Chapter 9
Oncodynamic Changes in Skeleton

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Abstract When cancers are present in bone, a number of complex changes occur that can alter the physiology and structure of the skeleton. To properly understand these *oncodynamic* processes—how the bone changes in response to cancer cell invasion—it is necessary to define the types of cells that are present in normal bone, to explore the main physiological functions of these cells and of the bone itself, and to describe the types of cancers that often grow in bone. To properly characterize the functional and anatomical responses of bone cells, a broader definition of what cell types are present in bone is required. Using a more comprehensive and inclusive definition of bone cells, adaptations that result from cancer cell invasion can be categorized on the basis of the signalled functional and structural changes that occur between all involved cells in the bone environment. These pathological responses will be integrated with what is known about the chemical mediators that may be involved. This analysis of the normal signalling environment in bone and the potential interactions between cell types will help to better characterize the complex oncodynamic processes that can occur when cancer invades bone and disrupts this carefully balanced microenvironment.

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Introduction

Cancers growing in bone, whether due to a primary tumour that develops in the bone or a distant cancer that has spread to the bone, elicit a variety of physiological and structural changes that can lead to significant clinical morbidity. The role of therapy in the majority of patients with metastatic bone disease is palliative [1], with a strong focus on symptoms such as pain, fractures, fatigue, or bone marrow suppression. As some patients present with bone-related symptoms *prior* to treatment, it is these directly *signalled* changes in bone that are what we consider as *oncodynamic* responses—how the body changes in response to cancer cell invasion—and they have been given little focus in the cancer literature. Oncodynamics is a conceptual framework that parallels that of pharmacodynamics, the study of how drugs affect the body. With the primary clinical motivation being to identify how to either alter or kill the cancer cells, we need to re-conceptualize what occurs in the cancer bone microenvironment to better understand how to prevent the changes that result, independently of those elicited by cancer therapy. Looking just at the host changes that are elicited by cancer cells allows us to better view these effects as normal responses to altered environmental conditions due to the unique physiological perturbation created by the cancer. With this perspective in mind, we can then begin to focus specifically on identifying what the underlying biological mechanisms are that initiate the pathology. Successful treatment depends on identifying and manipulating the most appropriate target that is causatively linked to the dynamic changes occurring in this complex environment. An understanding of the oncodynamic environment is just the first step in developing effective therapeutics for bone cancers.

This chapter is written from the perspective of the bone and its response to the invasion by cancer cells. The main goal of this review is to answer the question: "*What does cancer do to bone physiology and anatomy?*" To accomplish this, it is necessary to explore the main physiological functions of these cells, to define the types of cells that are present in normal bone, and to describe the cancers that frequently occur in bone. With this framework, bone adaptations that result from cancer cell invasion can then be categorized by way of the functional and structural changes that occur. These changes will also be integrated with what is known about the chemical mediators involved in these pathological processes. As with all oncodynamic effects, *context* is the critical feature to understand when examining the changes in normal physiology that occur due to cancer. Thus, the current local environment and normal functions are particularly important to consider. This chapter is all about context. What cancer cells do is determined as much by where they are located and what the normal physiological functions are in that location, as they are by the nature of the tumours themselves. As many changes in bone cell

functions are the natural and expected biological responses of these cell types to the perturbations caused by cancer, these fundamental mechanisms must be understood before that any effective approach to reversing or preventing the changes can be considered. To understand the scope of changes that can occur, it is necessary to characterize both the anatomical relationship between cell types in bone and their physiological/structural roles. In this respect, the most logical starting point for an understanding of bone is to identify the functions of the bone and the different types of cells of which it is comprised.

Functions of Normal Bone as a Tissue System

Bone is a very metabolically active and dynamic connective tissue system, and is composed primarily of a mineralized matrix and type I collagen. As reviewed by Wagner and Aspenberg [2] and others [3], internal skeletons may have evolved as an adaptation to provide enhanced movement and sensory capacity over the earlier exoskeleton format. Another important adaptation that likely favoured an internal skeleton is the use of calcium phosphate rather than calcium carbonate as a structural matrix. The reasoning for this is argued to be related to changes in the phosphate content of the oceans [4] or that calcium phosphate is more chemically stable in systems with higher metabolic activity [5]. It is agreed, however, that acellular mineralized bone evolved first, with cellular components emerging phylogenetically later [6]. The appearance of calcium sensing receptors appears to only have evolved in vertebrates, and the development of structures to detect extracellular calcium levels parallels the advent of G-protein coupled receptors and their correspondingly complex signal transduction mechanisms [7].

In addition to the mineralized structural components, there is a collection of cell types in bone that work in concert to perform a variety of functions. Those functions, however, are not limited to simply providing structural *support* for the body, to *protect* internal organs, and to allow for *movement*, but also to serve as a *storage* system for calcium and phosphate (and other factors such as sodium, potassium, magnesium, sulphur, copper, and fat), and a location for *hematopoiesis* [8]. An extensive body of work has also identified the bone as being an important endocrine regulator [9–12] that has significant impact on cellular energy metabolism [13], fertility [14–16], and neural functions [17].

Cell Types in Bone and Their Functions

When considering skeletal anatomy and physiology, three primary cell types are typically described as the ‘functional cells’ of bone. These are the osteoclasts (Oc), osteoblasts (Ob), and osteocytes (Ocyt). However, many cell types other than these *classic three* do in fact exist in bone, and each of these has very specific functions in bone homeostasis. Furthermore, each of the numerous cell types can respond

uniquely when cancer is present, and these responses are critical in determining the overall resulting pathological effects.

Bone remodelling occurs due to the coordinated actions of Oc, Ob, and Ocyt cells which together form the traditional *bone remodeling unit* or *basic multicellular unit* [18, 19]. Within this temporary anatomical structure, bone is formed by Ob, maintained by Ocyt, and degraded by Oc. These cells maintain a functional balance through a complex combination of paracrine [20–22] and physical interactions [18, 23]. However, by defining 'bone cells' only as those cells which are involved in making or breaking down of mineralized bone unnecessarily limits our ability to understand how the bone responds to cancer cell invasion. The bone is a complex microenvironment of multiple cell types, and each of these cellular partners contributes to the overall structure and function of the system. For example, all of the three classic bone cell types arise from osteoprogenitor cells—these progenitors are unequivocally present in the bone environment yet are often not mentioned when discussing bone physiology. Thus, osteoprogenitor cells represent an entire category of cells that are clearly resident in the bone environment, are critical for overall bone maintenance, but yet are often overlooked as major players in bone homeostasis. Florencio-Silva et al. [24] provide an excellent review of the major bone cell types and their functions and include a comprehensive set of histological images.

Widening the definition of what we call 'bone cells' is vital for a proper evaluation of oncodynamic effects, as numerous different cell types can be located in the bone environment at any single point in time, including those which may only be present transiently. Therefore, the following is an expanded list of cell types in bone that should be considered when evaluating oncodynamic effects. These cell types are *Oc*, *Ob*, *Ocyt*, bone lining cells (BLC), *stromal* or medullary cells (including osteoprogenitors, adipocytes, and fibroblasts), blood and *hematopoietic* stem cells (including macrophages), *chondrocytes*, *blood vessel-related* cells (e.g., endothelial cells, smooth muscle cells), and *neurons*. Although this list is admittedly incomplete and includes some overlap between categories, these cell types have been selected based on their abilities to respond to the physiological perturbations caused by cancer cell invasion.

