0163-769X/98/$03.00/0
Endocrine Reviews 19(1): 18–54

Cancer and Bone*

THERESA A. GUISE AND GREGORY R. MUNDY

Division of Endocrinology, Department of Medicine, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7877

I. Introduction

CANCER is associated with significant morbidity in the skeleton. This was evident as early as 1889 when Stephen Paget (1) observed that "in a cancer of the breast the bones suffer in a special way, which cannot be explained by any theory of embolism alone . . . the same thing is seen much more clearly in those cases of cancer of the thyroid body where secondary deposition occurs in the bones with astonishing frequency." He also noted that "a general degradation of the bones sometimes occurs in carcinoma of the breast, yet without any distinct deposition of cancer in them." These early observations were profound as it is now clear that cancer can involve bone through both metastatic and humoral mechanisms.

Since the time of Paget, it has become clear that cancer affects bone in several ways: 1) indirectly through elaboration of factors that act systemically on target organs of bone and kidney to disrupt normal calcium homeostasis; 2) locally and directly via secondary spread of tumor to bone; and 3) via direct involvement by primary bone tumors. As primary bone tumors comprise a small minority of all tumors affecting bone, this review will focus on the aspects of cancer and bone related to the former only.

The three most common neoplasms in humans, breast, prostate, and lung cancer, frequently affect the skeleton. Since the majority of patients dying of cancer have bone involvement either through metastatic spread or as a result of the systemic effects of tumor-produced factors on bone and kidney, this is not a trivial problem. In 1996 alone, the estimated number of new cancer cases in men included 317,000 cases of prostate cancer and 98,900 cases of lung cancer while 184,300 cases of breast cancer and 78,100 cases of lung cancer were diagnosed in women (2). Furthermore, in the same year, prostate and lung cancer were responsible for 41,400 and 94,400 deaths, respectively, in men while breast and lung cancer deaths in women totaled 44,300 and 64,300 individuals, respectively (2). Despite advances in cancer therapy, cancer statistics indicate that the mortality rate of lung cancer is still rising for women, even though 1996 is the first year that it has leveled off for men. Additionally, the age-adjusted death rates of prostate cancer continue to rise, and although 1996 was the first year that a slight decrease in mortality due to breast cancer was observed, the age-adjusted death rate for breast cancer remains similar to that of 1930 (2). Thus, to improve therapy and prevention, it is important to understand the pathophysiology of the effects of cancer on bone as it will be a continued source of morbidity for years to come. Although the topic is an expansive one, this review will attempt to detail scientific advances in this area regarding the pathophysiology of the effects of cancer on bone.

II. Normal Calcium and Bone Homeostasis

As tumor affects bone both through systemic mechanisms as well as via local mechanisms of metastatic spread, the topic of normal bone remodeling and calcium homeostasis will be reviewed.

A. Bone remodeling

Bone is unique among target tissues affected by cancer as it is being continually remodeled under the influence of systemic hormones and local bone-derived growth factors. Bone consists of two physically and biologically distinctive structures. The outer cortical bone is hard mineralized matrix in which cellular and metabolic activities are relatively low. Cortical bone makes up 85% of the total bone in the body and is most abundant in the long bones of the appendicular skeleton. The volume of cortical bone is regulated by the formation of periosteal bone, by remodeling within Haversian systems, and by endosteal bone resorption. Cancellous or trabecular bone constitutes the remaining 15% of the skel-

Address reprint requests to: Theresa A. Guise, M.D., Division of Endocrinology, Department of Medicine, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, Texas 78284-7877. E-mail: guise@uthscsa.edu.

