting bone fracture or functional impairment. In the case of bone fracture, a local treatment is required. Although bone injury and fracture can be treated with conventional approaches based on the self-healing nature of the bone, the regeneration process of the bone is usually slow and nonunion may occur because of poor blood circulation. In addition, bone grafts are required for the reconstruction of the lost bone. To resolve these problems, it is necessary to construct bone substitutes with living bone-forming cells and graft the substitutes to the injury sites. Several common approaches have been established and used for bone grafting and regeneration. These including: (1) identification and collection of osteogenic progenitor cells from the bone marrow or other sources, (2) expansion of the collected cells in vitro, (3) seeding and culture of expanded cells in porous bioceramic scaffolds (e.g., hydroxy-

apatite scaffolds) with a desired shape, and (4) reconstruction of fractured or injured bones with the cell-containing bone scaffold. Experimental investigations have consistently demonstrated that bone reconstruction with cell-containing scaffolds can significantly enhance bone regeneration and improve the healing process of injured bones. The grafted bone substitutes can be integrated into the natural bone when autogenous cells are used.

Paget's Disease

Pathogenesis, Pathology, and Clinical Features [22.11]. *Paget's disease* is a disorder characterized by excessive focal bone resorption due to increased activity of osteoclasts, subsequent fibrosis of bone marrow, and chaotic focal deposition of new bone matrix. Such activities often lead to focal bone distortion and weakening, rendering the bone fragile and prone to fracture. The incidence of Paget's disease is about 3% for the population over 40 years old. Aging is associated with an increase in the incidence of the disease. In the early stage, the disease may be asymptomatic and is often diagnosed by roentgenography for unrelated diseases. The etiology of Paget's disease is poorly understood. Since a treatment with glucocorticoids can relieve the symptoms of the disease, Paget's disease has been considered a disorder resulting from inflammatory reactions. Viral infection may contribute to the pathogenesis of the disorder. Potential viruses include respiratory syncitial, measles, and canine distemper viruses.

Pathological examinations demonstrate characteristic changes in Paget's disease, such as (1) bone mass reduction in association with enhanced angiogenic responses in the bone matrix during the early stage, (2) subsequent bone formation with irregular structure of the bone matrix, (3) an increase in the density and size of osteoclasts in association with an increase in the density of osteoblasts, (4) replacement of bone marrow with stromal tissue, and (5) reduction in the rate of bone resorption and formation during the late stage. Because of bone degeneration is associated with bone formation, which compensates for the loss of bone matrix, many patients may not show severe symptoms. During the late stage of the disease, clinical signs may occurs, including bone swelling, gait abnormalities due to unequal length of the long bones of the lower extremities (resulting from heterogeneous bone resorption and formation), and enlargement of the skull. Patients may also complain about headache, backache, and pain in other locations. The pain may be a result of nerve stimulation during bone resorption and formation. Some patients may experience hearing loss due to distortion of the ossicles and bones of the cochlea as well as the impingement of the hearing nerves by deformed bones. Brain tissue at the skull base (e.g., brainstem) may be compressed and impaired due to the distortion of the skull. The spinal cord may also be compressed by distorted vertebrae.

Conventional Therapy [22.11]. For patients without apparent symptoms, therapies are not usually necessary. When rapidly progressive manifestations are present, such as severe pains, signs of nervous compression, apparent bone distortions, and bone fractures, nonsteroid antiinflammatory drugs (such as indomethacin) and analgesics (such as aspirin) can be administrated. Although steroid antiinflammatory hormones, such as glucocorticoids, suppress the progression of Paget's disease, these hormones are not recommended because they cause osteoporosis and other disorders, such as reduction in cardiac output. In severe cases, surgical bone replacement may be recommended.

Molecular and Cellular Therapies [22.12]. Since the pathological changes in Paget's disease are similar to those of osteoporosis, all molecular and cellular therapies described for osteoporosis in Chapter 25 can be used for the treatment of Paget's disease.

Bone Tumors

Pathogenesis, Pathology, and Clinical Features [22.13]. Tumors are common disorders in the skeletal system. Bone tumors are classified into two types: benign tumors and malignant tumors. *Benign bone tumors* are those that do not invade neighboring tissues and do not metastasize. Examples of benign bone tumors include osteochondromas, endochondromas, benign giant cell tumors, and unicameral bone cysts. These tumors may arise from osteoblasts, osteoclasts, chondroblasts, and fibroblasts. Most benign bone tumors are not painful. Pathological examinations usually show restrained tumor cells, clearly identified tumor boundary, and bone enlargement and deformation. Clinical manifestations include slowly progressive enlargement of involved bones, bone deformation, and bone fractures.

Malignant bone tumors or cancers are tumors characterized by rapid invasion into neighboring tissues and metastasis. Common malignant bone tumors include multiple myeloma, osteosarcomas, chondrasarcomas, fibrosarcomas, and malignant giant cell tumors. Myeloma can arise from the bone marrow myeloid cells. Other bone cancers may be originated from osteoprogenitor cells, osteoblasts, chondroblasts, and fibroblasts. These malignant tumors are often found in the population at age of 20–40. Exposure to radiation, bone infarction, and Paget's disease may serve as predisposing factors for these tumors. Patients with malignant tumors experience severe pains, swelling, deformation, and destruction of the involved bones, which progress rapidly. Some tumors may destroy surrounding soft tissues. Laboratory tests often demonstrate an increase in the blood level of alkaline phosphatase. These tumors are usually associated with rapid metastasis. The primary target of metastasis is the lung. Bone malignant tumors often have poor prognosis.

Bone cancers often enhance the activity of osteoclasts, and stimulate the proliferation of these cells, promoting bone resorption. These events are possibly related to the production of parathyroid hormone and other mediators, such as interleukin-6 and interleukin-11, in cancer cells. These factors are known to activate osteoclasts and stimulate bone resorption. The bone-degrading activity of cancer cells facilitates cancer cell migration and metastasis.

Therapies for Bone Tumors. As for other types of tumor, conventional approaches, including surgical removal, chemotherapy, and radiotherapy, as well as molecular and cellular therapies can be used for the treatment of bone tumors. These approaches are discussed in detail in Chapter 25.

Rheumatoid Arthritis

Pathogenesis, Pathology, and Clinical Features [22.14]. *Rheumatoid arthritis* is a disorder characterized by inflammatory autoimmune reactions in the joints, resulting in chronic synovitis and progressive destruction of articular cartilage and bone. These pathological changes often lead to joint distortion, malfunction, pain, discomfort, and stiffening. The disorder is found in 0.5–1% of the human population. The incidence in the women

is about 2–3 times higher than that in the men. The etiology of arthritis is poorly understood. The disorder is increased with age and may be influenced by disorders in gene regulation. For instance, most patients with rheumatoid arthritis express a protein complex known as histocompatibility antigen HLA-DR4. This protein complex may contribute to immune reactions, which are responsible for the development of arthritis.

Although the cause of arthritis is not clear, immune reactions have been thought to contribute to the pathogenesis of the disorder. In the site of the disorder, several types of immune cell are often found. These include Tlymphocytes, Blymphocytes, and macrophages. Theses cells express and release cytokines, including interleukin (IL)1, IL6, IL11, IL13, IL17, interferons, and monocyte chemotactic factors, which promote inflammatory reactions and attract additional leukocytes. The presence of these cells supports the possibility that arthritis is a disorder induced by immune reactions. Such reactions activate osteoclasts, stimulate osteoclast formation, enhance cartilage and bone resorption, and induce bone and cartilage destruction. It remains to be investigated, however, whether exogenous antigens or autoimmune factors, such as extracellular matrix components, initiate the immune reactions. The presence of autoantibodies in patients with arthritis suggests that the disorder may involve autoimmune reactions.

Pathological examinations often demonstrate hyperplasia of synovial lining cells, infiltration of mononuclear cells, and angiogenesis during the early stage. The mononuclear cells often aggregate around small blood vessels. Granulation tissue can be found in the site of arthritis. This type of tissue contains blood vessels, fibroblasts, and macrophages. During the late stage, bone and cartilage destruction can be found in peripheral joints.

Patients with arthritis may show signs of fatigue, weakness, and anorexia during the early stage. With the progression of the disorder, arthritis-specific manifestations may appear, including pain, stiffening, and swelling of the hands, feet, wrists, and knees. During the late stage, the movement of the joints may be severely limited and apparent joint distortion can be found. In addition, distortion of ligaments and tendons as well as imbalance of skeletal muscles may occur, which contribute to joint distortion and malfunction. The manifestations described above are all attributed to inflammatory reactions, cell hyperplasia and hypertrophy, excessive production of fibrous tissue, and bone and cartilage destruction.

Conventional Therapy [22.14]. Rheumatoid arthritis is treated with several types of agents, including (1) antiinflammatory drugs, (2) analgesic drugs, (3) disorder-modifying drugs, and (4) immunosuppressive drugs. For the anti-inflammatory therapy, steroidal and nonsteroidal agents can be used. Steroidal agents primarily include glucocorticoid hormones. These hormones can effectively suppress inflammatory reactions. However, steroidal hormones do not remove the causative factors and do not significantly change the prognosis of the disorder. Nonsteroidal agents, such as aspirin, fenoprofen, indomethacin, and tolmetin, exert not onlyanti-inflammatory but also analgesic effects. Among these nonsteroidal agents, aspirin is the most effective agent for the treatment of arthritis. Several drugs, including d-penicillamine, antimalarials, and gold compounds, have been used for the treatment of arthritis. These drugs slow down the progression of the disorder, although the exact mechanisms remain poorly understood. Since arthritis is a disorder related to immune reactions, it is conceivable to administrate immunosuppressive agents. Common immunosuppressive drugs include azathioprine and cyclophosphamide. These drugs can effectively reduce immune responses at the site of disorder and exert therapeutic effects.

When drug therapies are not effective and arthritic joints are severely damaged, surgical approaches can be used to reconstruct or replace the joints. Such surgical procedures can correct joint distortions, relieve pain, and improve joint function to a certain degree. The reconstruction of the hips and knees is usually more successful than that of other joints.

Molecular Therapy [22.15]. While conventional approaches can be used to reduce the symptoms of rheumatoid arthritis, these approaches may not significantly alter the progression of the disorder. Furthermore, most agents used for the treatment of rheumatoid arthritis exert adverse effects. Thus it is necessary to develop effective approaches that prevent or reduce the progression of the disorder. Recent investigations have demonstrated that genetic manipulation may represent such an approach.

Rheumatoid arthritis is possibly induced by autoimmune inflammatory responses. The suppression of immune reactions is a critical approach for the prevention and treatment of rheumatoid arthritis. A number of cytokines, including interleukin (IL)1, tumor necrosis factor (TNF)α, IL4, IL10, and IL13, have been known to regulate the activity of T and B cells, which are involved in immune reactions. IL1 and TNFα stimulate inflammatory responses, whereas IL4, IL10, and IL13 are anti-inflammatory factors. The genetic manipulation of these factors may provide a means for the suppression of autoimmune inflammatory reactions. These molecules are briefly discussed as follows.

Interleukin-1 [22.16]. Interleukin (IL)1 (see list of IL1 isoforms and receptors in Table 22.6) is a family of inflammatory mediators, including 10 known members: IL1α (IL1F1), IL1β (IL1F2), IL1Ra (IL1F3), IL18 (IL1F4), IL1F5, IL1F6, IL1F7, IL1F8, IL1F9, and IL1F10. Among these members, IL1α, IL1β, and IL1Ra have been known to play critical roles in the mediation of inflammatory responses. The activity of other members has not been clearly identified. While IL1α and IL1β serve as proinflammatory mediators that stimulate autoimmune responses, IL1Ra is an IL1 receptor agonist (which gives the name IL1Ra) that competes for the IL1 receptor and blocks the effects of IL1α and IL1β. IL1α and IL1β contribute to the development of autoimmune disorders, including rheumatoid arthritis, by initiating inflammatory responses. Bacterial infection may induce autoimmune disorders, since some bacteria may contain antigens that are similar in structure to certain proteins in the host systems. When infected with bacteria, the host monocytes and macrophages are activated to produce and release IL1. IL1 can act on endothelial cells to stimulate the production of chemotactic proteins, such as monocyte chemotactic protein 1, and adhesion molecules, such as E-selectin, intercellular adhesion molecules (ICAM), and vascular cell adhesion molecule (VCAM). These molecules promote leukocyte activation and attachment to endothelial cells, leading to leukocyte infiltration to infected areas. Furthermore, IL1 can activate monocytes to release more proinflammatory cytokines. These activities all contribute to inflammatory responses, which potentially induce autoimmune disorders and destroy host cells and structure. Thus, IL1α and IL1β are the primary targets for the treatment of autoimmune disorders.

IL1α and IL1β are two cytokines which share about 22% identity. These factors exert similar effects and induce similar cellular activities by interacting with their receptors. There are two types of IL1 receptor: type I IL1 receptor (IL1RI, ~80 kDa, 552 amino acids) and type II IL1 receptor (IL1RII, 60–68 kDa, 385 amino acids). The IL1RI *has been found in* T cells, endothelium, fibroblasts, astrocytes, chondrocytes, keratinocytes, neurons, smooth muscle cells, whereas IL1RII is found primarily in leukocytes, including

TABLE 22.6. Characteristics of IL1 Isoforms and IL1 Receptors*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
IL1α	IL1α, hematopoietin 1, IL1F1	271	31	Monocytes, macrophages, brain, skin, lung	Regulating inflammatory and immune processes, mediating hematopoiesis, inducing apoptosis, mediating osteoclastogenesis, and inducing rheumatoid arthritis and Alzheimer's disease
IL1β	Catabolin, IL1F2	269	31	Macrophages, lung, skin	Similar to IL1α
Type I IL1 receptor	Interleukin-1 receptor α type 1, IL1Rα, IL1RA interleukin-1 receptor, IL1R, CD121A, antigen CD121a	569	65	T cell, monocyte, skin	Serving as a receptor for interleukin-1α (IL1α), interleukin-1β (IL1β), and mediating IL1 induced immune and inflammatory responses
Type II IL1 receptor	IL1RB, IL1Rβ, antigen CDw121b	398	45	T cell, B cell, monocytes, skin, nervous system, pancreas, testis	Serving as a receptor for interleukin-1 alpha (IL1α), interleukin-1β (IL1β), and acting as a decoy receptor that inhibits the activity of its ligands

*Based on bibliography 22.16.