Osteoclasts

Oc are the cells responsible for degrading mineralized bone matrix. These multinucleated cells are formed from the fusion of hematopoietic progenitor cells of the monocyte/macrophage lineage [25]. Oc cells generate an acidic environment by secreting protons onto the surface of the bone to demineralize the hydroxyapatite structure, while other secreted enzymes digest the non-mineralized components [26]. Specialized transport mechanisms within the Oc move the degraded material away from the bone surface for disposal—a process called transcytosis [27]. A number of helpful reviews are available that elegantly describe the functions and cellular anatomy of the Oc [28–32]. These cells work in balance with Ob cells to constantly maintain stable bone mass under normal conditions.

Osteoblasts

Ob cells primarily serve to build new bone. They are generally cuboidal in shape with a single nucleus and they are derived from mesenchymal stem cell precursors that exist in the bone marrow or the periosteum. Once the precursor cells begin to express alkaline phosphatase activity, they are classified as *preosteoblasts*. The preosteoblasts then proliferate and mature, and characteristically begin to secrete bone matrix proteins such as type I collagen, bone sialoprotein I and II, and osteocalcin [33]. Bone formation proceeds in a two-step process with the secretion of osteoid toward the surface followed by mineralization of the newly formed matrix. Several good reviews are also available which describe Ob cell differentiation and functions in new bone synthesis [34–37].

In addition to bone formation, Ob cells also function to actively modulate Oc cell formation and hematopoietic stem cell homeostasis via several different signalling systems. Secreted osteocalcin can act locally in the bone or in an endocrine manner to modulate other functions such as male fertility or whole-body energy metabolism [9, 34]. Once Ob cells have performed their bone synthesis role, some may senesce and die by apoptosis. However, Ob cells are actually the precursors to two other types of cells—the Ocyt and BLC. These cell types are vital to the overall maintenance and functioning of normal bone.

Osteocytes

Ocyt cells are terminally differentiated Ob that are incorporated directly into the matrix of newly formed bone [38, 39]. These cells were originally thought to be quiescent Ob with the limited role of holding the bone together, although they are now considered as the master coordinators of bone synthesis and resorption. They have also been described as integrators and transducers of mechanical information [10, 40–42]. These long-lived [41, 43] multifunctional cells comprise about 90 % of all bone cells [42]. Their cell bodies reside in the lacunae of the bone and have dendritic-like processes (usually $\sim$50 for each cell) that reach through the canaliculi to form a complex network with other cells via gap junctions [44]. It has been estimated that the total number of Ocyt in the human skeleton is $\sim$42 billion and that the total number of Ocyt dendritic projections from these cells is $\sim$3.7 trillion—thus leading to a staggering 23 trillion direct connections between cells [45].

In addition to their gap junctional connections, Ocyt cells secrete a variety of proteins that modulate both bone formation and bone degradation. For example, Ocyt cells express a protein called *sclerostin* that acts as an effective inhibitor of the Wnt signalling pathway and Ob bone mineralization [46]. Neuropeptide Y (NPY) is also secreted by the Ocyt and this peptide can directly repress Ob function [47]. To control bone resorption, Ocyt cells are a major source of receptor activator of nuclear factor kappa-B ligand (RANKL) [48], the predominant cytokine involved in

stimulating Oc differentiation [49]. They can also indirectly modulate Oc-mediated bone resorption, as parathyroid hormone (PTH) causes a decrease in sclerostin expression [50] which subsequently results in increased Ob mineralization and decreased Oc bone resorption.

Ocyt cells appear to act as mechanosensors to detect mechanical forces and they transmit this information to other cells in the bone environment for encoding as a structural change. Ocyt cells are the primary mechanosensory cells in bone and the sensation process likely involves Wnt/β-catenin signalling [51]. Mechanical transduction is also facilitated through paracrine mediation of bone cells via specific glutamate transporter [52–54] and receptor systems [55], in addition to nitric oxide, prostaglandins, and osteopontin [56]. Ocyt cells that are mechanically stimulated also begin to secrete factors that alter mesenchymal stem cell migration [57] and these prompt newly formed osteoprogenitor cells to migrate and replace the exhausted Ob.

Bone Lining Cells

BLC serve as a protection for the bone, and like Ocyt, these cells are derived from flattened Ob. BLC are quiescent cells that cover the bone surfaces wherever resorption and bone formation are not occurring. Coincident with their bone protection role, BLC are important in the regulation of calcium movement in and out of bone under the control of paracrine factors such as PTH and calcitonin [58, 59]. There are two types of BLC, based primarily on anatomical location—*endosteal* and *periosteal* cells. Endosteal cells line the marrow cavities, and as such, they maintain close contact with hematopoietic cells of the bone marrow. The endosteum has significantly less sympathetic innervation compared to the periosteum [60]. The highly innervated periosteum covers the entire surface of long bones except for the articular surfaces. The periosteum has an outer layer of fibroblasts, collagen, neurons, and microvessels, and an inner layer of mesenchymal progenitor cells, osteoprogenitor cells, Ob, fibroblasts, sympathetic neurons, and microvessels [61]. Both the periosteum and endosteum have numerous resident macrophages that are likewise involved in modulating bone metabolism at these surfaces [62]. For more detail on this cell type, Franz-Odendaal et al. [63] present a comprehensive review of how Ob become Ocyt.

Stromal Cells

This broad category incorporates a number of different cell types and their precursors and is essentially a definition of anatomical location, including many cell types residing in the medullary or bone marrow space. Arbitrarily defined, these are cell types which are not directly involved with the main function of bone marrow—

hematopoiesis. The bone marrow itself is a densely cellular heterogeneous tissue found in the interior of most bones. Bone marrow stromal cells (sometimes called mesenchymal stem cells) give rise to the non-hematopoietic cells [64, 65]. In this space you can also find the precursors to the Oc, Ob, and Ocyt—called the osteoprogenitor cells. Among many others, the major types that are found in the marrow are adipocytes, fibroblasts, and macrophages. Although many of these cell types have functions critical to bone maintenance, some may only temporarily reside in the bone marrow.

Osteoprogenitor Cells

Although not often considered as true bone cells, the osteoprogenitors that differentiate into Oc, Ob, Ocyt, and BLC cells are significant players in overall bone functioning. As described above, Oc progenitors are formed in bone marrow from hematopoietic stem cells of the monocyte/macrophage lineage. Bone marrow stromal cells are the precursors to Ob cells, which can further differentiate into Ocyt or BLC. Although it is difficult to identify osteoprogenitor cells on the basis of anatomic features alone, these cells can be defined by their differential expression of a variety of surface marker proteins [66]. A good review of the history relating to the identification of osteoprogenitor cells is presented by Modder and Khosla [67].

Adipocytes

Bone marrow in particular has a large number of adipocytes. Adipocytes and Ob are derived from a common mesenchymal progenitor cell, with specific environmental conditions and transcriptional regulation factors determining the fate of the mesenchymal precursors. Several important factors can alter the differentiation pathway to switch between adipocytes or Ob, and these include *zinc finger protein 521* [68] and extracellular glutamate levels [69].

Fibroblasts

Fibroblasts are a heterogeneous group of differentiated cells of mesenchymal origin that synthesize precursors of the extracellular matrix—particularly collagen—and have different appearances depending on their anatomical location. Their primary function is to maintain the integrity of connective tissues [70]. In many parts of the body, fibroblasts generate robust cellular connections with other fibroblasts [71]. Although differentiated, fibroblasts can be reprogrammed to become other cell types via controlling the expression of specific transcription factors and growth conditions [72].