*Supported by NIH Grants CA-69158 and AR-01899 (to T.A.G.) and RR-01346, AR-07464, and CA-40035 (to G.R.M) as well as Grant DAMD17–94-J-4213 from the Department of Defense, U.S. Army (to T.A.G.).

eton and is most abundant in the vertebral bodies. The adult skeleton is in a dynamic state as the coordinated actions of osteoclasts and osteoblasts on trabecular surfaces and in Haversian systems result in continual bone resorption and formation. The normal mineralization of bone matrix is contingent upon adequate amounts of vitamin D, calcium, and phosphate. The mineralized bone matrix contains abundant amounts of growth factors, transforming growth factor-β (TGFβ) and insulin-like growth factor II (IGF-II) comprising the majority (3, 4). Such growth factors are released from the bone matrix as a result of osteoclastic bone resorption (5), a component of the normal remodeling process necessary to maintain the structural integrity of bone. The inner portion of bone consists of multicellular bone marrow in which hematopoietic stem cells, stromal cells, and immune cells reside. The hematopoietic stem cells have the potential to differentiate into the blood-forming elements and bone-resorbing osteoclasts, while the stromal cells support the differentiation of the hematopoietic cells as well as from bone-producing osteoblasts. Cells in the bone marrow, stromal and immune in particular, produce cytokines and growth factors that mediate cell-to-cell interactions in autocrine, paracrine, and/or juxtacrine fashions (6). Thus, tumor secretion of hormones that act systemically on bone may disrupt normal calcium homeostasis and bone remodeling to result in hypercalcemia and bone loss while tumor-produced phosphaturic factors may result in osteomalacia. Likewise, once cancer cells arrest in bone, the high concentrations of growth factors and cytokines in the bone microenvironment provide a fertile soil on which the cells can grow. Furthermore, when the tumor cells stimulate osteoclastic bone resorption, this bone microenvironment is even more enriched with bone-derived growth factors that enhance survival of the cancer and similarly disrupt normal bone remodeling to result in bone destruction.

B. Calcium homeostasis

Blood-ionized calcium concentrations are remarkably stable in normal individuals due to a complex regulatory system involving the actions of three calciotropic hormones on the target organs of bone, gut, and kidney. Calcium exchanged between the extracellular fluid and these target organs normally remains in zero balance (Fig. 1). Normal calcium homeostasis is dependent on the interactions of PTH, 1,25-$(OH)_2D_3$, and calcitonin on these organs to maintain the ionized calcium concentration within a very narrow range. Regulation of normal calcium homeostasis has been extensively reviewed by Chattopadhyay *et al.* (7) as well as by Parfitt (8, 9).

1. PTH. PTH is synthesized by the chief cells of the parathyroid gland, and its secretion is highly dependent on the ionized calcium concentration in the extracellular fluid. The serum PTH concentration decreases as the serum calcium concentration increases (Fig. 2) and represents a simple negative feedback loop (10). Similar to other endocrine hormones, such as those secreted by the anterior pituitary, PTH is secreted in a pulsatile fashion in the normal state as well as in states of primary hyperparathyroidism (12). The calcium-sensing receptor that mediates this negative feedback has been cloned from bovine parathyroid cells (13) and is mutated in the disorders of familial benign hypocalciuric hypercalcemia (13–19) and autosomal dominant hypocalcemia (20, 21). Active vitamin D metabolites decrease PTH synthesis *in vitro* and *in vivo* (22, 23) as well.

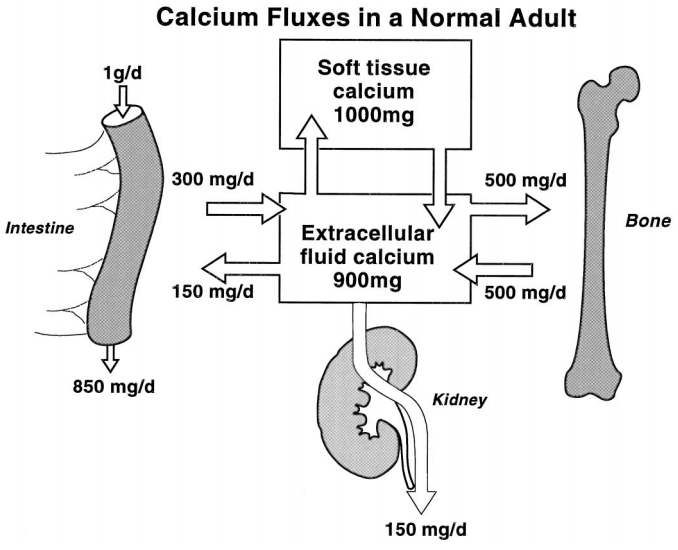

FIG. 1. Calcium homeostasis for a normal adult in zero calcium balance. The numbers are estimates of the amount of calcium exchanged between the extracellular fluid and gut, kidney, and bone each day. The exchange system between bone fluid and the extracellular fluid is not taken into account. [Adapted from G. R. Mundy, Bone Remodeling and Its Disorders (228).]