B cells, T cells, monocytes, and neutrophils. Both receptors are composed of an extracellular region with three immunoglobulin-like domains, and share about 28% identity for these domains. However, the cytoplasmic domain differs considerable between the two types of receptor. The IL1RI possesses a 213-amino acid cytoplasmic region, whereas the IL1RII has only a 29-amino acid cytoplasmic region. The structural difference may influence the function of these receptors. IL1RI has been shown to interact with a transmembrane glycoprotein known as IL1 RI accessory protein (IL1 RAcP, ~66 kDa, 550 amino acids), which induces the internalization of the IL1/IL1 RI complex, transducing active signals to the intracellular signaling pathways. In contrast, IL1RII cannot interact with IL1 RAcP and serve as a decoy or dummy receptor that does not transduce active signals into the cytoplasm. Both IL1RI and IL1RII can interact with ligands IL1α and IL1β, but with different affinities. IL-1α preferentially binds to IL1RI, whereas IL1β preferentially binds to IL1RII. Because the binding of IL1 to IL1RII does not activate the intracellular signaling pathways, the role of IL1RII is to downregulate the activity of IL1.

There exist soluble forms of IL1RI (~60 kDa) and IL1RII (47 kDa and 57 kDa, representing two forms of soluble IL1RII) in the extracellular space. The soluble form of IL1RI (soluble type I IL1 receptor) can bind to IL1Ra (IL1 receptor agonist) with high affinity, thus reducing the binding of IL1Ra to the cell membrane IL1RI (membrane type I IL1 receptor) and leaving more cell membrane IL1RI available for IL1α and IL1β. These processes enhance the activity of IL1. In contrast, soluble IL1RII (soluble type II IL1 receptor) preferentially binds to IL1α and IL1β. The affinity of soluble IL1RII to IL1Ra is reduced considerably compared to its membrane-bound form, leaving more IL1Ra available for competitive binding to IL1RI, which reduces the effect of IL1α and IL1β. This is another mechanism by which IL1RII downregulates the activity of IL1α and IL1β.

Molecular therapies have been developed to reduce the inflammatory effect of the IL1 system. Major strategies include (1) enhance the expression of IL1Ra and (2) enhance the expression of IL1RII and promote the formation of soluble IL1RII. As discussed above, both IL1Ra and IL1RII exert an inhibitory effect on the activity of IL1. Thus the overexpression of the genes for these proteins potentially suppresses the activity of IL1 and reduces inflammatory responses, which are beneficial for the treatment of rheumatoid arthritis.

Tumor Necrosis Factors (TNFs) [22.17]. Tumor necrosis factors (see Table 22.7 for a list of TNF isoforms and receptors) are a family of proteins that mediate inflammatory and immune reactions. There are two types of TNFs: TNFα and TNFβ. TNF-α exists in the form of either a membrane protein (~26 kDa, 233 amino acids) or soluble protein (~17-kDa, 157 amino acids). The membrane form of TNFα is composed of extracellular, transmembrane, and cytoplasmic domains. The soluble form of TNFα is produced by cleaving the membrane form by TNFα converting enzyme (TACE) and exists in the form of homodimer. Both membrane and soluble forms are biologically active, but the soluble form is more potent. In contrast to TNFα, TNFβ (25 kDa, 171 amino acids) exists only in the form of soluble protein. TNFα and TNFβ share about 28% identity in the amino acid structure and both bind to the same types of receptor.

There are two types of TNF receptors: type I TNF receptor (TNFRI, 55 kDa, 455 amino acids) and type II TNF receptor (TNFRII, 75 kDa, 461 amino acids). TNFRI is a receptor that can interact with both TNFα and TNFβ. This receptor possesses double-sided functions. While TNFRI can induce cell apoptosis via the activation of the "death domain" in

TABLE 22.7. Characteristics of Tumor Necrosis Factor Isoforms and Receptors*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
Tumor necrosis factor α	TNFα, tumor necrosis factor, TNFA, cachectin	233	26	Leukocyte, macrophage	Mediating inflammatory reactions, and regulating cellular activities including cell proliferation, differentiation, apoptosis
Tumor necrosis factor β	TNFB, TNF superfamily member 1, lymphotoxin α, lymphotoxin, lymphotoxin A, lyphotoxin α (LTα), lymphocyte-derived TNF, tumor necrosis factor ligand superfamily member 1	205	22	Lymphocyte, monocyte, dendritic cell, placenta	Mediating inflammatory and immune responses, regulating the formation of lymphoid organs, and inducing cell apoptosis
Type I tumor necrosis factor receptor	TNFR1, TNFR1α, tumor necrosis factor receptor superfamily, member 1A, p55 TNFR, TNF-R55, TNFR, 55-kDa, TNFR, 60-kDa	455	50	Lymphocytes, heart, tonsil	Interacting with tumor necrosis factor α, mediating inflammatory reactions, inducing apoptosis, and activating nuclear factor κB
Type II tumor necrosis factor receptor	TNFRSF1B, TNFR2, TNFβ receptor, TNFBR, CD120b	461	48	T cell, monocyte, dendritic cell, brain, heart, uterus, lung, thymus, intestine, kidney	Possibly serving as a decoy receptor; the soluble form reduces the activity of TNF α and β by binding to these ligands

*Based on bibliography 22.17.

its cytoplasmic region, it can also activate the nuclear factor κB mitogenic signaling pathway. However, how TNFRI selectively activates different signaling pathways remains poorly understood. TNFRI also exists in the form of soluble protein, which is generated by the cleavage of the membrane TNFRI. Soluble TNFRI can bind to and block the activity of TNFα. A number of cell types express TNFRI, including monocytes, neutrophils, endothelial cells, and hepatocytes.

Compared to TNFRI, TNFRII shows relatively low affinity to TNFα. Thus, the activity of TNFα is thought to be regulated primarily by TNFRI. TNFRII can interact with TNFβ. However, the binding of TNFβ to TNFRII does not induce activation of intracellular signaling pathways, suggesting that TNFRII may serve as a decoy receptor. There exists a soluble form of TNFRII, which is generated by cleaving the membrane form by a metalloproteinase known as TNF-receptor releasing enzyme (TRRE). The soluble form can bind to TNFα and TNFβ, which reduces the activity of these factors.

Molecular therapies can be developed on the basis of the observations described above. The overexpression of TNFRII can impose an inhibitory effect on the inflammatory effect of TNFα and TNFβ. Such a strategy can be achieved by transferring the TNFRII gene into target cells. Overexpressed TNFRII can effectively inhibit collagen-induced arthritis in mouse models. Furthermore, since soluble TNFRI binds to and block the activity of TNFα and reduces inflammatory reactions, the promotion of soluble TNFRI production helps to reduce arthritis. In particular, the cotransfer of a soluble TNF receptor-IgG1 fusion protein gene and the IL10 gene significantly suppresses the development of collagen-induced arthritis in animal models. Experimental investigations have demonstrated positive results for the genetic treatment of autoimmune disorders by manipulating the TNFR gene.

Antiinflammatory Cytokines [22.18]. There are a number of cytokines, including IL4, IL10, and IL13 (see Table 22.8 for IL13 characteristics), which exert antiinflammatory effects (see page 631 for the characteristics of these factors). These factors inhibit the release of proinflammatory factors, such as interferon-γ, IL1α, IL1β, and tumor necrosis factor, and stimulate the production and release of IL1Ra, which is known to competitively suppress the inflammatory effect of IL1α and IL1β. In particular, the structure and function

TABLE 22.8. Characteristics of IL13*

Proteins	Alternative Names	Amino Acids	Molecular Weight (kDa)	Expression	Functions
IL13	—	132	14	Lymphocyte, primarily activated Th2 cells, skin	Regulating B-cell differentiation, suppressing monocyte and macrophage activity, and inhibiting the production of proinflammatory cytokines and chemokines

*Based on bibliography 22.18.

of IL-10 have been studied extensively. Here, IL-10 is used as an example to demonstrate how the antiinflammatory cytokines inhibit the activity of proinflammatory factors.

Interleukin-10 is a protein (~18 kDa, 178 amino acids) present in the form of homodimer. IL10 is expressed in a umber of cell types, including monocytes, macrophages, T cells, B cells, natural killer cells, microglial cells, dendritic cells, eosinophils, and keratinocytes. The concentration of circulating IL10 is about 0.5 pg/mL in humans and other mammals. IL10 exerts its anti-inflammatory effect via interacting with the IL10 receptor. The IL10 receptor is a transmembrane glycoprotein complex composed of heterodimers α and β chains. The α chain is responsible for the binding of ligands, whereas the β chain transduces signals to the cytoplasmic signaling pathways. Each chain is composed of an extracellular, transmembrane, and cytoplasmic region. The binding of IL-10 to its receptor induces a number of antiinflammatory activities, including the inhibition of interferon-γ production and release from the T cells, suppression of chemokine secretion from neutrophils (e.g., MIP1α, MIP1β, and IL8), blockade of proinflammatory effects of IL1 and TNFα, reduction of inflammatory mediator generation in monocytes (e.g., IL8), and promotion of IL1Ra production. These activities results in the suppression of T-cell activation and immune responses, exerting beneficial effects for the treatment of rheumatoid arthritis. For therapeutic purposes, the genes encoding IL10 and IL10 receptor can be transferred into target cells in rheumatoid arthritis. Preliminary studies have demonstrated promising results for the treatment of arthritis by using IL10.

Dominant-Negative Mutant Ras Gene [22.19]. Ras is a protein that mediates the transduction of mitogenic factor signals. Acivated Ras stimulates cell proliferation and inflammatory reactions. Thus, Ras activation enhances the development of arthritis. The suppression of the Ras activity is an effective approach for the treatment of arthritis. One memthod for suppressing the Ras activity is to construct and transfer a dominant-negative mutant ras gene into the target cells. The dominant-negative mutant ras gene can be integrated into a gene-carrying vector such as a replication-deficient adenovirus vector. The vector can be delivered to the site of arthritis. Experimental investigations with such an approach have demonstrated that the delivered dominant-negative mutant ras gene can be expressed in target cells, resulting in a significant reduction in the level of inflammatory reactions in the joints (Fig. 22.12).

Osteoprotegerin [22.20]. Osteoprotegerin (OGP) is a protein of the cytokine tumor necrosis factor (TNF) receptor superfamily. This proetin serves as a decoy receptor for the osteoprotegerin ligand (OPGL) and can bind and inactivate OPGL. Osteoprotegerin ligand is a factor that stimulates the differentiation and proliferation of osteoclasts, which induce bone resorption and degeneration. Thus, osteoprotegerin can inhibit the activity of OPGL, suppress bone resorption, and enhance bone mineral deposition and bone formation. In the skeletal system, bone growth and resorption are controlled to a certain extent by the balance between OPGL and its decoy receptor osteoprotegerin. In transgenic animal models, the overexpression of osteoprotegerin is associated with eenhanced bone growth and reduced bone resorption. In contrast, the genetic disrutpion or knock out of the osteoprotegerin gene enhances bone resorption, resulting in osteoporosis-like alterations. Osteoprotegerin is present in the circulation and interstitial fluids of various tissues and organs. The direct delivery of osteoprotegerin or the transfer of the osteoprotegerin gene into the skeletal system prevents bone and cartilage resorption and destruction in inflammation and arthritis.

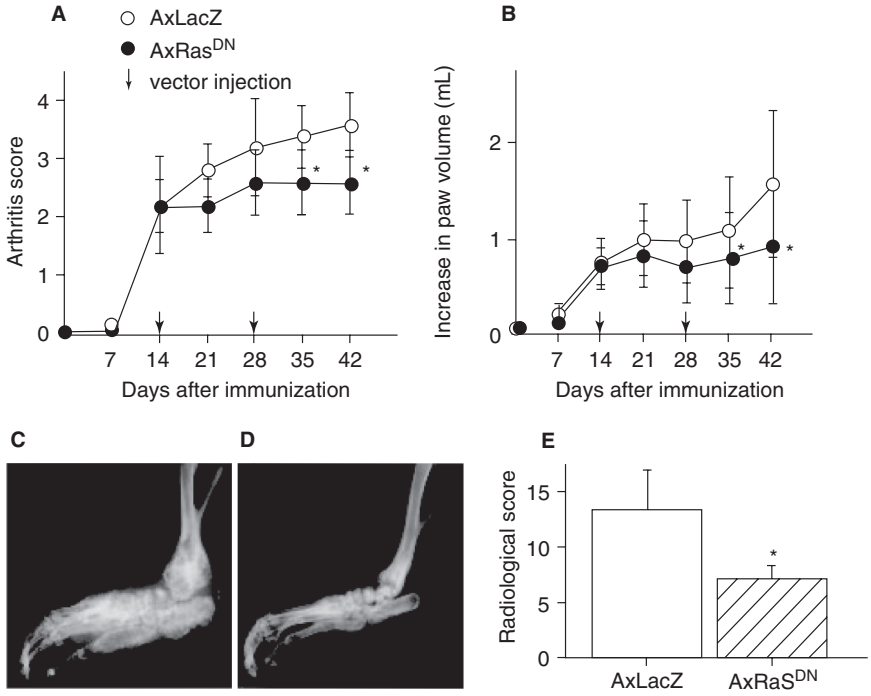


Figure 22.12. Therapeutic effects of replication-deficient adenovirus vector carrying the dominant-negative mutant of the Ras gene (AxRasDN) on rat adjuvant arthritis. All rats were immunized with a subcutaneous injection of adjuvant in the base of the tail (day 0). Viruses were then intraarticularly injected into the right ankles on days 7 and 14. Bars show the mean ± SD of 10 rats per group. (A) Effects of AxRasDN injection, evaluated by arthritis score. The arthritis score of the AxRasDN group was significantly lower than that of the control AxLacZ group on days 35 and 42. (B) Effects of AxRasDN injection, evaluated by the increase in paw volume. The increase in paw volume of the AxRasDN group was significantly less than that of the AxLacZ group on days 35 and 42. (C) The radiologic findings in the right ankles of AxLacZ-injected rats indicate severe joint destruction. (D) The radiologic findings in the right ankles of AxRasDN-injected rats show minimal destructive changes in the joint. (E) The radiologic score of the AxRasDN-injected ankles was significantly decreased in comparison with that of the AxLacZ group. * = $P < 0.01$ versus AxLacZ-injected joints. (Reprinted with permission of Wiley-Liss, Inc, a subsidiary of John Wiley & Sons, Inc., from Yamamoto A et al: Suppression of arthritic bone destruction by adenovirus-mediated dominant-negative Ras gene transfer to synoviocytes and osteoclasts, *Arthritis Rheum* 48:2682–92, copyright 2003.)