Blood and Hematopoietic Stem Cells

This diverse category includes red blood cells, macrophages/monocytes, lymphocytes (Natural killer cells, T cells, and B cells), and hematopoietic stem cells that differentiate into some of the cell categories discussed previously. Although many of the cells in this group have their primary functions in other parts of the body, important functional interactions between bone-resident cells and hematopoietic cells occur in the bone. A review by Taichman [8] provides the context to understand the many two-way interactions between classical bone cells and the processes relating to blood cell synthesis.

Macrophages are derived from hematopoietic precursors in the bone and are found throughout the body. These cells typically function as immune surveillance cells and will actively phagocytose cellular debris, regardless of their location. Macrophages that remain in bone, usually called *osteomacs*, are anatomically found near to the periosteal or endosteal BLC [62]. These cells are involved in both the degradation and synthesis processes for maintenance or repair of damaged bone [73, 74]. Osteomacs form a temporary and protective canopy-like cover over active Ob to aid in their generation of mineralized bone [70, 75]. Macrophages phagocytose old red blood cells and thus also serve as a regulator of iron levels for haemoglobin production [76].

Lymphocytes, or white blood cells of the immune system, include natural killer cells, T cells, and B cells. These originate in the bone marrow space and interact frequently with other cells in this environment. A number of factors secreted by lymphocytes are known to alter bone synthesis and degradation. For example, RANKL produced by activated T cells is important in normal bone metabolism by stimulating Oc differentiation [48], at least in young animals.

Hematopoietic stem cells are the precursors for the synthesis of virtually all blood cells, including myeloid cells, lymphoid cells, red blood cells, and platelets (or thrombocytes) [77, 78]. In adults, they reside primarily in the bone marrow space near blood vessels and the endosteum, with some evidence suggesting that their location may be partly related to oxygen availability [79]. These stem cells maintain typical stem cell features such as the abilities to self-replicate and to differentiate into non-hematopoietic cell types [4]. Regulation and maintenance of hematopoietic stem cells, however, appears to be under the control of bone marrow stromal cells [80], further demonstrating how the bone microenvironment can operate as a highly interconnected network.

Chondrocytes

Chondrocytes are derived from mesenchymal stem cell precursors and are important cells for the generation of cartilage and fully formed bone. The long bones of most vertebrates develop primarily through a process called endochondral

ossification, in which new cartilage is formed by hypertrophic chondrocytes then subsequently mineralized by the addition of hydroxyapatite crystals. How the mineralization occurs is still controversial, although Ob secretion of osteoid against new cartilage may be involved [81]. Another view is that chondrocytes differentiate into Ob-like cells [82], which then switch from collagen synthesis to begin expressing alkaline phosphatase [83]. The switchover from synthesis of collagen to the development of alkaline phosphatase activity may be related to the redox balance of chondrocytes [84]. Chondrocytes can either respond to external signals themselves or produce them to control other cell types, and it has been noted that they have all the appropriate mechanisms needed for fully functional glutamate signalling [85]. Vesicular glutamate release has been demonstrated to be signalled by activation of AMPA receptors on chondrocytes [86], and glutamate can inhibit chondral mineralization via enhancement of chondrocyte apoptosis [87].

Blood Vessel-Related Cells

All the cell types associated with blood vessels and the lymphatic system are represented in this category. These include vascular smooth muscle, endothelial cells, pericytes, etc. [88]. Although the blood vessel adventitia is primarily composed of collagen and connective tissue, it incorporates many cellular components of the types discussed above—macrophages, mast cells, progenitor cells, T cells, microvascular endothelial cells, and adipocytes [89]. Although there is some evidence that lymphatic vessels appear in normal bone, they are restricted to the outer fibrous layers of the periosteum [90]. It is likely that lymph vessels do not play a major role in bone function.

Neurons

Often forgotten, neurons are clearly present in bone and are particularly important for the regulation of bone metabolism. The bone has a very dense network of sensory [60] and sympathetic neurons [91] that are closely associated with blood vessels, trabecular bone, and near hematopoietic cells [92]. The periosteum, specifically, has an exceptionally high neuron density [60]. The sympathetic nervous system (SNS) exerts primary control over bone metabolism [93], with evidence of catecholamine signalling to Ob being well established [94]. Some bone compartments may also use cholinergic signalling systems [93]. Bone, bone marrow, and periosteum are densely innervated with peptide-rich sensory neurons/C-fibres (unmyelinated) (substance P and CGRP) [95]. Myelinated Aβ- and Aδ-fibres also are present [96].

Demonstrating the impact of the SNS, sympathectomy after administration of guanethidine to neonatal rats resulted in significantly increased numbers of Oc at

the surface of mineralized bone; removing just the sensory C-fibre innervation (with capsaicin treatment) caused a decreased number of Oc in the same locations [97]. These experiments suggest that sensory functions, in addition to sympathetic regulation, may also contribute to the feedback control over bone remodelling. In fact, the neurotransmitter glutamate is highly expressed particularly near bone cells, suggesting that glutamatergic control over bone functioning may be essential in normal bone metabolism [92], perhaps independently of the sympathetic modulation. Many of the primary afferent sensory neurons innervating mineralized bone express acid-sensing ion channels such as the vanilloid receptor, and these may be present to respond to the acidic microenvironment caused by osteoclast functions [60]. It is interesting to note that, although most bone structures deteriorate over time, the sensory neuron density apparently does not decline with age [98].

What Cancers Appear in Bone?

Many cancer types can appear in bone, and they may originate from a variety of sources. Cancers may originate in skeletal structures (primary cancers such as chondrosarcoma, osteoma, multiple myeloma), others migrate from other distant sites (metastatic cancers such as breast, prostate, lung cancers), while some may invade into bone from nearby structures (e.g., head and neck cancers). Once in the bone, many cancers cause similar alterations in the bone microenvironment as they interact with the same host cell environments, although in different ways.

Primary Bone Cancers

The most frequent primary bone cancers can be divided into solid tumour and non-solid (haematological) tumour types. This categorization is purely arbitrary, although it does allow the haematological cancers to be defined as a bone cancer type mostly due to the location of the affected cells in the bone marrow. The three most common solid bone tumours are osteosarcoma, Ewing's sarcoma, and chondrosarcoma. Osteosarcomas develop from uncontrolled proliferation of osteoprogenitors, and it has been argued that Ocyt cells may actually be the aberrant progenitor [99]. Ewing's sarcoma is thought to be of ectodermal origin although new evidence suggests that this cancer may derive from mesenchymal stem cells in the bone marrow [100]. Furthermore, genomic analysis demonstrates a potential relationship to chondrocyte progenitor cells [101]. Ewing's sarcoma is associated with severe bone pain with significant periosteal reaction but with little evidence for cortical bone changes. Similarly, chondrosarcomas are cancers of the chondroid matrix-producing cells. Although typically a primary cancer, chondrosarcoma can also become metastatic and move to other sites [102, 103].

Several non-solid tumours or haematological cancers develop partly in the bone marrow space, and these include leukaemia, lymphoma, and multiple myeloma. Leukaemias are cancers of myeloid or lymphoid cell lines, while multiple myeloma is a cancer originating in the white blood cell type called *plasma cells* [104], an important mediator of adaptive immunity. Although these cancer cells are present in the bone marrow for only some portion of their life cycle, the most prominent bone-related symptoms that occur are likely due to their overgrowth and disruption of bone marrow functioning.