The biological actions of PTH include: 1) stimulation of osteoclastic bone resorption and release of calcium and phosphate from bone; 2) stimulation of calcium reabsorption and inhibition of phosphate reabsorption from renal tubules; and 3) stimulation of renal production of 1,25-$(OH)_2D_3$, which increases intestinal absorption of calcium and phosphate. The amino terminus of the PTH molecule binds to the PTH receptor, a member of the family of G protein-coupled receptors that contain seven transmembrane-spanning domains (24), to elicit these biological responses. Activating mutations of this receptor have been demonstrated in the rare hypercalcemic disorder of Jansen's metaphyseal dysplasia (25, 26). Recently, other receptors for PTH have been identified. A distinct G protein-coupled receptor that is exclusively activated by PTH, and not PTH-related protein (PTHrP), has been cloned. This PTH-2 receptor is abundant in brain and pancreas, although its function is not yet clear (27). Additionally, functional evidence for a receptor that binds only the carboxyl-terminal portion of PTH exists (28).

Metabolism of PTH is complex, and the intact and biologically active peptide has a half-life of less than 4 min (29). Intact PTH is cleared rapidly by kidney and liver (30–34). Carboxy-terminal fragments circulate significantly longer than the intact hormone, mainly because they are cleared exclusively by glomerular filtration (35, 36). Highly sensitive and specific immunoradiometric assays for intact PTH are now widely available (37) and are extremely useful when employed in the differential diagnosis of hypercalcemia.

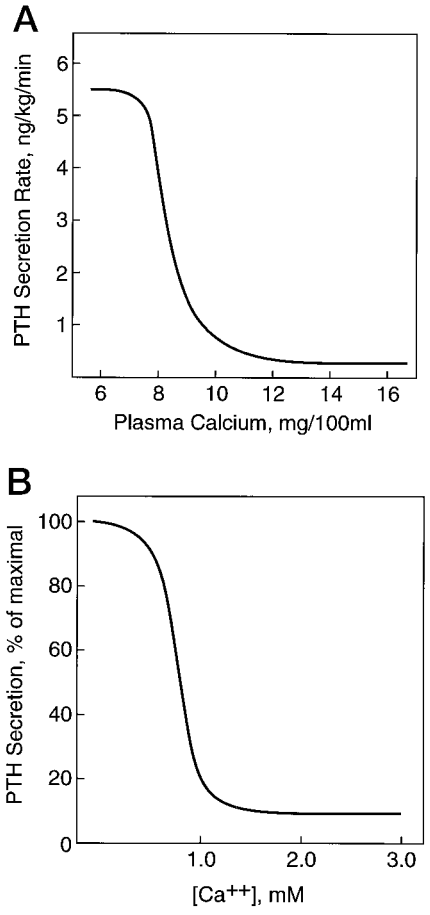

FIG. 2. PTH secretion as a function of calcium *in vivo* and *in vitro*. A, Secretory response of bovine parathyroid glands to induced alterations of plasma calcium. [Modified from G. P. Mayer and J. G. Hurst (11). © The Endocrine Society.] B, PTH secretion by dispersed parathyroid cells in culture as a function of extracellular calcium concentration. [Adapted from E. M. Brown, *Mineral and Electrolyte Metabolism* 8:130–150, 1982, with permission of S. Karger AG, Basel.]

2. Calcitonin. Plasma-ionized calcium concentration is the most important regulator of calcitonin secretion (38). Increases in plasma-ionized calcium result in an increase in calcitonin secretion and, conversely, a fall in the ambient calcium concentration inhibits calcitonin secretion. These changes are likely mediated through the calcium-sensing receptor, as the parafollicular cells of the thyroid gland express the same calcium-sensing receptor that is expressed in the parathyroid and kidney (39). Gastrointestinal peptide hormones, gastrin in particular, are potent calcitonin secretagogues. Although the physiological significance of this observation remains unclear, it is the basis for the pentagastrin stimulation test, a provocative test to determine the capacity of a patient to secrete calcitonin (40).