Viral Thymidine Kinase [22.21]. Rheumatoid arthritis often affects the synovium, inducing synoviocyte proliferation and synovium hypertrophy. These pathological changes often impose harmful effects on the function of the involved joints. One of the treatments for such a disorder is to remove altered synovium by surgical synovectomy. However, surgical trauma often induces inflammatory reactions and scar formation, which facilitate the distortion and destruction of the joint tissue. Alternatively, genes encoding growth inhibiting proteins can be used for the suppression of synoviocyte proliferation. One of such genes is the herpes viral thymidine kinase gene. This gene encodes a thymidine kinase protein, which can convert nucleosides to nucleotides by phosphorylation. In

dividing or proliferating cells, nucleotides are taken up by cells for DNA synthesis. When nucleoside analogues (e.g., ganciclovir) are present, the viral thymidine kinase can covert the nucleoside analogues to nucleotides, which can be used for DNA synthesis. However, the incorporation of the nucleotide analogs results in the termination of DNA synthesis, because no more nucleotides can be added to the incorporated nucleotide analogues. By such a mechanism, the viral thymidine kinase gene, together with nucleoside analogues, can be used to suppress the proliferation of synoviocytes and reduce pathological changes in rheumatoid arthritis.

Bone and Cartilage Injury

Pathogenesis, Pathology, and Clinical Features [22.22]. Bone and cartilage injury occurs due to mechanical overload in sports and accidents. Various types of injury can be induced, depending on the magnitude and direction of the mechanical load. The most common injury is bone fracture. Such an injury is followed by several remodeling processes, including inflammatory, reparative, and modeling processes. The understanding of these processes is critical to the treatment of bone injury. Here, bone fracture is used as an example to discuss these remodeling processes.

Inflammatory Responses. Inflammation is a process that occurs in response to injury. Such a process is necessary for the self-healing of injured tissues or organs. While inflammation is common to all types of tissue, there are several unique features for bone injury. Bone injury induces transport and deposition of calcium and phosphorus, which do not occur in the inflammation of other tissues; also, since bone is subject to large mechanical loads, bone inflammation and recovery are influenced by mechanical forces.

Bone injury induces a series of inflammatory reactions. Blood vessel injury usually occurs with bone fracture, inducing hemorrhage and the formation of hematoma. At the same time, necrotic and injured cells can release cytokines and growth factors, which stimulate the infiltration of leukocytes and the migration of fibroblasts and other cell types to the injury site. Cytokines and growth factors also stimulate the proliferation of these cells and the production of extracellular matrix. All these reactions contribute to the formation of granulation tissue, which replaces the necrotic tissue and hematoma. Furthermore, angiogenesis occurs near the injury site, generating new blood vessels that provide oxygen and nutrients necessary for cell and tissue regeneration. Within about two weeks, calcium and phosphorus start to deposit to the injured tissue, resulting in the formation of immature membranous bone structure, which is referred to as a *callus.* A callus can be further mineralized to form a mature bone as described in the following section.

Reparative Reactions. Reparative reactions are initiated for the formation of mature bones based on calluses. Following the inflammatory phase, callus formation occurs at several locations. One is formed near the cortical surface of the bone at the injury site by the periosteum and adjacent skeletal muscle cells. In the medullary cavity at the injury site, the bone marrow cells can form a callus, which seals the fracture. Another type of callus forms between the two fracture-ends, serving to bridge the gap between the calluses at the ends. Additional calluses can be formed to join all separate calluses. Within about a month, a bone fracture can be filled with joined calluses.

Modeling Process. Modeling is a process by which the newly formed bone is further matured, organized, and aligned along the direction of the principal mechanical forces. The modeling process is thought to be regulated by mechanical stress. There are several events for the modeling phase. First, the newly generated bone structure is reshaped in response to the distribution of the mechanical stress. In regions with sufficient mechanical stress, the new bone is strengthened with additional mineralization, whereas in regions without sufficient mechanical stress new bone may be absorbed and degraded. Second, the medullary cavity and bone marrow are gradually restored. Third, the restoration of the natural form of bone structure (also referred to as *bone reconstitution*) is accomplished by coordinated bone resorption and regeneration. Bone resorption is induced by osteoclasts, whereas bone regeneration is induced by osteoblasts. Each type of cell may be activated in response to mechanical stress. A mechanical stress below a critical level may activate osteoclasts, initiating bone resorption. In contrast, a mechanical stress above a critical level may activate osteoblasts, resulting in bone regeneration. With such a stress-regulated process, the reconstituted bone can be eventually shaped to the original natural form. Bone regeneration is a long-term process. The entire reconstitution process may take about several years.

Complications of Bone Injury. There are several complications that may occur during bone healing. The most common complications include fibrous union and nonunion. Fibrous union is a form of bone reconstitution with the establishment of a fibrous tissue bridge without mineralization between the fractured bones. A major cause for the formation of the fibrous tissue bridge is the lack of blood supply to the injured bone. A poor blood supply negatively influences the formation of calluses while promoting the formation of fibrous scar tissue, resulting in the formation of fibrous tissue bridges. Fibrous union often occurs in the injury of the distal pretibia and carpal navicular bone, which are associated with scarce blood vessels and insufficient blood circulation. Nonunion is a form of incomplete bone reconstitution, leaving a boneless gap between the ends of healed bone. Several factors, including bone loss, dislocation of fractured bone, infection, and severe soft tissue damage, may contribute to the bone nonunion. Such a consequence may occur in long-bone fracture.

Conventional Therapy [22.22]. The treatment of bone fracture and other types of bone injury is primarily dependent on the ability of the bone in self-healing and self-regeneration. Several approaches can be used to assist fractured bones in the healing processes. These include the realignment and restriction of dislocated bones to their natural positions by using external fixation devices, which ensures the anatomical reconstitution of the bones, stop of hemorrhage promptly, protection of injured bones from infection by administrating antibiotics, and treatment of bone non-union, if any, by bone transplantation or grafting. These approaches are usually effective for the treatment of bone fracture.

Molecular Regenerative Engineering [22.23]. The strategies for the molecular treatment of bone injury are to enhance bone regeneration and prevent bone nonunion or fibrous union. Although injured bones can be self-healed and conventional therapies are effective for most cases, bone reconstitution may take several months or longer. Furthermore, a fraction about 10% of bone fractures may experience delayed union or form nonunion structures. Bone regeneration can be greatly enhanced by using molecular regenerative

approaches. One effective approach is delivering bone formation-stimulating proteins or their genes into the cells responsible for bone regeneration. Typical factors that stimulate bone formation are bone morphogenetic proteins (BMPs), which can be used to enhance bone regeneration.

Bone morphogenetic protein 2, a member of the bone morphogenetic protein family, has been characterized and studied extensively in experimental bone injury models established in the rat, rabbit, and dog. The delivery of bone morphogenetic protein-2 or its gene to fractured bone enhances bone regeneration (Fig. 22.9). Clinical trials have demonstrated the effectiveness of locally delivered bone morphogenetic protein-2 in promoting bone regeneration. Bone morphogenetic protein 4 has also been used to enhance bone formation in experimental models of bone injury, demonstrating similar therapeutic effects as bone morphogenetic protein 2. The genes of these proteins can be used for gene therapy for improving bone regeneration. In addition to the bone morphogenetic protein genes, genes encoding angiogenic factors and vascular endothelial growth factor have been used for the promotion of bone regeneration. These genes can be delivered directly to the injury sites or indirectly delivered via the mediation of a matrix scaffold. Adenovirus vectors have been primarily used for mediating bone gene delivery.

Other growth factors, such as fibroblast growth factors (FGFs) and insulin-like growth factor (IGF), regulate the proliferation and differentiation of bone and cartilage cells (Fig. 22.13). These growth factors can be used to enhance the repair of injured bone and cartilage. In particular, fibroblast growth factors represent a family of potent growth factors for bone regeneration. The FGF family contains 23 known members. Among these members, FGF1 (~18 kDa, 155 amino acids) and FGF2 (~18 kDa, 155 amino acids) have been known to play an important role in the regulation of cell proliferation and differentiation. These growth factors are produced by many cell types, including the fibroblasts, endothelial cells, macrophages, hepatocytes, and keratinocytes. Both FGF1 and FGF2 can interact with FGF receptor tyrosine kinases and induce the proliferation of many cell

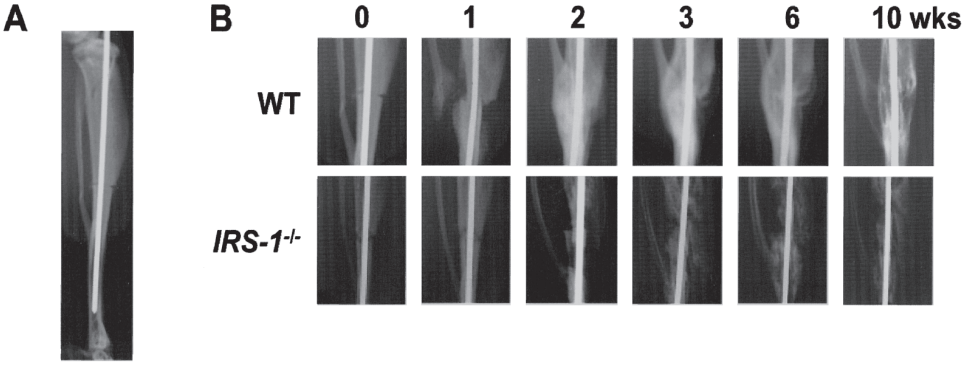


Figure 22.13. X-ray features of bone healing in WT and *IRS-1*$^{-/-}$ mice (IRS: insulin receptor substrate-1)**.** (A) The fracture model used in this study. After exposing the right tibiae of 8-week-old mice, a transverse osteotomy was performed at the midshaft with a bone saw. The bone marrow cavity was then stabilized with an intramedullary nail. (B) Time course of the fracture healing in representative WT and *IRS-1*$^{-/-}$ mice. (Reprinted with permission from Shimoaka T et al: Impairment of bone healing by insulin receptor substrate-1 deficiency, *J Biol Chem* 279:15314–22, copyright 2004.)

types, including osteoblasts, chondrocytes, endothelial cells, and fibroblasts, thus enhacing bone and cartilage formation.

Cell Regenerative Engineering [22.24]. Bone regeneration involves the activation of osteoprogenitor cells, which differentiate to osteoblasts and other types of bone cells. It is thus conceivable to transplant osteoprogenitor cells or stem cells to promote bone formation. The bone marrow is known to contain osteoprogenitor cells. Bone marrow-derived cells have been used for the enhancement of bone regeneration in animal models as well as in humans. The transplantation of these cells to injured bones significantly facilitates bone formation and injury recovery. Other types of stem cells, such as embryonic, fetal, muscle-derived adult, and fat-derived adult stem cells, may also be used for bone regenerative engineering. These stem cells may be induced to differentiate to osteoblasts and other types of bone cells, when appropriate experimental conditions and extracellular environment are provided.

For the treatment of cartilage injury, several types of cells, including chondrocytes and osteochondrocytes, can be used for cell transplantation. The cells can be seeded in a scaffold constructed with an appropriate material (e.g., hyaluronan-based biopolymers or collagen matrix). A cell-seeded scaffold can be used to replace injured cartilage. In addition to chondrocytes, other cell types, such as embryonic stem cells and bone marrow stromal cells or mesenchymal stem cells, can be used for cartilage regeneration.

The type of matrix scaffolds may influence the differentiation of progenitor and stem cells. For instance, when bone marrow stem cells are cultured in a hyaluronan matrix with the supplement of transforming growth factor β1, the stem cells can differentiate into chondrocytes, forming a cartilage-like structure. When bone marrow stem cells are seeded in a porous calcium phosphate scaffold, the stem cells are transformed into osteoblasts, forming a bone-like structure. Bone morphogenetic proteins and growth factors can be used in cartilage constructs to facilitate cartilage formation. These approaches have been successfully used in experimental models for cartilage regeneration.

Progenitor and stem cells for bone and cartilage regeneration can be genetically transfected with genes encoding bone regeneration-promoting factors, such as the bone morphogenetic protein and vascular endothelial growth factor genes. Such cells may exhibit enhanced capability of differentiation and proliferation, thus facilitating bone regeneration and recovery. Alternatively, the bone formation-stimulating genes can be delivered by using gene carriers. Fibroblasts derived from soft connective tissue have been used as such gene carriers. Fibroblasts can be collected, cultured, and transfected with a selected gene in vitro, and used for cell transplantation in vivo. These approaches have been successfully used in experimental models. In human trials, mesenchymal stem cells have been used as gene carriers for bone-injury therapy. These trials have demonstrated encouraging results.

Tissue Regenerative Engineering [22.25]. While molecular and cellular therapies enhance bone regeneration, engineering manipulations at the tissue level is also important for the recovery from bone injury, especially in delayed bone union and nonunion. A major type of engineering manipulation is bone grafting or reconstruction with bone substitutes. Such a manipulation is necessary in the case of bone loss and destruction. There are several types of materials that can be used for such a purpose. These include autogenous cancellous bone specimens, metal prostheses, calcium phosphate ceramics, and polymeric materials. Among these materials, the autogenous cancellous bone is the gold standard material. The cancellous bone contains osteoprogenitor cells and osteoblasts, which play a critical role in bone repair and regeneration. However, in the case of large bone

destruction, it is difficult to collect sufficient cancellous bone specimens. It is necessary to use other types of materials, such as synthetic materials and allogenic bones.

Synthetic materials have been used and tested for bone reconstruction. Osteoprogenitor or stem cells can be seeded in scaffolds of synthetic materials. A cell-containing scaffold can be tailored into a desired shape and used for bone grafting and reconstruction. However, synthetic materials cannon be integrated into the natural skeletal system. It is often difficult for cells and blood vessels to grow into the synthetic bone substitute. These limitations hinder the use of synthetic materials for bone reconstruction. Allogenic bone specimens are alternative materials for bone reconstruction. However, allogenic bones with living cells induce acute immune rejection reactions. It is necessary to administrate immune suppressive agents for patients with allogenic bone grafting. The removal of living cells from allogenic bone grafts can significantly reduce immune responses. Decellularized allogenic bone specimens can be used as bone substitutes for bone reconstruction.

BIBLIOGRAPHY

22.1. Anatomy and Physiology of the Bone and Cartilage

Li YC, Kong J, Wei M, Chen ZF, Liu SQ et al: 1,25-Dihydroxyvitamin D3 is a negative endocrine regulator of the renin-angiotensin system, *J Clin Invest* 110:229–38, 2002.

Suda T, Ueno Y, Fujii K, Shinki T: Vitamin D and bone, *J Cell Biochem* 88(2):259–66, 2003.

Guyton AC, Hall JE: *Textbook of Medical Physiology*, 11th ed, Saunders, Philadelphia, 2006.

McArdle WD, Katch FI, Katch VL: *Essentials of Exercise Physiology*, 3rd ed, Lippincott Williams & Wilkins, Baltimore, 2006.

