Skeletal Complications of Malignancy

Supplement to Cancer

The Development and Function of the Skeleton and Bone Metastases

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Bone is a frequent site of metastases of the most common tumors, e.g., breast carcinoma and prostate carcinoma. The functions of the skeleton, calcium homeostasis and mechanical support, are carried out by the continuous destruction and rebuilding of small packets of this tissue called bone remodeling. Multinucleated, hemopoietically derived osteoclasts, which are related to macrophages, digest the bone, and mesenchymal-derived osteoblasts rebuild it. This process is kept in balance by finely regulated processes whereby osteoblast lineage cells respond to homeostatic signals and release factors that regulate osteoclast generation and activity. Cells that participate in inflammation and immunity also can stimulate osteoclast formation and lead to bone destruction. Tumor cells most likely subvert these physiologic processes to lodge in bone and cause metastases.


DOI 10.1002/cncr.11147

KEYWORDS: bone remodeling, calcium homeostasis, mechanical support, osteoblasts, osteoclasts.

This is an overview of skeletal development and functions in the mammalian organism and may serve as background for the articles in this issue on skeletal metastases. It covers briefly the process of bone remodeling, through which the skeleton carries out its functions in the adult, and the participating cells: the bone destroying osteoclasts and the bone rebuilding osteoblasts. It points to similarities between bone remodeling and inflammation with respect to the sequence of events, the participating cell types, and regulatory molecules. Finally, it concludes that tumor cells localize and grow in bone to form metastases by subverting the normal physiologic processes described above.

Skeletal Functions and the Role of the Mammalian Skeleton in Evolution

The functions of the skeleton and the importance of the skeleton for general well being, which are both impacted negatively by bone metastases, can be traced back to evolution. A major function of the skeleton is calcium homeostasis. For the marine organisms that gave rise to current mammalian species, a mineralized skeleton provided no selective advantage for calcium regulation, because calcium was plentiful in the sea. Bones do increase the efficiency of muscle action for locomotion and other activities, another major function of the skeleton; and, in many fish, jawbones are critical for feeding. However, a cartilaginous skeleton (e.g., in sharks) seems to perform these functions with similar efficiency.

What could have been the selective advantage of a mineralized skeleton in marine organisms? It has been speculated that the min-
eralized skeleton evolved for the storage of phosphate. This would be advantageous for fish feeding on plankton that is dependent on sunlight and fluctuates substantially between seasons. Further evidence on phosphate homeostasis in fish and its regulation is needed to support this hypothesis.

However, on land, the calcium storage of the vertebrate skeleton was crucial for survival, providing a continuous source of calcium between meals. A steady calcium level is required for a myriad of physiologic functions. Bone is part of a finely regulated system that keeps calcium levels in the circulation within very narrow limits—around 2.5 mM—regardless of intake from the outside. A primary feedback loop involves the release of parathyroid hormone (PTH) in response to lowering calcium levels. PTH, in turn, stimulates the release of calcium from the skeleton through bone resorption (destruction), increases calcium absorption from the gut through increased synthesis of 1,25(OH)2D3, and increases calcium reabsorption in the kidney. PTH-related protein (PTHrP), which acts on the same receptor and is produced by certain tumors, can have effects similar to those of PTH. Elevated calcium levels block PTH secretion, and excess calcium after meals is either deposited in the skeleton and/or excreted in the urine.

The second major skeletal functions, providing mechanical support of soft tissues and levers for muscle action, also played an important role in many stages of mammalian evolution. Limb development enabled locomotion that helped terrestrial mammals cover increasingly larger distances and populate vast areas. Mammals, including human ancestors, became adept in climbing trees. Locomotion in trees was facilitated by the evolution of the apposing thumb in upper limbs, which enabled a firmer grip of branches. This was followed by bipedalism (locomotion on posterior limbs only), liberating the grabbing-competent upper extremities, a musculoskeletal function, for other tasks. Then came the invention and use of tools, allotment of large parts of the brain to fine hand motion, and the control of other muscles, and so on. Thus, through its role in muscle action, the skeleton was involved intimately in the evolutionary changes that led to our species.

In addition to serving as a storehouse for calcium and other ions and as levers for muscles, the skeleton has two other functions: 1) It protects the central nervous system and the spinal cord from trauma; and 2) it houses the bone marrow, source of the hemopoietic cells in the adult. To carry out the latter function, the skeleton has the ability to increase its medullary capacity when needed, for example, at high altitudes, through bone resorption. This intimate relation, in which local bone marrow stromal cells provide a suitable environment for blood cell development and bone cells respond to stimuli that control differentiation of hemopoietic lineages, can be exploited by inflammatory cells and tumor cells seeding in bone.