The precise biological role of calcitonin in the overall schema of calcium homeostasis is uncertain. Calcitonin directly inhibits osteoclastic bone resorption (41), and the effect is rapid, occurring within minutes of administration. This inhibition is accompanied by the production of cAMP (42), as well as an increase in cytosolic calcium (43) in the osteoclast, and results in contraction of the osteoclast cell membrane (44). These effects are transient and likely have little role in chronic calcium homeostasis. Clinical observations support this since neither calcitonin-deficient patients (athyroid) nor patients with medullary thyroid cancer and excess calcitonin production experience alterations in calcium homeostasis. The calcitonin receptor (45) is a G protein-coupled receptor with seven-transmembrane domains that is structurally similar to the PTH/PTHrP and secretin receptors. The half-life of calcitonin is measured in minutes and metabolism occurs predominantly in the kidney (38). Clinical abnormalities of calcitonin secretion include medullary thyroid carcinoma, small cell lung cancer, and carcinoids and islet cell tumors of the pancreas.

3. Calcitriol. The steroid hormone calcitriol or 1,25-$(OH)_2D_3$ is the major biologically active metabolite of the vitamin D sterol family. Vitamin D precursor (previtamin D_3) is either ingested in the diet or synthesized in the skin from 7-dehydrocholesterol through exposure to sunlight (46, 47). Hydroxylation occurs in the liver at the C-25 position to form 25-hydroxyvitamin D [25(OH)D], the precursor of the more potent metabolite, 1,25-$(OH)_2D_3$. 25(OH)D is hydroxylated at the C-1 position in the kidney by 1α-hydroxylase, a complex cytochrome P_{450} mitochondrial enzyme system located in the proximal nephron (48), to form 1,25-$(OH)_2D_3$ (49–51). The renal 1α-hydroxylation of 25(OH)D is the major recognized control point in vitamin D metabolism, responding to ambient phosphorus, PTH, and calcium concentrations. PTH and low serum phosphate concentrations independently increase 1,25-$(OH)_2D_3$ production, while hypercalcemia and 1,25-$(OH)_2D_3$ inhibit renal 1α-hydroxylase activity. Under physiological conditions, the kidney is the sole source of 1,25-$(OH)_2D_3$. The other known important extrarenal sites of 1,25-$(OH)_2D_3$ production are the placenta and granulomatous tissue (52–54). The half-life of 1,25-$(OH)_2D_3$ in the circulation is approximately 5 h in humans. Fifteen percent is excreted as urinary metabolites and 50% as fecal metabolites.

1,25-$(OH)_2D_3$ increases plasma calcium and phosphate concentrations by increasing the absorption of calcium and phosphate from the gastrointestinal tract (51). It also increases bone resorption (55) and enhances the capacity for PTH to promote renal tubular calcium reabsorption in the nephron. It is a powerful differentiation agent for committed osteoclast precursors (56, 57), causing their maturation to multinucleated cells that are capable of resorbing bone. Thus, 1,25-$(OH)_2D_3$ ensures a supply of calcium and phosphate available at bone surfaces for the mineralization of bone matrix. Deficiency of 1,25-$(OH)_2D_3$ or of 1α-hydroxylase results in osteomalacia or rickets, as does resistance to 1,25-$(OH)_2D_3$, caused by mutations in the vitamin D receptor (58–61). Although the function of other vitamin D metabolites has been unclear, recent evidence from mice deficient in the 24-hydroxylase gene indicate that such metabolites have a role in normal bone metabolism. Deficiency of 24-hydroxylase results in lack of the vitamin D metabolites hydroxylated at the 24 position and abnormal bone structure consisting of accumulation of osteoid at sites of intramembranous ossification (62).