Germann WJ, Stanfield CL (with contributors Niles MJ, Cannon JG), *Principles of Human Physiology*, 2nd ed, Pearson Benjamin Cummings, San Francisco, 2005.

Thibodeau GA, Patton KT: *Anatomy & Physiology*, 5th ed, Mosby, St. Louis, 2003.

Boron WF, Boulpaep EL: *Medical Physiology*: *A Cellular and Molecular Approach*, Saunders, Philadelphia, 2003.

Ganong WF: *Review of Medical Physiology*, 21st ed, McGraw-Hill, New York, 2003.

22.2. Pathogenesis, Pathology, and Clinical Features of Osteoporosis

Kanis JA, Melton III LJ, Christiansen C, Johnston CC, Khaltaev N: The diagnosis of osteoporosis, *J Bone Miner Res* 9:1137–41, 1994.

Downey PA, Siegel MI: Bone biology and the clinical implications for osteoporosis, *Phys Ther* 86(1):77–91, 2006.

Raisz LG: Pathogenesis of osteoporosis: Concepts, conflicts, and prospects, *J Clin Invest* 115(12):3318–25, 2005.

Rosen CJ, Brown SA: A rational approach to evidence gaps in the management of osteoporosis, *Am J Med* 118(11):1183–9, 2005.

Feng X: Regulatory roles and molecular signaling of TNF family members in osteoclasts, *Gene* 350(1):1–13, 2005.

Chien KR, Karsenty G: Longevity and lineages: Toward the integrative biology of degenerative diseases in heart, muscle, and bone. *Cell* 120(4):533–44, 2005.

Bassett JH, Williams GR: The molecular actions of thyroid hormone in bone, *Trends Endocr Metab* 14(8):356–64, 2003.

Harada S, Rodan GA: Control of osteoblast function and regulation of bone mass, *Nature* 423(6937):349–55, 2003.

Boyle WJ, Simonet WS, Lacey DL: Osteoclast differentiation and activation, *Nature* 423(6937):337–42, 2003.

Schneider AS, Szanto PA: *Pathology*, 3rd ed, Lippincott Williams & Wilkins, Philadelphia, 2006.

Frazier MS, Drzymkowski JW: *Essentials of Human Diseases and Conditions*, 3rd ed, Elsevier Saunders, St Louis, 2004.

22.3. Vitamin D Receptor (VDR)

Morrison NA, Qi JC, Tokita A, Kelly P, Crofts L et al: Prediction of bone density from vitamin D receptor alleles, *Nature* 367:284–7, 1994.

Arai H, Miyamoto KI, Taketani Y, Yamamoto H, Iemori Y et al: A vitamin D receptor gene polymorphism in the translation initiation codon: Effect on protein activity and relation to bone mineral density in Japanese women, *J Bone Miner Res* 12:915–21, 1997.

Garnero P, Munoz F, Borel O, Sornay-Rendu E, Delmas PD: Vitamin D receptor gene polymorphisms are associated with the risk of fractures in postmenopausal women, independently of bone mineral density, *J Clin Endocr Metab* 90(8):4829–35, 2005.

Carling T, Rastad J, Akerstrom G, Westin G: Vitamin D receptor (VDR) and parathyroid hormone messenger ribonucleic acid levels correspond to polymorphic VDR alleles in human parathyroid tumors, *J Clin Endocr Metab* 83:2255–9, 1998.

Colin EM, Uitterlinden AG, Meurs JBJ, Bergink AP, van de Klift M et al: Interaction between vitamin D receptor genotype and estrogen receptor alpha genotype influences vertebral fracture risk, *J Clin Endocr Metab* 88:3777–84, 2003.

Ensrud KE, Stone K, Cauley JA, White C, Zmuda JM et al: Vitamin D receptor gene polymorphisms and the risk of fractures in older women, *J Bone Miner Res* 14:1637–45, 1999.

Ferrari S, Manen D, Bonjour JP, Slosman D, Rizzoli R: Bone mineral mass and calcium and phosphate metabolism in young men: Relationships with vitamin D receptor allelic polymorphisms, *J Clin Endocr Metab* 84:2043–8, 1999.

Houston LA, Grant SFA, Reid DM, Ralston SH: Vitamin D receptor polymorphism, bone mineral density, and osteoporotic vertebral fracture: Studies in a UK population, *Bone* 18:249–52, 1996.

Hughes MR, Malloy PJ, Kieback DG, Kesterson RA, Pike JW et al: Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets, *Science* 242:1702–5, 1988.

Hustmyer FG, Peacock M, Hui S, Johnston CC, Christian J: Bone mineral density in relation to polymorphism at the vitamin D receptor gene locus, *J Clin Invest* 94:2130–4, 1994.

Kelly PJ, Hopper JL, Macaskill GT, Pocock NA, Sambrook PN et al: Genetic factors in bone turnover, *J Clin Endocr Metab* 72:808–13, 1991.

Lorentzon M, Lorentzon R, Nordstrom P: Vitamin D receptor gene polymorphism is associated with birth height, growth to adolescence, and adult stature in healthy Caucasian men: A cross-sectional and longitudinal study, *J Clin Endocr Metab* 85:1666–71, 2000.

Malloy PJ, Eccleshall TR, Gross C, Van Maldergem L, Bouillon R et al: Hereditary vitamin D resistant rickets caused by a novel mutation in the vitamin D receptor that results in decreased affinity for hormone and cellular hyporesponsiveness, *J Clin Invest* 99:297–304, 1997.

Morrison NA, Qi JC, Tokita A, Kelly PJ, Crofts L et al: Prediction of bone density from vitamin D receptor alleles, *Nature* 367:284–7, 1994. Note: Erratum: *Nature* 387: 106 only, 1997.

Riggs BL: Vitamin D-receptor genotypes and bone density, [editorial], *New Engl J Med* 337:125–126, 1997.

Sainz J, Van Tornout JM, Loro L, Sayre J, Roe TF et al: Vitamin D-receptor gene polymorphisms and bone density in prepubertal American girls of Mexican descent, *New Engl J Med* 337:77–82, 1997.

Uitterlinden AG, Weel AEAM, Burger H, Fang Y, Van Duijn CM et al: Interaction between the vitamin D receptor gene and collagen type I-alpha-1 gene in susceptibility for fracture, *J Bone Miner Res* 16:379–85, 2001.

Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K et al: Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning, *Nature Genet* 16:391–6, 1997.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

22.4. Type I Collagen Gene

Aitchison K, Ogilvie D, Honeyman M, Thompson E, Sykes B: Homozygous osteogenesis imperfecta unlinked to collagen I genes, *Hum Genet* 78:233–6, 1988.

Bateman JF, Chan D, Walker ID, Rogers JG, Cole WG: Lethal perinatal osteogenesis imperfecta due to the substitution of arginine for glycine at residue 391 of the alpha-1(I) chain of type I collagen, *J Biol Chem* 262:7021–7, 1987.

Bateman JF, Lamande SR, Dahl HHM, Chan D, Cole WG: Substitution of arginine for glycine 664 in the collagen alpha-1(I) chain in lethal perinatal osteogenesis imperfecta, *J Biol Chem* 263:11627–30, 1988.

Boedtker H, Fuller F, Tate V: The structure of collagen genes, *Int Rev Connect Tissue Res* 10:1–63, 1983.

Bonadio J, Ramirez F, Barr M: An intron mutation in the human alpha-1(I) collagen gene alters the efficiency of pre-mRNA splicing and is associated with osteogenesis imperfecta type II, *J Biol Chem* 265:2262–8, 1990.

Cabral WA, Mertts MV, Makareeva E, Colige A, Tekin M et al: Type I collagen triplet duplication mutation in lethal osteogenesis imperfecta shifts register of alpha chains throughout the helix and disrupts incorporation of mutant helices into fibrils and extracellular matrix, *J Biol Chem* 278:10006–12, 2003.

Chamberlain JR, Schwarze U, Wang PR, Hirata RK, Hankenson KD et al: Gene targeting in stem cells from individuals with osteogenesis imperfecta, *Science* 303:1198–1201, 2004.

Chu ML, de Wet W, Bernard M, Ramirez F: Fine structural analysis of the human pro-alpha-1(I) collagen gene: Promoter structure, AluI repeats, and polymorphic transcripts, *J Biol Chem* 260:2315–20, 1985.

Constantinou CD, Nielsen KB, Prockop DJ: A lethal variant of osteogenesis imperfecta has a single base mutation that substitutes cysteine for glycine 904 of the alpha-1(I) chain of type I procollagen: The asymptomatic mother has an unidentified mutation producing an overmodified and unstable type I procollagen, *J Clin Invest* 83:574–84, 1989.

Dalgleish R: The human type I collagen mutation database, *Nucleic Acids Res* 25:181–7, 1997.

Di Lullo GA, Sweeney SM, Korkko J, Ala-Kokko L, San Antonio JD: Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen, *J Biol Chem* 277:4223–31, 2002.

Grant SFA, Reid DM, Blake G, Herd R, Fogelman I et al: Reduced bone density and osteoporosis associated with a polymorphic Sp1 binding site in the collagen type I-alpha 1 gene, *Nature Genet* 14:203–5, 1996.

Long JR, Liu PY, Lu Y, Xiong DH, Zhao LJ et al: Association between COL1A1 gene polymorphisms and bone size in Caucasians, *Eur J Hum Genet* 12:383–8, 2004.

Pereira R, Khillan JS, Helminen HJ, Hume EL, Prockop DJ: Transgenic mice expressing a partially deleted gene for type I procollagen (COL1A1): A breeding line with a phenotype of spontaneous fractures and decreased bone collagen and mineral, *J Clin Invest* 91:709–16, 1993.

Uitterlinden AG, Burger H, Huang Q, Yue F, McGuigan FEA et al: Relation of alleles of the collagen type I-alpha-1 gene to bone density and the risk of osteoporotic fractures in postmenopausal women, *New Engl J Med* 338:1016–21, 1998.

Uitterlinden AG, Weel, AEAM, Burger H, Fang Y, Van Duijn CM et al: Interaction between the vitamin D receptor gene and collagen type I-alpha-1 gene in susceptibility for fracture, *J Bone Miner Res* 16:379–85, 2001.

Grant SFA, Reid DM, Blake G, Herd R, Fogelman I et al: Reduced bone density and osteoporosis associated with a polymorphic Sp1 site in the collagen type I alpha 1 gene, *Nat Genet* 14:203–5, 1996.

Mann V, Hobson EE, Li B, Stewart TL, Grant SF, Robins SP et al: A COL1A1 Sp1 binding site polymorphism predisposes to osteoporotic fracture by affecting bone density and quality, *J Clin Invest* 107:899–907, 2001.

Qureshi AM, McGuigan FEA, Seymour DG, Hutchison JD, Reid DM et al: Association between COLIA1 Sp1 alleles and femoral neck geometry, *Calcif Tissue Int* 69:67–72, 2001.

Garcia-Giralt N, Nogues X, Enjuanes A, Puig J, Mellibovsky L et al: Two new single nucleotide polymorphisms in the COLIA1 upstream regulatory region and their relationship with bone mineral density, *J Bone Miner Res* 17:384–93, 2002.

Niyibizi C, Wang S, Mi Z, Robbins PD: Gene therapy approaches for osteogenesis imperfecta, *Gene Ther* 11(4):408–16, 2004.

22.5. Estrogen Receptor

Rodan GA, Martin TJ: Therapeutic approaches to bone diseases, *Science* 289:1508–14, 2000.

Alves SE, Lopez V, McEwen BS, Weiland NG: Differential colocalization of estrogen receptor beta (ER-beta) with oxytocin and vasopressin in the paraventricular and supraoptic nuclei of the female rat brain: An immunocytochemical study, *Proc Natl Acad Sci USA* 95:3281–3286, 1998.

Bonnelye E, Aubin JE: Estrogen receptor-related receptor alpha: A mediator of estrogen response in bone, *J Clin Endocr Metab* 90(5):3115–21, 2005.

Rosen CJ: Clinical practice. Postmenopausal osteoporosis, *New Engl J Med* 353(6):595–603, 2005.

Kurita T, Medina R, Schabel AB, Young P, Gama P et al: The activation function-1 domain of estrogen receptor alpha in uterine stromal cells is required for mouse but not human uterine epithelial response to estrogen, *Differentiation* 73(6):313–22, 2005.

Vaskivuo TE, Maentausta M, Torn S, Oduwole O, Lonnberg A et al: Estrogen receptors and estrogen-metabolizing enzymes in human ovaries during fetal development, *J Clin Endocr Metab* 90(6):3752–6, 2005.

Bershtein LM, Poroshina TE, Zimarina TS, Tsyrlina EV, Zhil'tsova EK et al: Expression of estrogen receptors-alpha and -beta in primary breast neoplasms and tumors exposed to neoadjuvant hormonal therapy, *Bull Exp Biol Med* 138(5):494–6, 2004.

Poola I, Abraham J, Liu A: Estrogen receptor beta splice variant mRNAs are differentially altered during breast carcinogenesis, *J Steroid Biochem Mol Biol* 82(2–3):169–79, 2002.

Cartoni R, Leger B, Hock MB, Praz M, Crettenand A et al: Mitofusins 1/2 and ERRalpha expression are increased in human skeletal muscle after physical exercise, *J Physiol* 567(Pt 1):349–58, 2005.

Mansur Ade P, Nogueira CC, Strunz CM, Aldrighi JM, Ramires JA: Genetic polymorphisms of estrogen receptors in patients with premature coronary artery disease, *Arch Med Res* 36(5):511–7, 2005.

Albagha OME, Pettersson U, Stewart A, McGuigan FEA, MacDonald HM et al: Association of oestrogen receptor alpha gene polymorphisms with postmenopausal bone loss, bone mass, and quantitative ultrasound properties of bone, *J Med Genet* 42:240–6, 2005.

Bord S, Horner A, Beavan S, Compston J: Estrogen receptors alpha and beta are differentially expressed in developing human bone, *J Clin Endocr Metab* 86:2309–14, 2001.

Chu S, Mamers P, Burger HG, Fuller PJ: Estrogen receptor isoform gene expression in ovarian stromal and epithelial tumors, *J Clin Endocr Metab* 85:1200–5, 2000.

Colin EM, Uitterlinden AG, Meurs JBJ, Bergink AP, van de Klift M et al: Interaction between vitamin D receptor genotype and estrogen receptor alpha genotype influences vertebral fracture risk, *J Clin Endocr Metab* 88:3777–84, 2003.

Couse JF, Hewitt SC, Bunch DO, Sar M, Walker VR et al: Postnatal sex reversal of the ovaries in mice lacking estrogen receptors alpha and beta, *Science* 286:2328–31, 1999.