**Bone Development and Intramembranous and Endochondral Ossification**

The mammalian skeleton develops from two embryonic sources. The cranial bones develop from the branchial arches, and the axial and appendicular bones develop from the mesoderm in the somites. The somite mesenchyme also gives rise to muscle and skin. Multipotent mesenchymal cells, which have chondrogenic, osteogenic, adipogenic, and myogenic potential, have been isolated experimentally.

Limb development proceeds in a finely regulated fashion. It has been studied in detail in chick and, to some extent, in mice and is a model for organ morphogenesis. Interacting cell types (ectodermal and mesodermal) and interacting growth factors (e.g., fibroblast growth factors [FGFs], hedgehog, and bone morphogenic proteins [BMPs]) control one another and control specific transcription factors (e.g., dHand and Tbx) to generate the final anatomy of the limbs.

Skeletogenesis in the embryo starts with mesenchymal condensation in all prospective bones around Week 6–7 of gestation in humans. In cranial bones, which form by intramembranous ossification, this is followed directly by ossification centers at Weeks 7.0–8.5. Cells assume osteoblastic features and start depositing bone matrix that will go on to mineralize and form the flat cranial bones.

In the axial and appendicular bones derived from the sclerotomes of the somites, condensation leads to the formation of a complete cartilaginous skeleton that will eventually be replaced by bone through the process of endochondral bone formation. Ossification centers in this cartilaginous anlage also start appearing around fetal Week 7 and continue to Week 12 in the caudal vertebrae, Week 24 in the sacrum, and postnatally in the coccyx.

Endochondral bone formation starts with hypertrophy (enlargement) of the cartilage cells, changes in the composition of the extracellular matrix (e.g., the presence of Type X collagen), and matrix mineralization. The cartilage cells die, the area is invaded by blood vessels (probably in response to vascular endothelial growth factor [VEGF]), and bone-forming cells appear on the surface of the mineralized cartilage and start depositing bone. This area is called the primary spongiosa. At the same time, bone-resorbing cells, osteoclasts or chondroclasts, populate the area and clean up all remnants of cartilage, which eventually is
replaced fully by bone in the secondary spongiosa. This is the process responsible for initial replacement of the cartilage anlage in the embryo, for elongation of all bones at their epiphyseal extremities, and for fracture repair. The bone initially formed is replaced by remodeling (described below), giving rise to the new bone with an architecture that is better suited to serve its mechanical function.\textsuperscript{2}

**Bone Remodeling**

To fulfill its two most active functions in the adult, calcium mobilization from the skeleton and optimization of bone structure for mechanical support, the skeleton is continuously being broken down and rebuilt. This process is known as remodeling. To release calcium from the skeleton, the amount of bone containing that calcium has to be broken down (resorbed). Similarly, to generate the optimum architecture for mechanical usage, bone is being rebuilt under the influence of mechanical loads. It has been estimated that remodeling occurs at approximately 2 million microscopic sites in the adult skeleton.\textsuperscript{3} Macroscopically, bone can be divided, based on its structure, into cortical bone (the compact, dense, external envelope of each of the bones) and cancellous bone (the interior, honeycomb-like meshwork of interconnected plates). The cancellous bone has a huge surface on which bone destruction and rebuilding occurs and is estimated to remodel at the rate of about 30% per year. The space between the cancellous bone plates is filled with bone marrow. Cortical bone is traversed by blood vessels within canals that are recognized in histologic sections as Haversian lacunae. These canals are the sites of cortical bone remodeling (called Haversian remodeling), which proceeds at a rate of about 3% per year. It has been estimated that, in the adult skeleton, about 20% of the bone is cancellous, and 80% of the bone is cortical.\textsuperscript{3}

It has been debated whether remodeling occurs randomly in the skeleton or at specified sites. The overall rate of remodeling is stimulated by many factors, including calcium-mobilizing agents (such as PTH), immobilization, estrogen deficiency, and excess thyroid hormone, among others. Because mechanical changes (for example, decreased load\textsuperscript{4}) stimulate remodeling, one can assume that the site of remodeling is specified by mechanical factors, and the rate is determined by humoral factors, such as PTH, sex steroids, thyroid hormones, etc.