Metastatic Bone Cancers

Cancers that spread or metastasize from distant sites in the body can preferentially find refuge in the bone environment. The identity of the primary tumour is an important oncodynamic factor to consider since it may determine some of the metabolic properties of the metastatic cells and these properties determine the responses of local cells in the bone.

The most common metastatic cancers that spread to bone are breast and prostate [105]. Breast cancer's predilection to seek bone was initially described by Paget in 1899 in which he suggested that the properties of the cancer cells (the seed) were as important as the properties of the bone (the soil) in determining this preferential localization [106]. Frequent bone localization in breast cancer metastasis is therefore not a chance phenomenon. In fact, 73 % of breast cancer patients were found to have bone metastases on *post mortem* examination [107]. The same occurs in prostate cancer, with about 68 % of patients having bone metastases [107]. Although at a lower frequency (between 35 and 42 %), other tumours that spread to bone include lung [108], kidney [107], thyroid, and gastrointestinal cancers [107]. Some cancers can metastasize to bone but only do so rarely. These include melanoma [109], neuroblastoma [110], cervical [111], and ovarian cancers [112, 113].

What Bone Functions Change When Cancer Cells Are Present?

Virtually, all of the cell types residing in bone can respond to cancer invasion with changes in their normal physiological functions. The resulting clinical symptoms experienced by patients with bone cancer can include bone pain, fractures, impaired mobility, impaired haematological functions, and hypercalcemia [105]. How these symptoms occur in patients is based mostly on a composite of the individual cellular responses that transpire within the bone environment. Since the maintenance of bone requires a delicate balance between bone synthesis and bone degradation processes, one common response to cancer invasion is a change in the inherent structure of the bone that results from disruption in one or both of these

processes. Although there may be either increased bone loss or enhanced bone synthesis, many cancers result in simultaneous and sometimes quite subtle alterations in both processes.

Understanding oncodynamic effects is vital to enable clinicians to effectively identify and treat cancer in the bone. To demonstrate this more clearly, take as an example the presentation of a patient with breast cancer bone metastasis showing significant Oc-mediated bone resorption. A common therapeutic intervention for this apparent osteolysis problem, in addition to starting standard anticancer chemotherapy or endocrine therapy, is to inhibit Oc function with drugs such as a bisphosphonate (e.g., zoledronic acid) or reduce Oc differentiation using a RANKL inhibitor (e.g. denosumab) [114, 115]. However, even though it is agreed that there is increased bone degradation, the treatment choices for this metastatic bone disease may be better informed by determining the fundamental cellular signalling mechanisms driving the oncodynamic effects that result in the observed bone resorption. Many cancers may appear to present as a simple increase in Oc activity, yet the real effect could easily be due to a variety of factors—including inhibition of Ob differentiation [116], alteration of Ocyt control over the balance maintained between Oc and Ob functions [40], or an enhancement of Oc-precursor cell survival [117]. The overall osteolytic result would likely appear the same in each case. Although Oc inhibition has proven effectiveness in bone metastasis [118, 119], many clinicians agree that the solution to the problem is not to just get rid of the Oc effector cells, as this may miss what is really happening. By perceiving the system as an oncodynamic process, then identifying the actual changes that are occurring in bone, novel therapeutic targets may be identified. To better understand the specific oncodynamic effects that can occur in bone, the cellular responses will be described within the context of the essential functions of bone itself.

Changes in Bone Structure

Cancer-induced alterations in bone metabolism can directly impact the three structural functions of bone—namely support, protection, and movement. A disruption in homeostasis often leads to a reduction in bone strength and potential changes in its anatomical configuration. Considering that there are two distinct but interconnected processes used for bone maintenance—degradation and synthesis—theoretically, there are three possible changes that can be imagined. Although perhaps an oversimplification, these abstract categories are important for logically defining the problem. The three possibilities are (a) disruptions of the bone degradation processes alone, (b) changes in the processes of bone synthesis alone, or (c) alterations in both processes at the same time. Since Oc and Ob functions are normally very tightly coordinated, it appears most likely that both cell type functions are impacted by cancer at the same time, and that it is the sum of these effects that will be the determining factor in classifying the bone pathology as predominantly *osteolytic* (decreased bone mass), *osteosclerotic* (increased bone mass), or

mixed (having a combination of both processes). To further complicate this model of structural modification, each of these inherent processes can have two opposing directions—either an increase or a decrease.

Increases in Bone Degradation

If the overall 'symptom' of cancer invasion is a loss of bone, increases in the functions and numbers of Oc responsible for degrading bone may be the culprit. These effects can be due to direct mature cell functional changes or to alterations in the differentiation of bone cell progenitors. Many cancers present with a predominantly degradative bone phenotype, including breast cancers, leukaemias, lung, thyroid, renal, multiple myeloma, and metastatic neuroblastoma. However, a few of these are solely the result of enhancement of Oc functions. More commonly, cancers alter the survival or growth/differentiation rate of the Oc progenitor cells.

A more effective technique to achieve osteolysis is to induce alterations in both Oc and Ob functions simultaneously. An example is *metastatic neuroblastoma*. In this cancer, osteolysis is mostly due to stimulation of osteoclastogenesis, although there is also some inhibition of Ob precursor differentiation. As a consequence of the relative increase in numbers of Oc cells, the result is enhanced resorption of bone [120]. However, most osteolytic phenotypes are more complex than this. Multiple myeloma cells can indirectly achieve the same goal by stimulating the secretion of factors from host cells in the bone environment which in turn stimulate Oc functioning [121, 122]. These myeloma cells also can cause a direct physical disruption of the bone remodelling unit which prevents bone formation from occurring normally [18]. Other examples of complex mechanisms achieving and osteolytic phenotype include breast cancer, in which the cancer cells secrete cytokines that both enhance the development and the survival of Oc progenitor cells, and thus indirectly stimulate bone resorption by sustaining an increased number of Oc cells [123]. Non-metastatic neuroblastoma [120] cells take a different approach and do this by actively suppressing osteoblastogenesis from Ob precursors. Of course, breast cancer cells also secrete factors that reduce the survival of monocyte Ob progenitor cells [124], but this further supports that the reality that parallel oncodynamic effects often occur in both the synthesis and degradation processes. The result from each of these different mechanisms is the same—increased Oc-mediated bone resorption.

Increases in Bone Synthesis

Prostate cancer is widely considered as the best example of a tumour that frequently elicits a net increase in bone mass [125]. However, this newly formed bone in prostate cancer is usually atypical in appearance, with incomplete mineralization

and osteoid deposition that creates what is sometimes called woven bone [126]. Woven bone may not be as structurally sound as normal trabecular bone [127]. As a parallel to the multiple options described in cases of predominant osteolysis, the pathological enhanced bone deposition in prostate cancer may be due to higher Ob activity, an indirect increase in Ob number (due to changes in osteoblastogenesis), or via corresponding decreases in Oc activity and Oc numbers.