Kudwa AE, Bodo C, Gustafsson, JA, Rissman EF: A previously uncharacterized role for estrogen receptor-beta: Defeminization of male brain and behavior, *Proc Natl Acad Sci USA* 102:4608–12, 2005.

Gosden JR, Middleton PG, Rout D: Localization of the human oestrogen receptor gene to chromosome 6q24-q27 by in situ hybridization, *Cytogenet Cell Genet* 43:218–20, 1986.

Green S, Walter P, Kumar V, Krust A, Bornert, JM et al: Human oestrogen receptor cDNA: Sequence, expression and homology to v-erb-A, *Nature* 320:134–9, 1986.

Greene GL, Gilna P, Waterfield M, Baker A, Hort Y et al: Sequence and expression of human estrogen receptor complementary DNA, *Science* 231:1150–4, 1986.

Khosla S, Riggs BL, Atkinson EJ, Oberg AL, Mavilia C et al: Relationship of estrogen receptor genotypes to bone mineral density and to rates of bone loss in men, *J Clin Endocr Metab* 89:1808–16, 2004.

Korach KS: Insights from the study of animals lacking functional estrogen receptor, *Science* 266:1524–7, 1994.

Lee K, Jessop H, Suswillo R, Zaman G, Lanyon L: Bone adaptation requires oestrogen receptor-alpha, *Nature* 424: 389 (only), 2003.

Moore JT, McKee DD, Slentz-Kesler K, Moore LB, Jones SA et al: Cloning and characterization of human estrogen receptor beta isoforms, *Biochem Biophys Res Commun* 247:75–8, 1998.

Kuiper GGJM, Enmark E, Pelto-Huikko M, Nilsson S, Gustafsson JA: Cloning of a novel estrogen receptor expressed in rat prostate and ovary, *Proc Natl Acad Sci USA* 93:5925–30, 1996.

Mosselman S, Polman J, Dijkema R: ER-beta: Identification and characterization of a novel human estrogen receptor, *FEBS Lett* 392:49–53, 1996.

Rissman EF, Heck AL, Leonard JE, Shupnik MA, Gustafsson JA: Disruption of estrogen receptor beta gene impairs spatial learning in female mice, *Proc Natl Acad Sci USA* 99:3996–4001, 2002.

Shim GJ, Wang L, Andersson S, Nagy N, Kis LL et al: Disruption of the estrogen receptor beta gene in mice causes myeloproliferative disease resembling chronic myeloid leukemia with lymphoid blast crisis, *Proc Natl Acad Sci* 100:6694–9, 2003.

Wang L, Andersson S, Warner M, Gustafsson JA: Estrogen receptor (ER)-beta knockout mice reveal a role for ER-beta in migration of cortical neurons in the developing brain, *Proc Natl Acad Sci* 100:703–8, 2003.

Weihua Z, Saji S, Makinen S, Cheng G, Jensen EV et al: Estrogen receptor (ER)-beta, a modulator of ER-alpha in the uterus, *Proc Natl Acad Sci USA* 97:5936–41, 2000.

Zhu Y, Bian Z, Lu P, Karas RH, Bao L et al: Abnormal vascular function and hypertension in mice deficient in estrogen receptor beta, *Science* 295:505–8, 2002.

Weel AM, Uitterlinden AG, Burger H, Schuit SC, Hofman A et al: Estrogen receptor polymorphism predicts the onset of natural and surgical menopause, *J Clin Endocr Metab* 84:3146–50, 1999.

Albagha OM, McGuigan FEA, Reid DM, Ralston SH: Estrogen receptor alpha gene polymorphisms and bone mineral density: Haplotype analysis in women from the United Kingdom, *J Bone Miner Res* 16:128–34, 2001.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

22.6. Calcitonin

Rodan GA, Martin TJ: Therapeutic approaches to bone diseases, *Science* 289:1508–14, 2000.

Martin TJ et al: in *Metabolic Bone Disease*, Avioli LV, Krane SM, eds, Academic Press, New York, 1998, p 95.

Hoovers JMN, Redeker E, Speleman F, Hoppener JWM, Bhola S et al: High-resolution chromosomal localization of the human calcitonin/CGRP/IAPP gene family members, *Genomics* 15:525–9, 1993.

Mathe AA, Agren H, Lindstrom L, Theodorsson E: Increased concentration of calcitonin gene-related peptide in cerebrospinal fluid of depressed patients: A possible trait marker of major depressive disorder, *Neurosci Lett* 182:138–42, 1994.

New HV, Mudge AW: Calcitonin gene-related peptide regulates muscle acetylcholine receptor synthesis, *Nature* 323:809–11, 1986.

Olesen J, Diener HC, Husstedt IW, Goadsby PJ, Hall D et al: Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine, *New Engl J Med* 350:1104–10, 2004.

Saller B, Feldmann G, Haupt K, Broecker M, Janssen OE et al: RT-PCR-based detection of circulating calcitonin-producing cells in patients with advanced medullary thyroid cancer, *J Clin Endocr Metab* 87:292–6, 2002.

22.7. Osteoprotegerin (OPG)

Ulrich-Vinther M, Schwarz EM, Pedersen FS, Soballe K, Andreassen TT: Gene therapy with human osteoprotegerin decreases callus remodeling with limited effects on biomechanical properties, *Bone* 37(6):751–8, 2005.

Kostenuik PJ, Bolon B, Morony S, Daris M, Geng Z et al: Gene therapy with human recombinant osteoprotegerin reverses established osteopenia in ovariectomized mice, *Bone* 34(4):656–64, 2004.

Bolon B, Carter C, Daris M, Morony S, Capparelli C et al: Adenoviral delivery of osteoprotegerin ameliorates bone resorption in a mouse ovariectomy model of osteoporosis, *Mol Ther* 3(2):197–205, 2001.

Bucay N, Sarosi I, Dunstan CR, Morony S, Tarpley J et al: Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification, *Genes Dev* 12:1260–8, 1998.

Croucher PI, Shipman CM, Lippitt J, Perry M, Asosingh K et al: Osteoprotegerin inhibits the development of osteolytic bone disease in multiple myeloma, *Blood* 98:3534–40, 2001.

Cundy T, Davidson J, Rutland MD, Stewart C, DePaoli, AM: Recombinant osteoprotegerin for juvenile Paget's disease, *New Engl J Med* 353:918–23, 2005.

Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A et al: Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand, *Nature* 402:304–9, 1999.

Krane SM: Genetic control of bone remodeling—insights from a rare disease, *New Engl J Med* 347:210–2, 2002.

Mizuno A, Amizuka N, Irie K, Murakami A, Fujise N et al: Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin, *Biochem Biophys Res Commun* 247:610–5, 1998.

Morinaga T, Nakagawa N, Yasuda H, Tsuda E, Higashio K: Cloning and characterization of the gene encoding human osteoprotegerin/osteoclastogenesis-inhibitory factor, *Eur J Biochem* 254:685–91, 1998.

Ohmori H, Makita Y, Funamizu M, Hirooka K, Hosoi T et al: Linkage and association analyses of the osteoprotegerin gene locus with human osteoporosis, *J Hum Genet* 47:400–6, 2002.

Simonet WS, Lacey DL, Dunstan CR, Kelley M, Chang MS et al: Osteoprotegerin: A novel secreted protein involved in the regulation of bone density, *Cell* 89:309–19, 1997.

Soufi M, Schoppet M, Sattler AM, Herzum M, Maisch B et al: Osteoprotegerin gene polymorphisms in men with coronary artery disease, *J Clin Endocr Metab* 89:3764–8, 2004.

Tan KB, Harrop J, Reddy M, Young P, Terrett J et al: Characterization of a novel TNF-like ligand and recently described TNF ligand and TNF receptor superfamily genes and their constitutive and inducible expression in hematopoietic and non-hematopoietic cells, *Gene* 204:35–46, 1997.

Tsuda E, Goto M, Mochizuki S, Yano K, Kobayashi F et al: Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis, *Biochem Biophys Res Commun* 234:137–42, 1997.

Whyte MP, Obrecht SE, Finnegan PM, Jones JL, Podgornik MN et al: Osteoprotegerin deficiency and juvenile Paget's disease, *New Engl J Med* 347:175–84, 2002.

Yasuda H, Shima N, Nakagawa N, Mochizuki SI, Yano K et al: Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): A mechanism by which OPG/OCIF inhibits osteoclastogenesis in vitro, *Endocrinology* 139:1329–37, 1998.

Osteoprotegerin Ligand

Anderson DM, Maraskovsky E, Billingsley WL, Dougall WC, Tometsko ME et al: A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function, *Nature* 390:175–9, 1997.

Croucher PI, Shipman CM, Lippitt J, Perry M, Asosingh K et al: Osteoprotegerin inhibits the development of osteolytic bone disease in multiple myeloma, *Blood* 98:3534–40, 2001.

Fata JE, Kong YY, Li J, Sasaki T, Irie-Sasaki J et al: The osteoclast differentiation factor osteoprotegerin-ligand is essential for mammary gland development, *Cell* 103:41–50, 2000.

Glass DA II, Patel MS, Karsenty G: A new insight into the formation of osteolytic lesions in multiple myeloma, *New Engl J Med* 349:2479–80, 2003.

Ikeda F, Nishimura R, Matsubara T, Tanaka S, Ioune J et al: Critical roles of c-Jun signaling in regulation of NFAT family and RANKL-regulated osteoclast differentiation, *J Clin Invest* 114:475–84, 2004.

Kim N, Odgren PR, Kim DK, Marks SC Jr, Choi Y: Diverse roles of the tumor necrosis factor family member TRANCE in skeletal physiology revealed by TRANCE deficiency and partial rescue by a lymphocyte-expressed TRANCE transgene, *Proc Natl Acad Sci USA* 97:10905–10, 2000.

Koga T, Inui M, Inoue K, Kim S, Suematsu A et al: Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis, *Nature* 428:758–63, 2004.

Kong YY, Feige U, Sarosi I, Bolon B, Tafuri A et al: Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand, *Nature* 402:304–9, 1999.

Lacey DL, Timms E, Tan HL, Kelley MJ, Dunstan CR et al: Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation, *Cell* 93:165–76, 1998.

Pearse RN, Sordillo EM, Yaccoby S, Wong BR, Liau DF et al: Multiple myeloma disrupts the TRANCE/osteoprotegerin cytokine axis to trigger bone destruction and promote tumor progression, *Proc Natl Acad Sci USA* 98:11581–6, 2001.

Sezer O, Heider U, Zavrski I, Kuhne CA, Hofbauer LC: RANK ligand and osteoprotegerin in myeloma bone disease, *Blood* 101:2094–8, 2003.

Takayanagi H, Kim S, Matsuo K, Suzuki H, Suzuki T et al: RANKL maintains bone homeostasis through c-Fos-dependent induction of interferon-B, *Nature* 416:744–9, 2002.

Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL: IL-1 mediates TNF-induced osteoclastogenesis, *J Clin Invest* 115:282–90, 2005.

Wong BR, Rho J, Arron J, Robinson E, Orlinick J et al: TRANCE is a novel ligand of the tumor necrosis factor receptor family that activates c-Jun N-terminal kinase in T cells, *J Biol Chem* 272:25190–4, 1997.

Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M et al: Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL, *Proc Natl Acad Sci USA* 95:3597–602, 1998.

22.8. Integrin-Binding Proteins

McLane MA, Sanchez EE, Wong A, Paquette-Straub C, Perez JC: Disintegrins, *Curr Drug Targets Cardiovasc Haematol Disord* 4(4):327–55, 2004.

Mercer B, Markland F, Minkin C: Contortrostatin, a homodimeric snake venom disintegrin, is a potent inhibitor of osteoclast attachment, *J Bone Miner Res* 13(3):409–14, 1998.

Kumar CC, Nie H, Rogers CP, Malkowski M, Maxwell E et al: Biochemical characterization of the binding of echistatin to integrin alphavbeta3 receptor, *J Pharmacol Exp Ther* 283(2):843–53, 1997.

22.9. Growth Factors

Hiltunen MO, Ruuskanen M, Huuskonen J, Mahonen AJ, Ahonen M et al: Adenovirus-mediated VEGF-A gene transfer induces bone formation in vivo, *FASEB J* 17(9):1147–9, 2003.

Langdahl BL, Knudsen JY, Jensen HK, Gregersen N, Eriksen EF: A sequence variation: 713-8delC in the transforming growth factor-beta 1 gene has higher prevalence in osteoporotic women than in normal women and is associated with very low bone mass in osteoporotic women and increased bone turnover in both osteoporotic and normal women, *Bone* 20:289–94, 1997.

Yamada Y, Miyauchi A, Takagi Y, Tanaka M, Mizuno M, Harada A: Association of the C-509→T polymorphism, alone of in combination with the T869→C polymorphism, of the transforming growth factor-beta1 gene with bone mineral density and genetic susceptibility to osteoporosis in Japanese women, *J Mol Med* 79:149–56, 2001.

Ralston SH: Genetic determinants of susceptibility to osteoporosis, *Curr Opin Pharmacol* 3:286–90, 2003.

Niyibizi C, Wang S, Mi Z, Robbins PD: Gene therapy approaches for osteogenesis imperfecta, *Gene Ther* 11(4):408–16, 2004.

Rodan GA, Martin TJ: Therapeutic approaches to bone diseases, *Science* 289:1508–14, 2000.

22.10. Cell Therapy for Bone Regeneration

Allen TD, Dexter TM, Simmons PJ: Marrow biology and stem cells, *Immunol Ser* 49:1–38, 1990.

Caplan AI: Mesenchymal stem cells, *J Orthop Res* 9:641–50, 1991.

Bianco P, Cossu G: Uno, nessuno e centomila: Searching for the identity of mesodermal progenitors, *Exp Cell Res* 251:257–63, 1999.

Caplan AI: Mesenchymal stem cells, *J Orthop Res* 9:641–50, 1991.

Caplan AI: Review: Mesenchymal stem cells: Cell-based reconstructive therapy in orthopedics, *Tissue Eng* 11(7–8):1198–211, 2005.

Cancedda R, Bianchi G, Derubeis A, Quarto R: Cell therapy for bone disease: A review of current status, *Stem Cells* 21:610–19, 2003.

Quarto R, Mastrogiacomo M, Cancedda R et al: Repair of large bone defects with the use of autologous bone marrow stromal cells, *New Engl J Med* 344:385–6.

Goan SR, Junghahn I, Wissler M et al: Donor stromal cells from human blood engraft in NOD/SCID mice, *Blood* 96:3971–8, 2000.

Pereira RF, Halford KW, O'Hara MD et al: Cultured adherent cells from marrow can serve as long-lasting precursor cells for bone, cartilage, and lung in irradiated mice, *Proc Natl Acad Sci USA* 92:4857–61, 1995.

Horwitz EM, Prockop DJ, Fitzpatrick LA et al: Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta, *Nat Med* 5:309–13, 1999.