Remodeling is a coordinated, long-term process carried out by the bone multicellular unit (BMU),\textsuperscript{3} which contains the participating cell types (Fig. 1). It probably is initiated by osteocytes, cells buried inside the bone, or lining cells on the surface of cancellous bone trabeculae or cortical bone Haversian canals in response to stimuli elicited by a change in bone strain, hormones, or cytokines. The osteocytes supposedly sense a change in bone strain through their attachments to the matrix and signal to the lining cells through long cellular extensions that criss-cross the bone.\textsuperscript{3} The lining cells prepare the surface for osteoclastic bone resorption by secreting collagenase, which digests a protective-coating layer of matrix that covers the mineral.\textsuperscript{6} These cells contract and move
away, exposing the mineral surface and, along with other cells, release signals that promote osteoclast formation. Osteoclastogenic signals also are released by other cells of the osteoblastic lineage in response to PTH, thus determining the rate of bone resorption. Osteoclastogenic signals also can be released by macrophages and T-cells, which often are present in the vicinity of tumors, initiating resorption in the absence of mechanical changes. Initiation of resorption and remodeling at a particular site probably is determined by a fine balance between positive and negative stimuli on the relevant cells, as described below.

Osteoclasts or their precursors may be attracted chemotactically to the exposed mineral surface or may recognize it the way macrophages recognize a foreign body. Multiple osteoclasts often are found at sites of active bone resorption.

Resorption at the BMU lasts 2–4 weeks. The signals that terminate bone resorption during the remodeling process have not been identified firmly. Stimuli that can block osteoclast activity or can reduce their numbers are discussed below, although it is uncertain whether these stimuli actually regulate the extent of resorption in the BMU. Considering the adaptation of bone architecture to mechanical forces, it is attractive to assume that increased strain in the bone conveys signals for arresting resorption.

Resorption is followed by a brief transition phase called reversal during which mononucleated cells clean up the surface and prepare it for bone formation. The secreted glycoprotein, osteopontin, which was discovered independently in bone matrix and during tumor induction,7 is a histologic marker for the reversal of resorption to formation.9 Osteopontin null mice have a normal skeleton but are resistant to many stimuli of bone resorption.7,9,10 A mechanistic role for osteopontin in reversal has not yet been established. During the next step, the resorbed surface is covered with a contiguous layer of osteoblasts in a cobblestone fashion. These cells synthesize the matrix that goes on to mineralize and replace the bone that had been resorbed. This bone has a new orientation of the collagen fibers (detectable microscopically) and possibly a new trabecular thickness, presumably determined by the prevailing mechanical loads.

The bone formation period lasts about 4 months. The coordinate fashion whereby formation follows resorption in the BMU is referred to as coupling and is aimed at maintaining bone balance. This coupling also is apparent in bone metastases, in which active bone destruction is accompanied by local bone formation. The inherently faster rate of bone resorption easily overwhelms the balance, causing osteolytic lesions. However, suppression of resorption can result in complete restoration of the bone structure, as seen in patients with Paget disease after they receive bisphosphonate (BP) treatment.

The molecular basis of coupling still is under investigation. There probably are multiple signals linking resorption to formation. It has been suggested that the growth factors present in bone that are released and/or activated during resorption, specifically IGF and transforming growth factor β, stimulate formation. Many agents that increase resorption, including PTH, prostaglandin E (PGE), FGF, and others, stimulate formation as well, apparently by acting on a separate set of cells. To summarize this section, through remodeling, on a continuous basis, the skeleton provides the necessary calcium to the rest of the organism and optimizes the structure of the bones to provide maximum strength for a minimum amount of weight and material.

**Bone Cells: Osteoclasts and Pharmacologic Control of their Activity**

Early in the remodeling process, osteoclasts are being generated and are attracted to the bone surface, where they actively digest the bone, as discussed above. Osteoclasts are cells of hemopoietic origin, most likely derived from granulocyte macrophage colony-forming units. It also has been shown that B220 cells are capable of producing osteoclasts. In many respects, the osteoclasts are the tissue macrophages of bone.11,12

Spontaneous mutations and genetic experiments have identified many factors that are essential for osteoclast differentiation and function. The receptor activator of nuclear factor κB (RANK) ligand (RANK-L) is essential for osteoclast differentiation. A null mutation of RANK-L or RANK in mice eliminates all osteoclasts. Deletion of the following factors also resulted in the absence of osteoclasts: macrophage colony-stimulating factor 1 (CSF1), the transcription factor for hemopoietic cells (PU-1), the two subunits (p50 and p52) of nuclear factor κB, and c-fos. Many cytokines involved in inflammation, produced by macrophages and other cells that often are found in the vicinity of metastases, can stimulate osteoclast formation. These include interleukin 1 (IL-1), IL-11, IL-6, PGE2, and PThrP. It has been shown that these osteoclast-inducing agents stimulate the expression of RANK-L in osteoblast lineage cells or in other target cells. RANK-L also is produced by lymphocytes and synovial cells.11