In vitro evidence supports that a soluble secreted protein from prostate cancer cells called prostatic acid phosphatase (PAP) causes direct activation of bone mineralization by mature Ob cells [128], separate from effects on Ob differentiation. Rabbani et al. also identified that urokinase-type plasminogen activator (uPA) secreted by prostate cancer cells acted as a mitogenic agent for Ob cells to stimulate their mineralization functions [129]. Similarly, we observed that the amino acid glutamate results in increases of alkaline phosphatase activity and bone mineralization in Ob cells independently of cell differentiation or proliferation [130]. Although perhaps not as common, direct inhibition in Oc functioning has been observed. When in contact with bone, prostate cancer cells secrete *endothelin-1* (ET-1) and this peptide will signal to the Oc cells and reduce their functioning by directly impairing cell mobility [131].

More subtle indirect changes can occur, with prostate cancer cells enhancing Ob precursor differentiation via secreted factors that stimulate this process—leading eventually to enhanced bone formation [132]. Although inhibiting Oc activity directly, the secreted ET-1 is also received by Ob and will stimulate their functions [133], with the specific endothelin-A receptor mediating this effect [134] through a secondary cytokine-based signalling mechanism. Secondary activation of bone formation by reducing Oc proliferation rather than by direct inhibition of functioning is very well established in the literature. *Osteoprotegerin* (OPG), a soluble decoy receptor for RANKL, is produced by Ob, and this factor inhibits RANKL stimulation of Oc differentiation, thus decreasing Oc numbers [135, 136].

Uncoupling of Ob and Oc

As seen above, many cancer-signalled changes in bone metabolism involve simultaneous changes in both Oc and Ob cell functions. Being so delicately balanced, the functioning of these two cell types appears to be easily perturbed when cancer cells invade. The homeostasis of normal bone is maintained by complex intercellular communications between all the cellular partners in the bone environment, and anything that disrupts this signalling can lead to uncoupling of the Oc and Ob and result in the observed bone pathologies. In such a complex system, it is quite unlikely that a unilateral change in a single cell type will result in changes in bone structure.

Prostate cancer can be used as an example to emphasize this point more clearly. Although prostate tumours typically result in an overall osteosclerotic phenotype, there is considerable evidence that Oc-mediated bone degradation is still critical in

the development of the net bone formation effect [137]. Without degradation, the tumour-enhanced Ob cells may have few locations at which to build new bone. In fact, a purely osteoblastic phenotype of prostate cancer may be a rarity. In a study of prostate cancer patient samples, not a single patient had bone lesions that could be categorized into either purely osteosclerotic or purely osteolytic [126]. Other cancers that generally show a predominant phenotype also show evidence of changes in both degradation and synthesis. This includes breast cancers that typically evoke an osteolytic response—these cells actually secrete factors such as bone morphogenetic proteins (BMPs) that enhance the mineralization activities of Ob [138]. BMPs in turn can alter both Ob and Oc differentiation, although the overall result in breast cancer bone metastases is osteolytic. In multiple myeloma, bone marrow stromal cells maintain their capacity to differentiate into Ob cells (given the correct environmental signals) even though this is almost always an osteolytic disorder [139], suggesting that uncoupling of Ob and Oc in this case may be related to disruption of communication between cells rather than pathological changes to the individual cell types directly. This type of effect is the basis of our hypothesis that the cancer cell secreted factor glutamate may be acting to uncouple Oc and Ob by disruption of critical glutamate signalling mechanisms between cell types in the bone [130, 140].

Changes in Other Bone Functions

Bone performs many functions other than as a connective tissue. The bone's storage, endocrine, and hematopoiesis activities are also important tasks that can be perturbed when cancer invades. Thus, identifying oncodynamic effects relating to these functions is critical to fully understand the implications of cancer invasion of bone.

Changes in Storage Functions

The bone stores a variety of different factors within its hydroxyapatite matrix, including calcium, phosphorous, and numerous growth factors. When bone is degraded by Oc, whether pathological or not, these factors may be released to have local autocrine or paracrine effects or distant endocrine-like responses. This is a normal process that occurs all of the time, with the bone acting as a primary storage mechanism for a number of different substances. When cancer induces oncodynamic effects, a number of changes occur in bone degradation and formation to result in a different rate of release of stored components than normal. Pathologies can result when the rate of release of those stored factors is important for the normal balance maintained in the bone. These altered release dynamics are typically observed to result in direct pathological effects in the bone or at distant sites in the body.

Calcium

Being the principal mineral component of the bone matrix and a critical ionic signalling molecule throughout the body, the maintenance of calcium balance is a tightly regulated multisystem process. Vitamin D enhances intestinal absorption of calcium in response to low circulating calcium levels. If absorption from the diet is insufficient to maintain the proper circulating levels, the high vitamin D will signal the bone to move calcium into circulation [141]. This signalling leads to Oc, Ob, and Ocyt cells all responding together to achieve a net increase in bone resorption either directly, or indirectly via stimulation of hormone signalling from the parathyroid glands [142, 143]. When cancers invade bone and drive an overall increased bone resorption, hypercalcemia can become a significant clinical problem. Although mostly due to Oc-related bone resorption, additional signalling by endocrine factors secreted by the cancer cells that are received by the kidney can cause increased renal reabsorption of calcium, resulting in even higher circulating levels. A dysregulated serum calcium level, regardless of the aetiology, can have profound neurological and psychiatric consequences [144, 145].

Phosphate

Like calcium, phosphorous is stored as a component of the calcium phosphate bone matrix and its availability in circulation is regulated through a number of endocrine and paracrine mechanisms. In addition to being a structural component of bone, phosphate is also critically important for all phases of cellular energy metabolism. Phosphate balance relies on several systems and endocrine factors secreted primarily by Oc and Ocyt cells [146] and the parathyroid glands will regulate phosphate secretion by the kidney. For a review of what is known about normal phosphate homeostasis, see a review by Eleanor Lederer [147]. When cancer appears in bone, additional factors secreted by the tumour cells disturb this delicate balance and often leads to hypophosphatemia and tumour-induced osteomalacia—a 'softening' of the bones as a result of inadequate bone mineralization. This change in bone strength can also be accompanied by pain, fatigue, and muscle weakness. A recent review of cancer-induced osteomalacia describes some of the factors involved in the development of this disruption in phosphate homeostasis [148].

Other Stored Factors

Numerous growth factors, hormones, and cytokines are stored within bone matrix and these can be released during normal bone remodelling or cancer-induced bone resorption. Much of the literature on bone metastasis emphasizes the paracrine

stimulatory effects of these factors on the invading tumour cells directly, although these released substances evoke important responses from host cells in the bone environment. An example here is transforming growth factor β (TGF-β) which is released from bone in a latent form [149], and the acidic conditions accompanying Oc-mediated matrix resorption will activate it [150]. Although TGF-β has effects on the growth of cancer cells, it also directly and indirectly alters the functions of Oc and Ob. Specific TGF-β receptors are expressed by Oc and the resulting stimulation of this receptor may secondarily lead to other growth factors being secreted by the Oc [151]. There is some evidence that suggests that TGF-β signalling of Oc also assists in stimulating Ob-mediated bone formation [152], as part of the coupling mechanism that normally regulates Oc and Ob functions.

We have taken advantage of the storage function of bone in our own work. Since we anticipated that metastatic breast cancer cells would prompt Oc to degrade the bone, we pre-administered the tetracycline drug *doxycycline* and allowed it to accumulate in the bone matrix. When the expected oncodynamic responses occurred, we observed an overall decrease in bone resorption and a reduced tumour burden as the high local concentrations of doxycycline released from its storage in the matrix effectively inhibited both tumour growth and osteolysis [153–155].