Horwitz EM, Prockop DJ, Gordon PL et al: Clinical responses to bone marrow transplantation in children with severe osteogenesis imperfecta, *Blood* 97:1227–31, 2001.

Kadiyala S, Young RG, Thiede MA et al: Culture expanded canine mesenchymal stem cells possess osteochondrogenic potential in vivo and in vitro, *Cell Transplant* 6:125–34, 1997.

Krebsbach PH, Kuznetsov SA, Satomura K et al: Bone formation in vivo: Comparison of osteogenesis by transplanted mouse and human marrow stromal fibroblasts, *Transplantation* 63: 1059–69, 1997.

Ohgushi H, Goldberg VM, Caplan AI: Repair of bone defects with marrow cells and porous ceramic. Experiments in rats, *Acta Orthop Scand* 60:334–9, 1989.

Krebsbach PH, Mankani MH, Satomura K et al: Repair of craniotomy defects using bone marrow stromal cells, *Transplantation* 66:1272–8, 1998.

Casabona F, Martin I, Muraglia A et al: Prefabricated engineered bone flaps: An experimental model of tissue reconstruction in plastic surgery, *Plast Reconstr Surg* 101:577–81, 1998.

Mankani MH, Krebsbach PH, Satomura K et al: Pedicled bone flap formation using transplanted bone marrow stromal cells, *Arch Surg* 136:263–70, 2001.

Bruder SP, Kraus KH, Goldberg VM et al: The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects, *J Bone Joint Surg Am* 80:985–96, 1998.

Kon E, Muraglia A, Corsi A et al: Autologous bone marrow stromal cells loaded onto porous hydroxyapatite ceramic accelerate bone repair in critical-size defects of sheep long bones, *J Biomed Mater Res* 49:328–37, 2000.

Petite H, Viateau V, Bensaid W et al: Tissue-engineered bone regeneration, *Nat Biotechnol* 18:959–63, 2000.

Quarto R, Mastrogiacomo M, Cancedda R et al: Repair of large bone defects with the use of autologous bone marrow stromal cells, *New Engl J Med* 344:385–6, 2001.

Reddi AH: Role of morphogenetic proteins in skeletal tissue engineering and regeneration, *Nat Biotechnol* 16(3):247–52, 1998.

22.11. Pathogenesis, Pathology, and Clinical Features of Paget's Disease

Roodman GD, Windle JJ: Paget disease of bone, *J Clin Invest* 115(2):200–8, 2005.

Pierie JP, Choudry U, Muzikansky A, Finkelstein DM, Ott MJ: Prognosis and management of extramammary Paget's disease and the association with secondary malignancies, *J Am Coll Surg* 196(1):45–50, 2003.

Hadjipavlou AG, Gaitanis IN, Kontakis GM: Paget's disease of the bone and its management, *J Bone Joint Surg Br* 84(2):160–9, 2002.

Roodman GD: Studies in Paget's disease and their relevance to oncology, *Semin Oncol* 28(4 Suppl 11):15–21, 2001.

Dickinson CJ: The possible role of osteoclastogenic oral bacterial products in etiology of Paget's disease, *Bone* 26(2):101–2, 2000.

22.12. Molecular and Cellular Therapies

Cundy T, Davidson J, Rutland MD, Stewart C, DePaoli AM: Recombinant osteoprotegerin for juvenile Paget's disease, *New Engl J Med* 353:918–23, 2005.

22.13. Pathogenesis, Pathology, and Clinical Features of Bone Tumors

Rodan GA, Martin JT: Therapeutic approaches to bone diseases, *Science* 289:1508–14, 2000.

22.14. Pathogenesis, Pathology, and Clinical Features of Rheumatoid Arthritis

Kavanaugh ALP: in *Rheumatoid Arthritis*, 3rd ed, Gallin JI, Snyderman R, eds, Lippincott Williams and Wilkins, 1999.

Firestein GS: Immunologic mechanisms in the pathogenesis of rheumatoid arthritis, *J Clin Rheumatol* 11(3 Suppl):S39–44, 2005.

Walsh NC, Crotti TN, Goldring SR, Gravallese EM: Rheumatic diseases: The effects of inflammation on bone, *Immunol Rev* 208:228–51, 2005.

Rosenstein ED, Greenwald RA, Kushner LJ, Weissmann G: Hypothesis: The humoral immune response to oral bacteria provides a stimulus for the development of rheumatoid arthritis, *Inflammation* 28(6):311–8, 2004.

Sakaguchi S, Sakaguchi N: Animal models of arthritis caused by systemic alteration of the immune system, *Curr Opin Immunol* 17(6):589–94, 2005.

Choileain NN, Redmond HP: Regulatory T-cells and autoimmunity, *J Surg Res* 130(1):124–35, 2006.

Ma Y, Pope RM: The role of macrophages in rheumatoid arthritis, *Curr Pharm Des* 11(5):569–80, 2005.

Meinecke I, Rutkauskaite E, Gay S, Pap T: The role of synovial fibroblasts in mediating joint destruction in rheumatoid arthritis, *Curr Pharm Des* 11(5):563–8, 2005.

22.15. Molecular Therapy

Furlan R, Butti E, Pluchino S, Martino G: Gene therapy for autoimmune diseases, *Curr Opin Mol Ther* 6(5):525–36, 2004.

22.16. Interleukin-1

Wieser C, Stumpf D, Grillhosl C, Lengenfelder D, Gay S et al: A Regulated and constitutive expression of anti-inflammatory cytokines by nontransforming herpesvirus saimiri vectors, *Gene Ther* 12(5):395–406, 2005.

IL-1α

Bensen JT, Langefeld CD, Hawkins GA, Green LE, Mychaleckyj JC et al: Nucleotide variation, haplotype structure, and association with end-stage renal disease of the human interleukin-1 gene cluster, *Genomics* 82:194–217, 2003.

Cox A, Camp NJ, Cannings C, di Giovine FS, Dale M et al: Combined sib-TDT and TDT provide evidence for linkage of the interleukin-1 gene cluster to erosive rheumatoid arthritis, *Hum Mol Genet* 8:1707–13, 1999.

Du Y, Dodel RC, Eastwood BJ, Bales KR, Gao F et al: Association of an interleukin 1-alpha polymorphism with Alzheimer's disease, *Neurology* 55:480–4, 2000.

Furutani Y, Notake M, Fukui T, Ohue M, Nomura H et al: Complete nucleotide sequence of the gene for human interleukin 1 alpha, *Nucleic Acids Res* 14:3167–79, 1986.

Green EK, Harris JM, Lemmon H, Lambert JC, Chartier-Harlin MC et al: Are interleukin-1 gene polymorphisms risk factors or disease modifiers in AD? *Neurology* 58:1566–8, 2002.

Grimaldi LME, Casadei VM, Ferri C, Veglia F, Licastro F et al: Association of early-onset Alzheimer's disease with an interleukin-1-alpha gene polymorphism, *Ann Neurol* 47:361–5, 2000.

Hogquist KA, Nett MA, Unanue ER, Chaplin DD: Interleukin 1 is processed and released during apoptosis, *Proc Natl Acad Sci USA* 88:8485–89, 1991.

Lord PCW, Wilmoth LMG, Mizel SB, McCall CE: Expression of interleukin-1 alpha and beta genes by human blood polymorphonuclear leukocytes, *J Clin Invest* 87:1312–21, 1991.

Nicoll JAR, Mrak RE, Graham DI, Stewart J, Wilcock G et al: Association of interleukin-1 gene polymorphisms with Alzheimer's disease, *Ann Neurol* 47:365–8, 2000.

Wei S, Kitaura H, Zhou P, Ross FP, Teitelbaum SL: IL-1 mediates TNF-induced osteoclastogenesis, *J Clin Invest* 115:282–90, 2005.

Werman A, Werman-Venkert R, White R, Lee JK, Werman B et al: The precursor form of IL-1-alpha is an intracine proinflammatory activator of transcription, *Proc Nat Acad Sci* 101:2434–9, 2004.

IL1β

Auron PE, Webb AC, Rosenwasser LJ, Mucci SF, Rich A et al: Nucleotide sequence of human monocyte interleukin 1 precursor cDNA, *Proc Natl Acad Sci USA* 81:7907–11, 1984.

Cameron P, Limjuco G, Rodkey J, Bennett C, Schmidt JA: Amino acid sequence analysis of human interleukin 1 (IL-1): Evidence for biochemically distinct forms of IL-1, *J Exp Med* 162:790–801, 1985.

El-Omar EM, Carrington M, Chow WH, McColl KEL, Bream JH et al: Interleukin-1 polymorphisms associated with increased risk of gastric cancer, *Nature* 404:398–402, 2000.

Langdahl BL, Lokke E, Carstens M, Stenkjaer LL, Eriksen EF: Osteoporotic fractures are associated with an 86-base pair repeat polymorphism in the interleukin-1-receptor antagonist gene but not with polymorphisms in the interleukin-1 beta gene, *J Bone Miner Res* 15:402–14, 2000.

March CJ, Mosley B, Larsen A, Cerretti DP, Braedt G et al: Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs, *Nature* 315:641–7, 1985.

Nicklin MJH, Weith A, Duff GW: A physical map of the region encompassing the human interleukin-1-alpha, interleukin-1-beta, and interleukin-1 receptor antagonist genes, *Genomics* 19:382–4, 1994.

Ohmura K, Johnsen A, Ortiz-Lopez A, Desany P, Roy M et al: Variation in IL-1-beta gene expression is a major determinant of genetic differences in arthritis aggressivity in mice, *Proc Natl Acad Sci USA* 102:12489–94, 2005.

Patterson D, Jones C, Hart I, Bleskan J, Berger R et al: The human interleukin-1 receptor antagonist (IL1RN) gene is located in the chromosome 2q14 region, *Genomics* 15:173–6, 1993.

Samad TA, Moore KA, Sapirstein A, Billet S, Allchorne A et al: Interleukin-1-beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity, *Nature* 410:471–5, 2001.

Voronov E, Shouval DS, Krelin Y, Cagnano E, Benharroch D et al: IL-1 is required for tumor invasiveness and angiogenesis, *Proc Natl Acad Sci USA* 100:2645–50, 2003.

Type I IL1 Receptor

Copeland NG, Silan CM, Kingsley DM, Jenkins NA, Cannizzaro LA et al: Chromosomal location of murine and human IL-1 receptor genes, *Genomics* 9:44–50, 1991.

Dale M, Nicklin MJ: Interleukin-1 receptor cluster: Gene organization of IL1R2, IL1R1, IL1RL2 (IL-1Rrp2), IL1RL1 (T1/ST2), and IL18R1 (IL-1Rrp) on human chromosome 2q, *Genomics* 57:177–9, 1999.

Dower SK, Kronheim SR, Hopp TP, Cantrell M, Deeley M et al: The cell surface receptors for interleukin-1(alpha) and interleukin-1(beta) are identical, *Nature* 324:266–8, 1986.

Sims JE, Acres RB, Grubin CE, McMahan CJ, Wignall JM et al: Cloning the interleukin 1 receptor from human T cells, *Proc Natl Acad Sci USA* 86:8946–50, 1989.

Colotta F, Re F, Muzio M et al: Interleukin-1 type II receptor: A decoy target for IL-1 that is regulated by IL-4, *Science* 261:472–5, 1993.

Burger D, Chicheportiche R, Giri JG, Dayer JM: The inhibitory activity of human interleukin-1 receptor antagonist is enhanced by type II interleukin-1 soluble receptor and hindered by type I interleukin-1 soluble receptor, *J Clin Invest* 96:38–41, 1995.

Dinarello CA: Interleukin-1, interleukin-1 receptors and interleukin-1 receptor antagonist, *Int Rev Immunol* 16(5–6):457–99, 1998.

Type II IL1 Receptor

Colotta F, Re F, Muzio M, Bertini R, Polentarutti N et al: Interleukin-1 type II receptor: A decoy target for IL-1 that is regulated by IL-4, *Science* 261:472–5, 1993.

Dale M, Nicklin MJ: Interleukin-1 receptor cluster: Gene organization of IL1R2, IL1R1, IL1RL2 (IL-1Rrp2), IL1RL1 (T1/ST2), and IL18R1 (IL-1Rrp) on human chromosome 2q, *Genomics* 57:177–9, 1999.

McMahan CJ, Slack JL, Mosley B, Cosman D, Lupton SD et al: A novel IL-1 receptor, cloned from B cells by mammalian expression, is expressed in many cell types, *EMBO J* 10:2821–32, 1991.

22.17. Tumor Necrosis Factor (TNF)

Burstein H Gene therapy for rheumatoid arthritis. Curr Opin Mol Ther. 3(4):362–74, 2001.

Kim KN, Watanabe S, Ma Y, Thornton S, Giannini EH, Hirsch R: Viral IL-10 and soluble TNF receptor act synergistically to inhibit collagen-induced arthritis following adenovirus-mediated gene transfer, *J Immunol* 164(3):1576–81, 2000.

TNF α

Aggarwal BB, Eessalu TE, Hass PE: Characterization of receptors for human tumour necrosis factor and their regulation by gamma-interferon, *Nature* 318:665–7, 1985.

Balding J, Kane D, Livingstone W, Mynett-Johnson L, Bresnihan B et al: Cytokine gene polymorphisms: Association with psoriatic arthritis susceptibility and severity, *Arthritis Rheum* 48:1408–13, 2003.

Bouwmeester T, Bauch A, Ruffner H, Angrand PO, Bergamini G et al: A physical and functional map of the human TNF-alpha/NF-kappa-B signal transduction pathway, *Nature Cell Biol* 6:97–105, 2004.

Garcia-Ruiz C, Colell A, Mari M, Morales A, Calvo M et al: Defective TNF-alpha-mediated hepatocellular apoptosis and liver damage in acidic sphingomyelinase knockout mice, *J Clin Invest* 111:197–208, 2003.

Kamata H, Honda S, Maeda S, Chang L, Hirata H et al: Reactive oxygen species promote TNF-alpha-induced death and sustained JNK activation by inhibiting MAP kinase phosphatases, *Cell* 120:649–61, 2005.

Marino MW, Dunn A, Grail D, Inglese M, Noguchi Y et al: Characterization of tumor necrosis factor-deficient mice, *Proc Natl Acad Sci USA* 94:8093–8, 1997.

Obeid LM, Linardic CM, Karolak LA, Hannun YA: Programmed cell death induced by ceramide, *Science* 259:1769–71, 1993.

Pennica D, Nedwin GE, Hayflick JS, Seeburg PH, Derynck R et al: Human tumour necrosis factor: Precursor structure, expression and homology to lymphotoxin, *Nature* 312:724–9, 1984.