The precursor cells go on to differentiate into mononuclear osteoclasts that contain virtually all of the osteoclastic proteins and are capable of resorption, at least in vitro. On the bone surface, these cells fuse to form multinucleated osteoclasts that have abundant membrane and can resorb bone most effi-
ciently. Resorption starts with osteoclast activation and polarization on the bone surface (Fig. 2), most likely mediated by the highly abundant osteoclast integrin αvβ3. A ring-shaped structure of the membrane that is rich in actin attaches firmly to bone to form a sealing zone that defines a closed compartment in which resorption takes place. The osteoclast membrane that faces this compartment becomes highly convoluted through the insertion of lysosome-type vesicles. A vacuolar ATPase, similar to that found in other cells but containing a unique subunit, starts acidifying this compartment, which resembles a giant lysosome. Mutations in a unique subunit of this enzyme are among the few identified molecular changes in congenital human osteopetrosis. Inside the cell, carbonate anhydrase (CA) helps maintain the pH. A mutation of CA II is another identified cause of human osteopetrosis. The pH in the resorption lacuna is below 4.0, and the acid solubilizes the mineral.

At the same time, there is secretion of lysosomal enzymes into the resorption space, the most abundant of which is cathepsin K, which can digest collagen at acidic pH. Matrix metalloproteinase 9 also is expressed by osteoclasts. These proteinases digest collagen and the noncollagenous proteins of the matrix. All resorption products, including calcium, are transported through the osteoclast by transcytosis to be secreted at the basolateral membrane.

During bone resorption, osteoclasts move from site to site along the bone surface. These cells are differentiated terminally, they do not proliferate, and they die by apoptosis after an estimated lifespan of about 2 weeks. Most available evidence suggests that osteoclasts mediate the osteolysis associated with tumor metastases in bone. Increased osteoclast activity, thus, is responsible for the morbidity associated with bone metastases, and its inhibition is the common strategy to manage it. Each of the pathways that control osteoclast generation or activity may be targeted in principle for blocking osteoclastic bone resorption, provided it is sufficiently selective and can be modulated effectively. Osteoclast generation can be blocked effectively with a soluble receptor of RANK-L, osteoprotegerin, a 55-KD protein that dimerizes, binds to RANK-L, and strongly inhibits osteoclast formation. Osteoprotegerin derivatives currently are under development for the control of tumor-induced bone destruction.

To date, the most effective agents used clinically to inhibit bone resorption and the only agents approved so far for the treatment of patients with bone metastases are the BPs. Major aspects of the mode of action of these agents have been elucidated recently and are summarized briefly below.

BPs are analogs of pyrophosphate in which the oxygen that binds the two phosphates is replaced by carbon, and the carbon side chains generate a large number of compounds, some of which have bone resorption-inhibitory activity. All BPs bind to the bone mineral through the phosphate moiety, which localizes their action to the target tissue. Uptake is higher at sites of active bone resorption. During bone resorption, BPs are taken up by osteoclasts and, through their cellular effects, inhibit osteoclast activity and/or
induce apoptosis. From a mechanism point of view, BPs can be divided into two groups: BPs that contain nitrogen (N-BP) and BPs that do not.

The N-BPs inhibit the farnesyl diphosphate synthase enzyme in the cholesterol biosynthesis pathway. Consequently, there is a reduction in its downstream product, geranylgeranyl diphosphate, a lipid that is required for the prenylation of GTPases and is essential for cytoskeletal organization and vesicular traffic. This prevents the regulatory GTPases, such as Rho, Rac, Rab, and Cdc42, to bind to membranes; as a consequence, the osteoclasts are unable to form the ruffled border and to carry out resorption effectively. Eventually, the cells undergo apoptosis. It has been shown that the nonnitrogen-containing BPs, such as clodronate, incorporate into ATP and form cytotoxic compounds that lead to osteoclast apoptosis.19

Bone Cells: Osteoblasts
Embryologically, osteoblasts develop from multipotent mesenchymal cells that also give rise to chondrocytes, myocytes, adipocytes, tendon cells, and various types of fibroblasts. It has been demonstrated that clonally derived cells indeed can generate several of these cell types in culture.20 However, it is not clear to what the extent this multipotent capability is preserved in adult human bone or bone marrow cells or how to activate it. A dual osteogenic/adipogenic capability can be demonstrated in cell lines and fresh bone marrow.20 The osteogenic/chondrogenic transition of periosteal precursor cells is probably the source of cartilage during fracture repair.