Changes in Endocrine Functions

The storage and endocrine functions of bone are intimately linked, as many factors released by osteoclastic bone degradation lead to responses locally as well as elsewhere in the body. Many of the factors that are released by bone when it is degraded can also be considered as endocrine mediators. These or the cancer cells may secondarily stimulate host cells to produce other substances—all of these may lead to oncodynamic responses in other parts of the body. The prime example of such a factor is the calcium that is released from degrading bone—this calcium has well-established roles as a mediator in the parathyroid glands and the intestinal tract, as discussed above.

Changes in Hematopoiesis Functions

An important function of bone is to be a location for the development of blood cells. One of the most direct oncodynamic effects is the simple displacement of these cells from the marrow space by the cancer, and this essentially prevents the complex interactions between hematopoietic precursor cells and the bone environment from occurring. In addition to this compartment-based displacement, some cancer cells may lead to a paracrine factor-mediated “reprogramming” of bone marrow cells to produce a generalized immunosuppression—presumably by

altering the progression of hematopoietic stem cells preferentially toward the myeloid lineage [156]. Since the bone provides a safe environment for hematopoietic cells, other cancers like chronic myelogenous leukaemia take advantage of this relative safety to proliferate in the endosteal niche, enhancing the growth of Ob which directly support the growth of the cancer cell [157]. One example of a significant hematopoietic disruption by cancer is the disruption of the immune system cells that initially develops in the bone. Various cytokines (e.g., TGF-β) that cause functional responses in Ob and Oc also cause inhibition of T cell and natural killer cell proliferation, and this results in reduced immune surveillance in the bone [158]. Evading the immune system is an important factor in cancer cell survival.

Other Signalled Changes

A vital function of bone that is often overlooked relates to its sensory activities. Bone incorporates a number of different cell types with the ability to sense a variety of stimuli other than the secreted chemical signals. The bone changes its structural configuration in response to mechanical stimulation by altering the balance between Ob and Oc activities. Multiple signalling molecules are definitely involved, although glutamate intercellular communication appears prominently in the literature [51, 55, 57, 159]. The effector cells for the adaptive degradation or formation of bone are the Oc and Ob, but the Ocyt cells have been revealed as the master modulator of these changes [52]. We have proposed that the presence of cancer cells that secrete high concentrations of glutamate into the bone environment is able to disrupt this control system [140, 160], and have some evidence to demonstrate that this glutamate mechanism may be related to the sensation of bone pain in cancer [161].

Although the mediators of bone cancer pain are not well understood, the sensation of pain is strongly associated with cancer invasion into the bone. Acute lymphoblastic leukaemia [162], multiple myeloma [163], and metastatic breast [164] and prostate cancers [165] are all associated with significant bone pain. As described previously, the bone has numerous sensory fibres within its structure, and these sensory neurons can respond to chemical and mechanical stimuli which may be perceived as pain. In addition to traditional signalling, cancer cells can cause direct damage to neurons [96] and eventually lead to a neuropathic type of pain. Mechanistically, many believe the Oc to be critically involved in cancer-induced bone pain. However, although protons secreted by the Oc to demineralize bone are associated with pain sensation [166, 167], Oc are not the only players, as therapeutic ablation of Oc function does not stop pain in later stages of the disease [168]. It should be noted, though, that signalling from the bone to the nervous system is not the only direction possible—there is evidence demonstrating that substances released from sensory neurons also play a role in coordinating the functional adaptation of bone cells to strain and mechanical loading [169].

What Are the Signalling Mediators?

With all the anatomical and functional complexity of the skeletal system, it is not reasonable to expect that a single mediator molecule could be solely responsible for a specific oncodynamic effect. In fact, as many of the mediators can arise from numerous sources within the bone environment, it is often difficult to confirm whether the signals derive from the cancer or the host cells—or both at the same time. Combined with frequent secondary responses to the same or related mediators, the sophisticated interplay between cell types in the bone remains the greatest obstacle for understanding and treating bone cancers. By taking an oncodynamic approach, it allows a different perspective to be applied to this problem. An attempt at reviewing current knowledge of cancer-derived or cancer-induced signalling from the viewpoint of the cell types present in bone may help to make some sense of the complex interactions that can occur.

An admirable attempt at integrating the many mediator molecules controlling bone homeostasis in breast cancer metastasis is provided in a recent review by Rusz and Kahán (see Table 1 in Ref. [170]). This table identifies many of the mediators that are known to be involved in changing Oc, Ob, and tumour cell functions. However, the authors appear to approach the problem from the perspective of how the bone responses will continue the *vicious cycle* that many groups characterize as being a fundamental feature of bone metastasis [171–175]. This cycle directly connects the bone cell responses back to the growth and survival of the cancer cells in a positive feedback loop.

To better fit with our oncodynamic interpretation of bone cancer, and to concentrate primarily on changes induced by the cancer cells, we have developed a similar tabular format but have instead organized the signalling molecules by the cell types present in bone that are impacted by those mediators. This is clearly a non-exhaustive list (see Table 9.1, sorted alphabetically within each cell type), but it provides some of the signalling context for a better understanding of oncodynamic responses in bone.

By examining the list of mediators in Table 9.1, a few general patterns begin to emerge. The most striking pattern is how frequently some of the mediators appear as modulators of different cell types. For example, glutamate appears repeatedly as a mediator and it impacts almost all cell types in bone. This, however, should not be overly surprising since glutamate is a highly conserved chemical signalling molecule that is phylogenetically quite ancient. In fact, eukaryotes used glutamate (a simple and easily accessible amino acid) as a signalling molecule *before* they evolved discrete nervous systems [176]. Most cell types in bone, including cancer cells [177], express various glutamate receptors and transporters [160] and thus have the requisite capacity to communicate via glutamate signals. From the oncodynamic perspective, we have found that glutamate alters the differentiation and functions of Ob and the differentiation (but not the functions) of mature Oc [130]. Glutamate also appears to cause direct stimulatory and inhibitory effects in addition to the more enduring and slower to achieve effects on cell differentiation, further supporting its relevance to normal bone homeostasis.

Table 9.1 Oncodynamic mediators and effects by bone cell type

Cell type	Signalling substance	Source	Effect
Osteoclasts and progenitors	bFGF, FGF-1, FGF-2	Cancer, host, bone	Enhances proliferation
	BMP	Cancer, host, bone	Enhances differentiation
	ET-1	Cancer, host	Directly impairs mobility
	Glutamate	Cancer, host	Enhances differentiation
	Interleukins (multiple)	Cancer, host	Enhances differentiation, survival, and function
	MCP-1	Cancer, host (Ob)	Enhances maturation
	M-CSF	Cancer, host	Enhances differentiation and proliferation
	microRNA	Cancer, host	Enhances differentiation
	OPG	Cancer, host (Ob)	Indirectly inhibits differentiation
	PDGF	Cancer, host, bone	Enhances differentiation
	PTHrP	Cancer	Enhances differentiation
	RANKL	Cancer, host (Ocyt)	Enhances differentiation and survival
	sICAM1	Cancer	Enhances differentiation
	TGF-P	Cancer, host, bone	Enhances function
	TNF-a	Cancer, host	Enhances differentiation
	VEGF	Cancer, host	Enhances differentiation
Osteoblasts and progenitors	BMP	Cancer, host, bone	Directly enhances functions; enhances differentiation
	DKK1	Cancer	Inhibits terminal differentiation
	ET-1	Cancer, host (Ob)	Enhances proliferation and functions
	FGF23	Host (Ob, Ocyt)	Secondarily regulates mineralization
	Glutamate	Cancer, host	Direct activation of functions and differentiation
	Interleukins (IL-18)	Cancer, host (Ob)	Enhances functions
	microRNA	Cancer	Inhibits differentiation
	NPY	Cancer, host	Directly inhibits functions
	PAP	Cancer	Direct activation of functions
	PTH, PTHrP	Cancer, host	Inhibits Ob functions; inhibits differentiation
	Semaphorin 3A	Cancer	Enhances differentiation
	TGF-P	Cancer, host, bone	Indirectly enhances function
	uPA	Cancer	Direct activation of functions