C. Defenses against hyper- and hypocalcemia

The normal physiological defenses against hypercalcemia and hypocalcemia are listed in Table 1. The majority of these defense mechanisms are mediated through the hormonal actions of PTH and 1,25-$(OH)_2D_3$. Although the role of endogenous calcitonin is relatively modest in comparison to PTH and 1,25-$(OH)_2D_3$, pharmacological calcitonin therapy can be beneficial as discussed later.

PTH secretion increases in response to a fall in ionized calcium concentration. This results in 1) osteoclastic bone resorption and release of calcium and phosphate from bone into the extracellular fluid compartment; 2) renal tubular reabsorption of calcium and inhibition of phosphate uptake; and 3) synthesis of 1,25-$(OH)_2D_3$. If these mechanisms are intact, the extracellular calcium concentrations should return to normal.

In the converse situation, a rise in ionized calcium concentration results in decreased PTH secretion from the parathyroid glands. Thus, renal tubular calcium reabsorption is decreased, as is osteoclastic bone resorption. Synthesis of 1,25-$(OH)_2D_3$ and, subsequently, gastrointestinal absorption of dietary calcium and phosphate are decreased. Thus, the normal response to increases in ionized calcium is an increase in renal calcium excretion and a decrease in intestinal absorption of calcium.

In general, these hormonal responses are more effective in protecting against hypocalcemia than hypercalcemia. Perturbations in these mechanisms, as exemplified by excessive increases in bone resorption, deficiencies or excess of PTH or 1,25-$(OH)_2D_3$, and defects in renal capacity to handle calcium and phosphate, will result in either hypercalcemia or hypocalcemia.

III. Humoral Mechanisms by Which Solid Tumors Affect the Skeleton

A. Hypercalcemia

Hypercalcemia is defined as a total serum calcium, adjusted for protein concentration, above 10.2 mg/dl (2.55 mmol/liter) in adults (63). Ionized calcium is a more precise measure of calcium concentration, the normal plasma concentrations ranging from 1.12–1.23 mmol/liter (63). With the advent of automated biochemical testing, hypercalcemia is now recognized to be more common than once realized. By far, the most common causes of hypercalcemia are primary hyperparathyroidism and malignancy.

1. Clinical features of hypercalcemia. The clinical features of hypercalcemia are listed in Table 2. Symptoms may vary in individual patients and are related both to the absolute concentration of serum calcium and to the rate of rise in serum calcium. Symptoms also reflect the underlying cause of the hypercalcemia as well as intercurrent medical conditions. In older or critically ill patients, symptoms of hypercalcemia may be more prominent with relatively small increases in serum calcium concentration. Hypercalcemia most often results in neuromuscular, gastrointestinal, and renal manifestations. Severe hypercalcemia is likely the result of a vicious cycle. The hypercalcemic effects of anorexia, nausea, vomiting, and impaired renal concentrating ability lead to dehydration and, subsequently, altered mental status. This, in turn, may promote immobilization and lead to worsening hypercalcemia. In addition to the symptoms of hypercalcemia, clinical features of hypercalcemia of malignancy include signs and symptoms of the underlying cancer. Generally, the cancer is well advanced when hypercalcemia occurs, and the prognosis is poor. Survival beyond 6 months is uncommon (64–66).

2. Humoral mediators of hypercalcemia in malignancy. Malignancy is the most common cause of hypercalcemia in the hospitalized patient, and malignancy-associated hypercalcemia is one of the more common paraneoplastic syndromes. The relative frequencies of malignancies associated with hypercalcemia are listed in Table 3 (67). Hypercalcemia occurring in the setting of malignancy may be due to 1) humoral factors secreted by tumors that act systemically on target organs of bone, kidney, and intestine to disrupt normal calcium homeostasis; 2) local factors secreted by tumors in bone, either metastatic or hematological, which directly stimulate osteoclastic bone resorption; and 3) coexisting primary hyperparathyroidism.