Steed PM, Tansey MG, Zalevsky J, Zhukovsky EA, Desjarlais JR et al: Inactivation of TNF signaling by rationally designed dominant-negative TNF variants, *Science* 301:1895–8, 2003.

Takahashi JL, Giuliani F, Power C, Imai Y, Yong VW: Interleukin-1-beta promotes oligodendrocyte death through glutamate excitotoxicity, *Ann Neurol* 53:588–95, 2003.

Vielhauer V, Stavrakis G, Mayadas TN: Renal cell-expressed TNF receptor 2, not receptor 1, is essential for the development of glomerulonephritis, *J Clin Invest* 115:1199–1209, 2005.

Wang AM, Creasey AA, Ladner MB, Lin LS, Strickler J et al: Molecular cloning of the complementary DNA for human tumor necrosis factor, *Science* 228:149–54, 1985.

TNF β

Aggarwal BB, Eessalu TE, Hass PE: Characterization of receptors for human tumour necrosis factor and their regulation by gamma-interferon, *Nature* 318:665–7, 1985.

Balding J, Kane D, Livingstone W, Mynett-Johnson L, Bresnihan B et al: Cytokine gene polymorphisms: Association with psoriatic arthritis susceptibility and severity, *Arthritis Rheum* 48:1408–13, 2003.

Gray PW, Aggarwal BB, Benton CV, Bringman TS, Henzel WJ et al: Cloning and expression of cDNA for human lymphotoxin, a lymphokine with tumour necrosis activity, *Nature* 312:721–4, 1984.

Knight JC, Keating BJ, Kwiatkowski DP: Allele-specific repression of lymphotoxin-alpha by activated B cell factor-1, *Nature Genet* 36:394–9, 2004.

Type I TNF Receptor

Baker E, Chen LZ, Smith CA, Callen DF, Goodwin R et al: Chromosomal location of the human tumor necrosis factor receptor genes, *Cytogenet Cell Genet* 57:117–8, 1991.

Brockhaus M, Schoenfeld HJ, Schlaeger EJ, Hunziker W, Lesslauer W et al: Identification of two types of tumor necrosis factor receptors on human cell lines by monoclonal antibodies, *Proc Nat Acad Sci USA* 87:3127–31, 1990.

Castellino AM, Parker GJ, Boronenkov IV, Anderson RA, Chao, MV: A novel interaction between the juxtamembrane region of the p55 tumor necrosis factor receptor and phosphatidylinositol-4-phosphate 5-kinase, *J Biol Chem* 272:5861–70, 1997.

Chan FKM, Chun HJ, Zheng L, Siegel RM, Bui KL et al: A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling, *Science* 288:2351–4, 2000.

Engelmann H, Novick D, Wallach D: Two tumor necrosis factor-binding proteins purified from human urine: Evidence for immunological cross-reactivity with cell surface tumor necrosis factor receptors, *J Biol Chem* 265:1531–6, 1990.

Fuchs P, Strehl S, Dworzak M, Himmler A, Ambros, PF: Structure of the human TNF receptor 1 (p60) gene (TNFR1) and localization to chromosome 12p13, *Genomics* 13:219–24, 1992.

Gray PW, Barrett K, Chantry D, Turner M, Feldmann M: Cloning of human tumor necrosis factor (TNF) receptor cDNA and expression of recombinant soluble TNF-binding protein, *Proc Nat Acad Sci USA* 87:7380–4, 1990.

Loetscher H, Pan YCE, Lahm HW, Gentz R, Brockhaus M et al: Molecular cloning and expression of the human 55 kd tumor necrosis factor receptor, *Cell* 61:351–9, 1990.

Micheau O, Tschopp J: Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes, *Cell* 114:181–190, 2003.

Nophar Y, Kemper O, Brakebusch C, Engelmann H, Zwang R et al: Soluble forms of tumor necrosis factor receptors (TNF-Rs): The cDNA for the type I TNF-R, cloned using amino acid sequence data of its soluble form, encodes both the cell surface and a soluble form of the receptor, *EMBO J* 9:3269–78, 1990.

Rothe J, Lesslauer W, Lotscher H, Lang Y, Koebel P et al: Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by Listeria monocytogenes, *Nature* 364:798–802, 1993.

Schall TJ, Lewis M, Koller KJ, Lee A, Rice GC et al: Molecular cloning and expression of a receptor for human tumor necrosis factor, *Cell* 61:361–70, 1990.

Schievella AR, Chen JH, Graham JR, Lin LL: MADD, a novel death domain protein that interacts with the type 1 tumor necrosis factor receptor and activates mitogen-activated protein kinase, *J Biol Chem* 272:12069–75, 1997.

Smith CA, Davis T, Anderson D, Solam L, Beckmann MP et al: A receptor for tumor necrosis factor defines an unusual family of cellular and viral proteins, *Science* 248:1019–23, 1990.

Zhang JY, Green CL, Tao S, Khavari, PA: NF-kappa-B RelA opposes epidermal proliferation driven by TNFR1 and JNK, *Genes Dev* 18:17–22, 2004.

Type II TNF Receptor

Baker E, Chen LZ, Smith CA, Callen DF, Goodwin R et al: Chromosomal location of the human tumor necrosis factor receptor genes, *Cytogenet Cell Genet* 57:117–118, 1991.

Beltinger CP, White PS, Maris JM, Sulman EP, Jensen SJ et al: Physical mapping and genomic structure of the human TNFR2 gene, *Genomics* 35:94–100, 1996.

Bruce AJ, Boling W, Kindy MS, Peschon J, Kraemer PJ et al: Altered neuronal and microglial responses to excitotoxic and ischemic brain injury in mice lacking TNF receptors, *Nature Med* 2:788–94, 1996.

Chan, FK-M, Chun HJ, Zheng L, Siegel RM, Bui KL et al: A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling, *Science* 288:2351–4, 2000.

Kemper O, Derre J, Cherif D, Engelmann H, Wallach D et al: The gene for the type II (p75) tumor necrosis factor receptor (TNF-RII) is localized on band 1p36.2-p36.3, *Hum Genet* 87:623–4, 1991.

Li X, Yang Y, Ashwell JD: TNF-RII and c-IAP1 mediate ubiquitination and degradation of TRAF2, *Nature* 416:345–9, 2002.

Santee SM, Owen-Schaub LB: Human tumor necrosis factor receptor p75/80 (CD120b) gene structure and promoter characterization, *J Biol Chem* 271:21151–9, 1996.

Sashio H, Tamura K, Ito R, Yamamoto Y, Bamba H et al: Polymorphisms of the TNF gene and the TNF receptor superfamily member 1B gene are associated with susceptibility to ulcerative colitis and Crohn's disease, respectively, *Immunogenetics* 53:1020–7, 2002.

Schall TJ, Lewis M, Koller KJ, Lee A, Rice GC et al: Molecular cloning and expression of a receptor for human tumor necrosis factor, *Cell* 61:361–70, 1990.

Van Ostade X, Vandenabeele P, Everaerdt B, Loetscher H, Gentz R et al: Human TNF mutants with selective activity on the p55 receptor, *Nature* 361:266–9, 1993.

Vielhauer V, Stavrakis G, Mayadas, TN: Renal cell-expressed TNF receptor 2, not receptor 1, is essential for the development of glomerulonephritis, *J Clin Invest* 115:1199–1209, 2005.

Oligino T, Ghivizzani S, Wolfe D et al: Intra-articular delivery of a herpes simplex virus IL-1Ra gene vector reduces inflammation in a rabbit model of arthritis, *Gene Ther* 6:1713–20, 1999.

Chernajovsky Y, Adams G, Podhajcer OL et al: Inhibition of transfer of collagen-induced arthritis into SCID mice by ex vivo infection of spleen cells with retroviruses expressing soluble tumor necrosis factor receptor, *Gene Ther* 2:731–35, 1995.

Quattrocchi E, Walmsley M, Browne K et al: Paradoxical effects of adenovirus-mediated blockade of TNF activity in murine collagen-induced arthritis, *J Immunol* 163:1000–1009, 1999.

Le CH, Nicolson AG, Morales A, Sewell KL: Suppression of collagen-induced arthritis through adenovirus-mediated transfer of a modified tumor necrosis factor alpha receptor gene, *Arthritis Rheum* 40:1662–1669, 1997.

22.18. Antiinflammatory Cytokines

Wieser C, Stumpf D, Grillhosl C, Lengenfelder D, Gay S et al: Regulated and constitutive expression of anti-inflammatory cytokines by nontransforming herpesvirus saimiri vectors, *Gene Ther* 12(5):395–406, 2005.

Neumann E, Judex M, Kullmann F, Grifka J, Robbins PD et al: Inhibition of cartilage destruction by double gene transfer of IL-1Ra and IL-10 involves the activin pathway, *Gene Ther* 9(22): 1508–19, 2002.

Blackburn MR, Lee CG, Young HWJ, Zhu Z, Chunn JL et al: Adenosine mediates IL-13-induced inflammation and remodeling in the lung and interacts in an IL-13-adenosine amplification pathway, *J Clin Invest* 112:332–44, 2003.

Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F et al: Requirement for IL-13 independently of IL-4 in experimental asthma, *Science* 282:2261–3, 1998.

Heinzmann H, Mao XQ, Akaiwa M, Kreomer RT, Gao PS et al: Genetic variants of IL-13 signalling and human asthma and atopy, *Hum Mol Genet* 9:549–59, 2000.

Howard TD, Koppelman GH, Xu J, Zheng SL, Postma DS et al: Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma, *Am J Hum Genet* 70:230–6, 2002.

Howard TD, Whittaker PA, Zaiman AL, Koppelman, GH Xu J et al: Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population, *Am J Resp Cell Molec Biol* 25:377–84, 2001.

Kelly-Welch AE, Hanson EM, Boothby MR, Keegan AD: Interleukin-4 and interleukin-13 signaling connections maps, *Science* 300:1527–28, 2003.

Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM et al: Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma, *Nature Med* 8:885–9, 2002.

Lacy DA, Wang, Z-E, Symula DJ, McArthur CJ, Rubin EM et al: Faithful expression of the human 5q31 cytokine cluster in transgenic mice, *J Immun* 164:4569–74, 2000.

Loots GG, Locksley RM, Blankespoor CM, Wang ZE, Miller W et al: Identification of a coordinate regulator of interleukins 4, 13, and 5 by cross-species sequence comparisons, *Science* 288: 136–40, 2000.

McKenzie ANJ, Culpepper JA, de Waal Malefyt R, Briere F, Punnonen J et al: Interleukin 13, a T-cell-derived cytokine that regulates human monocyte and B-cell function, *Proc Nat Acad Sci USA* 90:3735–9, 1993.

Minty A, Chalon P, Derocq JM, Dumont X, Guillemot JC et al: Interleukin-13 is a new human lymphokine regulating inflammatory and immune responses, *Nature* 362:248–50, 1993.

Morgan JG, Dolganov GM, Robbins SE, Hinton LM, Lovett M: The selective isolation of novel cDNAs encoded by the regions surrounding the human interleukin 4 and 5 genes, *Nucleic Acids Res* 20:5173–9, 1992.

Vladich FD, Brazille SM, Stern D, Peck ML, Ghittoni R: IL-13 R130Q, a common variant associated with allergy and asthma, enhances effector mechanisms essential for human allergic inflammation, *J Clin Invest* 115:747–54, 2005.

Wang M, Xing ZM, Lu C, Ma YX, Yu DL: A common IL-13 arg130-to-gln single nucleotide polymorphism among Chinese atopy patients with allergic rhinitis, *Hum Genet* 113:387–90, 2003.

Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY et al: Interleukin-13: central mediator of allergic asthma, *Science* 282:2258–61, 1998.

Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP et al: Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production, *J Clin Invest* 103:779–88, 1999.

Zhu Z, Zheng T, Homer RJ, Kim YK, Chen NY et al: Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation, *Science* 304:1678–82, 2004.

Zurawski G, de Vries, JE: Interleukin 13 elicits a subset of the activities of its close relative interleukin 4, *Stem Cells* 12:169–74, 1994.

Bessis N, Honiger J, Damotte D et al: Encapsulation in hollow fibres of xenogeneic cells engineered to secrete IL-4 or IL-13 ameliorates murine collagen-induced arthritis (CIA), *Clin Exp Immunol* 117:376–82, 1999.

Bessis N, Chiocchia G, Kollias G et al: Modulation of proinflammatory cytokine production in tumour necrosis factor-alpha (TNF-alpha)-transgenic mice by treatment with cells engineered to secrete IL-4, IL-10 or IL-13, *Clin Exp Immunol* 111:391–6, 1998.

Bessis N, Boissier MC, Ferrara P et al: Attenuation of collagen-induced arthritis in mice by treatment with vector cells engineered to secrete interleukin-13, *Eur J Immunol* 26:2399–2403, 1996.

Bessis N, Doucet C, Cottard V, Anne-Marie Douar AM, Firat H et al: Gene therapy for rheumatoid arthritis, *J Gene Med* 4:581–91, 2002.

Human protein reference data base, Johns Hopkins University and the Institute of Bioinformatics, at http://www.hprd.org/protein.

22.19. Dominant-Negative Mutant ras Gene

Yamamoto A et al: Suppression of arthritic bone destruction by adenovirus-mediated dominant-negative Ras gene transfer to synoviocytes and osteoclasts, *Arthritis Rheum* 48:2682–92, 2003.

22.20. Osteoprotegerin

Kong YY, Feige U, Sarosi I, Bolons B, Tafuri A et al: Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand, *Nature* 402, 304–309, 1999.

22.21. Viral Thymidine Kinase

Goossens PH, Schouten GJ, 't Hart B et al: Feasibility of adenovirus-mediated nonsurgical synovectomy in collagen-induced arthritis-affected rhesus monkeys, *Hum Gene Ther* 10:1139–49, 1999.

Honore P, Luger NM, Sabino MA, Schwei MJ, Rogers SD et al: Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain and pain-related neurochemical reorganization of the spinal cord, *Nat Med* 6(5):521–8, 2000.

Rodan GA, Martin TJ: Therapeutic approaches to bone diseases, *Science* 289:1508–14, 2000.

22.22. Pathogenesis, Pathology, and Clinical Features of Bone and Cartilage Injury

Lewis CL, Sahrmann SA: Acetabular labral tears, *Phys Ther* 86(1):110–21, Jan 2006.

Beris AE, Lykissas MG, Papageorgiou CD, Georgoulis AD: Advances in articular cartilage repair, *Injury* 36 (Suppl 4):S14–23, 2005.

Giannoudis PV, Pountos I: Tissue regeneration. The past, the present and the future, *Injury* 36 (Suppl 4):S2–5, 2005.