(continued)

Table 9.1 (continued)

Cell type	Signalling substance	Source	Effect
Osteocytes	Glutamate	Cancer, host	Disrupts control over Ob and Oc
	Interleukins (various)	Cancer, host	Stimulates FGF23 release
Bone lining cells	bFGF	Cancer, host, bone	Enhances endosteal bone formation
Stromal cells	bFGF	Cancer, host, bone	Alters functions
	GRP78	Cancer	Enhances activation
	VEGF	Cancer, host	Polarizes macrophages
Hematopoietic cells	DKK1	Cancer, host	Inhibits proliferation
	NPY	Cancer, host	Stabilizes and regulates (hibernation)
	TGF-P	Cancer, host, bone	Inhibits proliferation
	TNF-a	Cancer, host	Inhibits differentiation
	VEGF	Cancer, host	Activates macrophages
Chondrocytes	FGF23	Cancer	Alters cartilage formation
	Glutamate	Cancer, host	Inhibits endochondral ossification and enhances apoptosis
	Protons	Cancer, host	Enhances chondrocyte apoptosis
Blood vessel-related cells	ET-1	Cancer, host (Ob)	Contracts vascular smooth muscle
	NO	Cancer, host	Relaxes vascular smooth muscle; enhances angiogenesis
	VEGF	Cancer, host	Enhances angiogenesis
Neurons	Glutamate	Cancer, host	Nociception; stimulates neurogenesis
	NGF	Cancer, host	Enhances neuron growth
	NPY	Cancer, host	Alters signalling; prevents nerve injury
	Protons	Cancer, host	Nociception

This is a non-exhaustive list of signalling mediators known to alter bone cell functions when cancer invades bone, organized by bone cell type. The mediators are sorted alphabetically within each cell type and both the source(s) and the potential effect(s) of the mediator are noted. Many factors are generated by cancer cells (*cancer*) as well as by host bone cells (*host*), with several also being stored in the bone matrix (*bone*) and released upon bone degradation

Abbreviations: bFGF: basic fibroblast growth factor; BMP: Bone morphogenetic proteins; DKK1: dickkopf 1 protein (a Wnt inhibitor); ET-1: endothelin-1; FGF23: fibroblast growth factor-23; GRP78: glucose-regulated protein-78 (a heat-shock protein); MCP-1: monocyte chemoattractant protein-1; M-CSF: macrophage colony stimulating factor; NGF: nerve growth factor; NO: nitric oxide; NPY: neuropeptide Y; Ob: osteoblast; Oc: osteoclast; Ocyt: osteocyte; OPG: osteoprotegerin; PAP: prostatic acid phosphatase; PDGF: platelet-derived growth factor; PTH: parathyroid hormone; PTHrP: parathyroid hormone-related protein; RANKL: receptor activator of nuclear factor kappa-B ligand; sICAM1: soluble intercellular adhesion molecule-1; TGF-β: transforming growth factor-beta; TNF-α: tumour necrosis factor-alpha; uPA: urokinase-type plasminogen activator; VEGF: vascular endothelial growth factor

There are numerous growth factors and cytokines that appear in multiple locations on the list, and these include vascular endothelial growth factor (VEGF) [178], nerve growth factor (NGF) [179], tumour necrosis factor-alpha (TNF-α) [180], and fibroblast growth factor 23 (FGF23) [146]. These and other similar growth factors are derived from either the invading cancer cells or the various classes of host cells in the environment. Also, included are those that can also be stored in the bone matrix (thus being available from at least three separate sources), including *TGF-β* [158], basic fibroblast growth factor (bFGF) [181], platelet-derived growth factor (PDGF) [182], and BMP [138]. A good list of these bone matrix-derived growth factors is available in a review by Mohan and Baylink [183]. This class of mediators is involved in many of the fundamental processes of bone metabolism, but appears to be also critical for reactive processes related to immune responses and inflammation. In more general terms, many of these cytokines act as predominantly stimulatory or enhancing mediators, and often have effects on multiple cell types simultaneously, implying that they cause more generalized effects rather than being involved in specific cell-type homeostatic control. Furthermore, these effects also impact both mature cell functioning and differentiation of progenitors in very complex ways. A more detailed discussion of the oncodynamic implications of cytokines and growth factors is described in other chapters of this volume.

PTH and *PTHrP* are well-characterized endocrine factors that are secreted by either the cancer cells or the host (typically from the parathyroid glands) and have specific effects that relate to whole-body mineral homeostasis. The effects are described here as being more specific as they induce increases in Oc differentiation and they inhibit Ob both differentiation and function, thus leading to increased bone resorption and calcium mobilization from the skeleton [184]. This is particularly important in osteolytic metastatic breast cancer, as these cells are unable to alter Oc functions directly, and thus use PTHrP to inhibit the opposing cell type (Ob) to achieve the same net result. Roodman provides an excellent review of PTHrP in bone metastasis that is well worth reading [185].

Similar to PTHrP, where the control over functioning is accomplished by skewing the balance between Oc and Ob, the *RANKL* and *OPG* system is understood in considerable detail. In breast cancer, this system operates similarly in many ways to ensure an overall induction of Oc activity. Breast cancer cells can sometimes produce RANKL directly, and this leads to increased Oc differentiation secondarily through its interaction with the receptor RANK expressed on Oc precursors—this receptor–ligand interaction essentially permits other growth factors in the environment to elicit the required Oc differentiation [186]. However, in prostate cancer this system operates differently. Prostate cancer cells secrete OPG which acts as a decoy receptor for RANKL, preventing the soluble RANKL signal from binding to permit the growth factors from causing Oc differentiation. As discussed previously, prostate cancers often cause mixed osteoblastic and osteolytic lesions, and this is partly due to these cancer cells also producing the RANKL signal, interleukin-1, and TNF-α, all of which are associated with enhanced osteoclastogenesis [187]. Often, it is the ratio of RANKL to OPG in the bone environment that determines the resultant phenotype [188].

Another repeating pattern is that there appear to be several highly specific and direct effects on mature Oc and Ob functions that result from small protein-based signals arising from cancer cells. As discussed above, ET-1 is a protein produced by some cancers, which causes direct inhibition of Oc functions by interfering with cell mobility [134] along with a concomitant direct enhancement of Ob functions [133]. The previously described prostate cancer-derived factor uPA also has direct Ob enhancing properties [129]. Direct inhibition of Ob functioning can also be achieved by other small peptides, such as NPY [47] and PAP [128]. These specific and immediately functional responses from exogenous agents stand out as being an unusually precise effect in such a complex system with multiple redundant control systems. By recognizing this pattern, these highly specific proteins and their responses distinguish themselves as being potentially accessible and specific targets for future therapeutic strategies. More typical, however, are the innumerable examples of effector molecules that result in the slower, yet potentially longer lasting changes in cell numbers—that is, by the enhancement or inhibition of precursor differentiation processes.