Mechanistically, differentiation into specific lineages is determined by lineage specific transcription factors, many of which have been identified, although their regulation requires further study. The first to be discovered were the muscle specific myo D, myogenin, and myf-5, which raised the expectation that other members of the family of homeodomain proteins would direct differentiation of other mesenchyme-derived lineages. This has not proven true to date, although rate-limiting differentiation transcription factors for fat, bone, and cartilage cells have been found. Osteoblast differentiation requires the core binding protein from the runt family, CBF-A1,21 and downstream from it, osterix (OSX), which does not belong to a known family.22 In adipocytes, the nuclear receptor PPARγ and the transcription factors C/EBPs23 are key regulators. For cartilage, the family of SOX genes (SOX-9, SOX-5, and SOX-6), which have a highmobility group and are related to the sex determining factor SRY, are rate-limiting regulatory molecules.24

The function of these factors is complex, differs in the different lineages, and is now the topic of active investigation. Eventually, these and additional transcription factors induce or up-regulate the expression of tissue specific genes. For osteoblasts, it was shown that the lack of CBF-A1 or OSX resulted in the complete absence of ossification. CBF-A1 expression is stimulated by BMP and, in turn, possibly through OSX, up-regulates the expression of osteoblast specific genes, which include collagen Type 1, osteocalcin, alkaline phosphatase, matrix noncollagenous proteins, and others.

Osteoblast lineage cells can be divided into two groups: 1) the worker cells that lay down the sheets of bone matrix in a coordinated fashion on bone formation surfaces and 2) a group of regulatory cells. The latter include osteocytes, lining cells, and other fibroblast-like cells that are not on the bone surface. It is believed that osteocytes and lining cells are derived from bone-forming osteoblasts after incorporation into the matrix (osteocytes) or after cessation of bone formation (lining cells). Many osteoblasts probably undergo apoptosis, and it has been suggested that control of the osteoblast lifespan, for example, by PTH and PGE, contributes to the regulation of bone formation.25,26

The regulatory function of osteoblast lineage cells, as mentioned above, includes response to signals that regulate bone remodeling and control of osteoclastogenesis. Osteoblastic cells respond to osteoclast-stimulating signals, including PTH, IL-1, PGE2, and others, by expression of RANK-L on their surface, which leads to osteoclast formation through cell-cell interaction. Similarly, osteocytes and lining cells presumably perceive the strain in the matrix through integrin attachment and translate it into bone-forming or bone-resorbing signals. Cell-cell interaction through cell contact (e.g., gap junctions) or paracrine factors makes the whole system respond as an integrated unit.

Bone Destruction, Inflammation, and Tumor Metastases
The bone remodeling process described above bears several similarities to the events that occur in inflammation. Inflammation can be initiated by trauma that causes tissue damage. A change in bone strain may initiate an analogous cascade. The cells recruited in the first phase of remodeling, the osteoclasts, are essentially tissue specific macrophages of bone. The cytokines, which promote osteoclast formation, are proinflammatory cytokines: IL-1, IL-6, tumor necrosis factor α, and PGE. The process, as a whole, involves coordinated cell-cell interaction in both instances.

The next stage in inflammation is an attempt at containment or repair. Fibroblasts are active participants in that process. They will fill any gap left in the
repair of a specialized tissue by fibrosis (scar tissue formation). The mesenchymal osteoblastic cells are related closely to fibroblasts and carry out a similar function of renewal and repair. Bone remodeling and fracture repair continue in healthy individuals into very advanced age. The intimate relation of bone to the hematopoietic system and the shared regulatory factors and features probably are the basis of inflammation-related bone loss in rheumatoid arthritis and periodontal disease.

The lodging and growth of tumor cells in bone to form metastases requires the destruction of the bone they replace, which is carried out by osteoclasts spurred on by tumor cells and cells that reside in their vicinity. This process undoubtedly is exploiting the normal pathways of bone remodeling described above, subverted by factors released from the tumors themselves (e.g., IL-6 in myeloma) or by inflammatory cells associated with the tumor. These topics are discussed in great detail in the articles herein.

REFERENCES