Beynnon BD, Johnson RJ, Abate JA, Fleming BC, Nichols CE: Treatment of anterior cruciate ligament injuries, part I, *Am J Sports Med* 33(10):1579–602, 2005.

Schachter AK, Chen AL, Reddy PD, Tejwani NC: Osteochondral lesions of the talus, *J Am Acad Orthop Surg* 13(3):152–8, 2005.

Smith GD, Knutsen G, Richardson JB: A clinical review of cartilage repair techniques, *J Bone Joint Surg Br* 87(4):445–9, 2005.

Alford JW, Cole BJ: Cartilage restoration, part 2: Techniques, outcomes, and future directions, *Am J Sports Med* 33(3):443–60, 2005.

Ritchie PK, McCarty EC: Surgical management of cartilage defects in athletes, *Clin Sports Med* 24(1):163–74, 2005.

Dirschl DR, Marsh JL, Buckwalter JA, Gelberman R, Olson SA et al: Articular fractures, *J Am Acad Orthop Surg* 12(6):416–23, 2004.

22.23. Molecular Regenerative Engineering

Mont MA, Ragland PS, Biggins B, Friedlaender G, Patel T et al: Use of bone morphogenetic proteins for musculoskeletal applications. An overview, *J Bone Joint Surg Am* 86(Suppl 2):41–55, 2004.

Kofron MD, Laurencin CT: Orthopaedic applications of gene therapy, *Curr Gene Ther* 5(1):37–61, 2005.

Lee J, Qu-Petersen Z, Cao B et al: Clonal isolation of muscle-derived cells capable of enhancing muscle regeneration and bone healing, *J Cell Biol* 150:1085–1100, 2000.

Wozney J, Rosen V, Celeste A et al: Novel regulators of bone formation: Molecular clones and activities, *Science* 242:1528–34, 1988.

Bostrom M, Lane J, Tomin E et al: Use of bone morphogenetic protein-2 in the rabbit ulnar nonunion model, *Clin Orthop* 327:272–82, 1996.

Wang E, Rosen V, D'Assandro J et al: Recombinant human bone morphogenetic protein induces bone formation, *Proc Natl Acad Sci USA* 87:2220–4, 1990.

Cook S, Baffes G, Wolfe M et al: The effects of recombinant human osteogenic protein-1 on healing of large segmental bone defects, *J Bone Joint Surg* 76:827–38, 1994.

Sandhu H, Kanim L, Kabo J et al: Effective doses of recombinant human bone morphogenetic protein-2 in experimental spinal fusion, *Spine* 21:2115–22, 1996.

Boden S, Moskovitz P, Morone M et al: Video-assisted lateral intertransverse process arthrodesis. Validation of a new minimally invasive lumbar spinal fusion technique in the rabbit and non-human primate (rhesus) models, *Spine* 21:2689–97, 1996.

Mundy G: Regulation of bone formation by morphogenetic proteins and other growth factors, *Clin Orthop* 323:24–8, 1996.

Sampath T, Maliakai J, Hauschka P et al: 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: 20-352–20-362, 1992.

Sato M, Ochi T, Nakase T et al: Mechanical tension-stress induces expression of bone morphogenetic protein (BMP-2) and BMP-4, but not BMP-6, BMP-7, and GDF-f mRNA, during distraction osteogenesis, *J Bone Miner Res* 14:1084–95, 1999.

Fang J, Zhu YY, Smiley E et al: Stimulation of new bone formation by direct transfer of osteogenic plasmid genes, *Proc Natl Acad Sci USA* 93:5753–8, 1996.

Leong L, Brickell P: Molecules in focus: Bone morphogenetic protein-4, *Int J Biochem Cell Biol* 28:1293–6, 1996.

Sampath T, Rashka K, Doctor J et al: Drosophila transforming growth factor beta superfamily proteins induce endochondral bone formation in mammals, *Proc Natl Acad Sci USA* 90:6004–8, 1993.

Vainio S, Karavanova I, Jowett A et al: Identification of BMP-4 as a signal mediating secondary induction between epithelial and mesenchymal tissues during early tooth development, *Cell* 75:45–58, 1993.

Mori S, Yoshikawa H, Hashimoto J et al: Antiangiogenic agent (TNP-470) inhibition of ectopic bone formation induced by bone morphogenetic protein-2, *Bone* 22:99–105, 1998.

Ferguson C, Alpern E, Miclau T et al: Does adult fracture recapitulate embryonic skeletal formation? *Mech Dev* 87:57–66, 1999.

Nakagawa M, Kaneda T, Arakawa T et al: Vascular endothelial growth factor (VEGF) directly enhances osteoclastic bone resorption and survival of mature osteoclasts, *FEBS Lett* 473:161–4, 2000.

Goad D, Rubin J, Wang H et al: 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* 137:2262–68, 1996.

Gerber H, Vu T, Ryan A et al: VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation, *Nat Med* 5:623–8, 1999.

Hidaka M, Stanford W, Bernstein A: Conditional requirement for the Flk-1 receptor in the in vitro generation of early hematopoietic cells, *Proc Natl Acad Sci USA* 96:7370–5, 1999.

Mitola S, Sozzani S, Luini W et al: Tat-human immunodeficiency virus-1 induces human monocyte chemotaxis by activation of vascular endothelial growth factor receptor-1, *Blood* 90:1365–72, 1997.

Peng H, Wright V, Usas A et al: VEGF enhances bone formation and bone healing elicited by genetically engineered muscle derived stem cells expressing BMP-4, *J Clin Invest* 110:751–9, 2002.

Alden T, Pittman D, Hankins G et al: In vivo endochondral bone formation using a bone morphogenetic protein 2 adenoviral vector, *Hum Gene Ther* 10:2245–53, 1999.

Baltzer A, Lattermann C, Whalen J et al: Genetic enhancement of fracture repair: Healing of an experimental segmental defect by adenoviral transfer of the BMP-2 gene, *Gene Ther* 7:734–9, 2000.

Musgrave D, Bosch P, Ghivizzani S et al: Adenovirus-mediated direct gene therapy with bone morphogenetic protein-2 produces bone, *Bone* 24:541–7, 1999.

Okubo Y, Bessho K, Fujimura K et al: Osteoinduction by bone morphogenetic protein-2 via adenoviral vector under transient immunosuppression, *Biochem Biophys Res Commun* 267:382–7, 2000.

Fang J, Zhu Y, Smiley E et al: Stimulation of new bone formation by direct transfer of osteogenic plasmid genes, *Proc Natl Acad Sci USA* 93:5753–8, 1996.

Bonadio J, Smiley E, Patil P et al: Localized, direct plasmid gene delivery in vivo: Prolonged therapy results in reproducible tissue regeneration, *Nat Med* 5:753–9, 1999.

Goldstein S, Bonadio J: Potential role for direct gene transfer in the enhancement of fracture healing, *Clin Orthop* 355:S154–62, 1998.

22.24. Cell Regenerative Engineering

Steadman J, Rodkey W, Rodrigo J: Microfracture: Surgical technique and rehabilitation to treat chondral defects, *Clin Orthop* 39: S362–9, 2001.

Brittberg M, Lindahl A, Nilsson A et al: Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation, *New Engl J Med* 331:889–95, 1994.

Buckwalter J, Mankin H: Articular cartilage repair and transplantation, *Arthritis Rheum* 41: 1331–42, 1998.

Radice M, Brun P, Cortivo R et al: Hyaluronan-based biopolymers as delivery vehicles for bone-marrow-derived mesenchymal progenitors, *J Biomed Mater Res* 50:101–9, 2000.

Martin I, Padera RF, Vunjak-Novakovic G, Freed LE: In vitro differentiation of chick embryo bone marrow stromal cells into cartilaginous and bone-like tissues, *J Orthop Res* 16:181–9, 1998.

Gao J, Dennis JE, Solchaga LA, Awadallah AS, Goldberg VM et al: Tissue-engineered fabrication of an osteochondral composite graft using rat bone marrow-derived mesenchymal stem cells, *Tissue Eng* 7:363–71, 2001.

Arai Y, Kubo T, Kobayashi K et al: Adenovirus vector-mediated gene transduction to chondrocytes: In vitro evaluation of therapeutic efficacy of transforming growth factor-beta 1 and heat shock protein 70 gene transduction, *J Rheumatol* 24:1787–95, 1997.

Smith P, Shuler F, Georgescu H et al: Genetic enhancement of matrix synthesis by articular chondrocytes: Comparison of different growth factor genes in the presence and absence of interleukin-1, *Arthritis Rheum* 43:1156–64, 2000.

Wehling P, Schulitz K, Robbins P et al: Transfer of genes to chondrocytic cells of the lumbar spine. Proposal for a treatment strategy of spinal disorders by local gene therapy, *Spine* 22:1092–7, 1997.

Ikeda T, Kubo T, Nakanishi T et al: Ex vivo gene delivery using an adenovirus vector in treatment for cartilage defects, *J Rheumatol* 27:990–6, 2000.

Goomer R, Maris T, Gelberman R et al: Nonviral in vivo gene therapy for tissue engineering of articular cartilage and tendon repair, *Clin Orthop* 379: S189–200, 2000.

Mason J, Grande D, Barcia M et al: Expression of human bone morphogenic protein 7 in primary rabbit periosteal cells: Potential utility in gene therapy for osteochondral repair, *Gene Ther* 5:1098–1104, 1998.

Adachi N, Sato K, Usas A et al: Muscle derived cell based ex vivo gene therapy for the treatment of full-thickness articular cartilage defects, *J Rheumatol* 29:1920–30, 2002.

Bruder S, Kraus K, Goldberg V et al: The effect of implants loaded with autologous mesenchymal stem cells on the healing of canine segmental bone defects, *J Bone Joint Surg Am* 80:985–96, 1998.

Bruder S, Kurth A, Shea M et al: Bone regeneration by implantation of purified, culture-expanded human mesenchymal stem cells, *J Orthop Res* 16:155–62, 1998.

Lieberman J, Daluiski A, Stevenson S et al: The effect of regional gene therapy with bone morphogenetic protein-2-producing bone-marrow cells on the repair of segmental femoral defects in rats, *J Bone Joint Surg Am* 81:905–17, 1999.

Turgeman G, Pittman DD, Muller R et al: Engineered human mesenchymal stem cells: A novel platform for skeletal cell mediated gene therapy, *J Gene Med* 3:240–51, 2001.

Krebsbach P, Gu K, Franceschi R et al: Gene therapy-directed osteogenesis: BMP-7-transduced human fibroblasts form bone in vivo, *Hum Gene Ther* 11:1201–10, 2000.

Zuk P, Zhu M, Mizuno H et al: Multilineage cells from human adipose tissue: Implications for cell-based therapies, *Tissue Eng* 7:211–28, 2001.

Qu-Petersen Z, Deasy B, Jankowski R et al: Identification of a novel population of muscle stem cells in mice: Potential for muscle regeneration, *J Cell Biol* 157:851–64, 2002.

Lee J, Musgrave D, Pelinkovic D et al: Effect of bone morphogenetic protein-2-expressing muscle-derived cells on healing of critical-sized bone defects in mice, *J Bone Joint Surg Am* 83A:1032–9, 2001.

Bosch P, Musgrave D, Lee J et al: Osteoprogenitor cells within skeletal muscle, *J Orthop Res* 18:933–44, 2000.

22.25. Tissue Regenerative Engineering

Lohfeld S, Barron V, McHugh PE: Biomodels of bone: A review, *Ann Biomed Eng* 33(10):1295–311, 2005.

Yoshikawa H, Myoui A: Bone tissue engineering with porous hydroxyapatite ceramics, *J Artif Organs* 8(3):131–6, 2005.

Anderson DG, Albert TJ, Fraser JK, Risbud M, Wuisman P et al: Cellular therapy for disc degeneration, *Spine* 30(17 Suppl):S14–9, 2005.

Kang Y, Yang J, Khan S, Anissian L, Ameer GA: A new biodegradable polyester elastomer for cartilage tissue engineering, *J Biomed Mater Res A* 77(2):331–9, May 2006.

Mauney JR, Volloch V, Kaplan DL: Role of adult mesenchymal stem cells in bone tissue engineering applications: Current status and future prospects, *Tissue Eng* 11(5–6):787–802, 2005.

Mistry AS, Mikos AG: Tissue engineering strategies for bone regeneration, *Adv Biochem Eng Biotechnol* 94:1–22, 2005.

Sanchez C, Arribart H, Guille MM: Biomimetism and bioinspiration as tools for the design of innovative materials and systems, *Nat Mater* 4(4):277–88, 2005.

Hutmacher DW, Garcia AJ: Scaffold-based bone engineering by using genetically modified cells, *Gene* 347(1):1–10, 2005.

Wilson CJ, Clegg RE, Leavesley DI, Pearcy MJ: Mediation of biomaterial-cell interactions by adsorbed proteins: A review, *Tissue Eng* 11(1–2):1–18, 2005.

Lynn AK, Brooks RA, Bonfield W, Rushton N: Repair of defects in articular joints. Prospects for material-based solutions in tissue engineering, *J Bone Joint Surg Br* 86(8):1093–9, Nov 2004.

Heng BC, Cao T, Stanton LW, Robson P, Olsen B: Strategies for directing the differentiation of stem cells into the osteogenic lineage in vitro, *J Bone Miner Res* 19(9):1379–94, 2004.

Nussenbaum B, Teknos TN, Chepeha DB: Tissue engineering: The current status of this futuristic modality in head neck reconstruction, *Curr Opin Otolaryngol Head Neck Surg* 12(4):311–5, 2004.

Renner G, Lane RV: Auricular reconstruction: An update, *Curr Opin Otolaryngol Head Neck Surg* 12(4):277–80, 2004.

Liu X, Ma PX: Polymeric scaffolds for bone tissue engineering, *Ann Biomed Eng* 32(3):477–86, 2004.

Liu Y, de Groot K, Hunziker EB: Osteoinductive implants: The mise-en-scene for drug-bearing biomimetic coatings, *Ann Biomed Eng* 32(3):398–406, 2004.

Partridge KA, Oreffo RO: Gene delivery in bone tissue engineering: Progress and prospects using viral and nonviral strategies, *Tissue Eng* 10(1–2):295–307, 2004.

Derubeis AR, Cancedda R: Bone marrow stromal cells (BMSCs) in bone engineering: Limitations and recent advances, *Ann Biomed Eng* 32(1):160–5, 2004.

Sharma B, Elisseeff JH: Engineering structurally organized cartilage and bone tissues, *Ann Biomed Eng* 32(1):148–59, 2004.

Sander EA, Nauman EA: Permeability of musculoskeletal tissues and scaffolding materials: Experimental results and theoretical predictions, *Crit Rev Biomed Eng* 31(1–2):1–26, 2003.