There are many examples of mediators that serve to enhance Oc differentiation. This strategy for manipulating bone homeostasis may be viewed as a means of amplifying the effectiveness of a small quantity of signal to eventually generate an enduring and robust functional response. This is in contrast to the highly specific and direct effects on a very small number of cells discussed in the previous paragraph—where a small quantity of signal will achieve a small functional effect. In the small molecule category, various microRNA molecules derived from both host and cancer cells have been reported to enhance Oc differentiation. These small ribonucleotide molecules fulfil their communication goals by entering the receiving cell and altering or initiating transcriptional and translational processes in that cell. One specific example of this is *miRNA-223*, and this RNA fragment appears to be critical for Oc differentiation changes [189, 190]. Another small molecule called soluble intercellular adhesion molecule-1 [191] (sICAM1) similarly results in a generalized (but not rapid) increase in Oc number, eventually causing greater osteolysis. An example of a mediator working in the opposite direction to enhance Ob differentiation is the prostate cancer-derived molecule called *semaphorin 3A* [132]. The stimulation of Ob precursor proliferation effectively increases the number of Ob cells and thus increases bone formation. What is most interesting here is that *semaphorin 3a* is a member of a class of chemorepulsant protein inhibitors that are most often described in relation to the nervous system [192]. A protein that normally inhibits or repulses cell movement, in this case, acts as an activator of Ob precursor differentiation. Precursors to cell types other than Oc and Ob are also sensitive to oncodynamic manipulation. Glucose-regulated protein-78 (GRP78) is secreted from cancer cells and can stimulate/activate bone marrow fibroblasts to become cancer-associated fibroblasts [193]. GRP78 is also known as an endoplasmic reticulum chaperone and heat-shock protein when intracellularly located, so its effects (like that of microRNA), although specific, appear to not be a classic receptor–ligand interaction.

In contrast to the numerous enhancers of bone cell precursors, very few mediator molecules have been characterized that inhibit precursor differentiation, although, as described above, prostate cancer-derived OPG achieves this Oc-precursor inhibitory function indirectly. Since Oc cells are derived from hematopoietic progenitor cells in the busy bone marrow space, it is possible that the multipotent precursors that may eventually become Oc cells are not a very specific or practical target for such a subtle modulation. There are, however, a few molecules that can inhibit Ob progenitor development, and one example is dickkopf-1 (DKK1), a secreted protein known to be a Wnt signalling inhibitor. DKK1 is produced by myeloma and Ocyt cells, and it inhibits Ob differentiation to reduce the total number of Ob and thus decrease bone deposition [116]. This protein also has an 'enhancing' function with endothelial cell progenitors, causing these cells to have greater angiogenesis potential [194]. It is likely that precursor redundancy or anatomical location may be important factors in determining how easy it is to interfere with cell-specific precursor development.

Several nontraditional signalling molecules also are involved in oncodynamic bone cell responses. However, these molecules generate what may be described as more non-specific responses in comparison to the highly specific protein-based mediators described above. A good example is the response by bone cells to low levels of nitric oxide (NO), often generated by activated macrophages. This gaseous mediator can cause vessel relaxation and angiogenesis [195], which may sufficiently change the physiological environment to achieve functional responses. Even more atypical stimulation occurs from simple hydrogen atoms, or *protons*, which are liberated by many metabolic reactions in the bone. Chondrocytes possess G-protein coupled receptors that sense protons and this, in essence, becomes an acid-sensing system to detect levels of Oc-mediated bone resorption. These receptors, combined with the correct calcium environment, then promote chondrocyte apoptosis in advance of Ob bone mineralization [196]. Protons are also quite relevant to the sensory functions of neurons in the bone. It is commonly thought that the highly acidic environment (high numbers of protons) generated by Oc may be an initiator for the perception of bone pain due to excessive osteolysis [167, 197, 198]. This model suggests that acid-sensing ion channels present on the sensory neurons in bone receive these protons and respond electrically to be perceived eventually in the brain as pain [166]. However, we also suggest that the ubiquitous glutamate molecule secreted by cancer cells, also being an amino acid and a copious proton donor, could also serve the same function in nociception. This process could easily be signalled via acid-sensing ion channels and/or through specific glutamate receptor systems expressed by the peripheral sensory neurons present throughout the bone environment.

The overall patterns of mediators for oncodynamic processes in bone discuss herein seem to fall into at least five discrete categories. These are [1] simple and redundant amino acid signalling systems that are involved in normal bone homeostasis, but can be co-opted by cancer cells to disrupt effective communication between cells; [2] multifunctional but somewhat non-specific growth factor/cytokine-like mechanisms affecting many cells simultaneously; [3]

well-characterized endocrine and paracrine factors that are intimately involved with normal bone homeostasis but can be also be leveraged by cancer cells to change the environment; [4] specific and direct functional effects on mature bone cells by proteins (usually) that are produced by cancer cells or are already present in the bone environment (both inhibitory and stimulatory effects); and [5] enhancement of differentiation of bone cell precursors, frequently by small molecules, but only with limited examples of inhibition of bone cell precursor differentiation.

These emerging patterns suggest that there are some fundamental processes that may be more easily targeted in the bone, and depending on the nature of the invading cancer cells, these processes will be impacted differentially. Cell-type specific effects certainly can occur, but most frequent are enhancements of differentiation rather than interference with proliferation. This may be a result of anatomical or physiological barriers that make precursor inhibition a less controllable effect. Use of the precursor route for achieving functional changes can be viewed as an efficient adaptation that maximizes the response with the smallest mediator intervention. Although highly specific and direct functional responses occur, these may actually represent the most accessible targets for therapeutic interventions.

Conclusions

Oncodynamics, or how the body responds to the invasion of cancer cells, is a theoretical construct that parallels the concept of pharmacodynamics. By taking the view of examining the effects of cancer on normal physiological and anatomical processes from the perspective of the host cells, the oncodynamic approach may provide novel insights into how cancer may be treated. The bone is a frequent target of cancer, whether as a primary site for the development of a tumour, or a destination in which cancers take up residency. This chapter provides the basic context for the bone as an environment in which cancers can grow. This is first achieved by defining the types of cells that are in bone and by redefining which cell types should be included on that list. Followed by a brief description of the functions of bone as an organ/tissue system, it reviews the cancers that frequently are associated with the bone. The corresponding changes that occur in bone functions following cancer invasion are then characterized, based primarily on the functions of bone and the cell types involved in those processes. Perhaps, the most valuable aspect of the oncodynamic approach was to provide a fresh look at not only the chemical mediators that participate in bone cell responses, but also the emerging patterns of mediator-response associations that appear to occur with higher frequencies in bone cancers. This integration of dynamic bone responses and mediators revealed that there are several fundamental strategies that are used to realize functional changes in bone metabolism. These strategies may not have been recognized if a traditional cancer cell-centric viewpoint was used, since the advantage of oncodynamics is in

simplifying the variables to focus specifically on what a cancer cell does to the host. With these insights, novel therapeutic strategies may be more successful if they address the more readily targetable and specific disruptions in bone functions that occur, rather than the indirect and subtle changes that involve bone cell progenitor differentiation. Oncodynamics appears to be a very useful approach for identifying potential opportunities to exert control over pathological disruptions in bone homeostasis, and this is achieved by pursuing a better understanding of the cells, processes, and mediators that maintain normal bone structure and functions.

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