It is probably more accurate to think of the first two situations as a continual spectrum rather than as discrete groups. The pathophysiology of hypercalcemia is very different in patients with solid tumors and no bone metastases at one end of the spectrum, and myeloma associated with extensive local bone destruction adjacent to the tumor cells at the other. However, in between these two extremes are hypercalcemic patients with squamous cell carcinomas in which hypercalcemia may occur with some, but not extensive, osteolytic bone metastases and hypercalcemic patients with advanced breast carcinoma in which hypercalcemia almost never occurs in the absence of extensive osteolytic bone destruction. Separating hypercalcemia into subcatego-

TABLE 1. Defenses against hypocalcemia and hypercalcemia

Organ	Hypocalcemia	Hypercalcemia
Parathyroid	↑ PTH secretion	↓ PTH secretion
Kidney	↓ GFR → ↓ filtered Ca^{++} ↑ Ca^{++} reabsorption ↑ $1,25(OH)_2D_3$	↑ GFR → ↑ filtered Ca^{++} ↓ Ca^{++} reabsorption ↓ $1,25(OH)_2D_3$
Gastrointestinal tract	↑ Ca^{++} absorption	↓ Ca^{++} absorption
Skeleton	↑ Bone resorption	↓ Bone resorption

TABLE 2. Clinical features of hypercalcemia

Neurologic and psychiatric	Lethargy, drowsiness Confusion, disorientation Disturbed sleep, nightmares Irritability, depression Hypotonia, decreased deep tendon reflexes Stupor, coma
Gastrointestinal	Anorexia, vomiting Constipation Peptic ulceration Acute pancreatitis
Cardiovascular	Arrhythmias Synergism with digoxin ? Hypertension
Renal	Polyuria, polydipsia Hypercalciuria Nephrocalcinosis Impaired glomerular filtration

TABLE 3. Malignancies associated with hypercalcemia (67)

Malignancy	Frequency (%)
Lung	35
Breast	25
Hematologic	14
Head and neck	6
Renal	3
Prostate	3
Unknown primary	7
Others	7

ries based on the assumption that the underlying mechanisms are distinct is not entirely satisfactory because the mediators may be identical except that in one situation it is a local mediator while in another it is a humoral mediator. Additionally, if the tumor burden in bone is great, local tumor-produced mediators of bone resorption may be produced in sufficient quantities to have systemic effects. Although this review will discuss the effects of cancer on bone organized into such subcategories, it is important that the reader understand that a significant portion of cancer patients will fall into the middle of this spectrum, having both humoral and local effects of tumor on bone.

a. PTHrP: Although cancer has been associated with hypercalcemia since the 1920s, it wasn't until 1941 that Fuller Albright first proposed the syndrome of ectopic hormone production in a patient with hypercalcemia, renal carcinoma, and a solitary bone metastasis (68). As the patient had normal parathyroid glands at the time of the neck operation, Albright postulated that the tumor produced PTH but when he had Collip assay the tumor for PTH, it was not detected. Thereafter, the syndrome was often referred to as "pseudohyperparathyroidism" or "ectopic hyperparathyroidism." When more sensitive PTH assays became available, it was clear that the offending factor was not PTH, but rather an immunologically distinct factor that had PTH-like biological activity (69).

Biochemical features of malignancy-associated hypercalcemia share some similarities to those seen in primary hyperparathyroidism (1°HPT). Specifically, in addition to the hypercalcemia, hypophosphatemia can occur (depending on the patient's renal function) as well as hypercalciuria, hyperphosphaturia, and increased excretion of nephrogenous cAMP (69). Plasma-intact PTH concentrations are suppressed in this syndrome except in rare cases of ectopic PTH production or concomitant primary hyperparathyroidism. Plasma 1,25-$(OH)_2D_3$ concentrations are generally low except in certain hematological malignancies when the humoral mediator is 1,25-$(OH)_2D_3$.

Almost 50 yr after Albright's observations, this PTHrP was purified from human lung cancer (70), breast cancer (71), and renal cell carcinoma (72) simultaneously by several independent groups and was cloned shortly thereafter (73). It is now evident that PTHrP, and not PTH, is a major mediator of humoral hypercalcemia of malignancy (74, 75), although four cases of authentic tumor-produced PTH have been reported (76–79). PTHrP has 70% homology to the first 13 amino acids of the N-terminal portion of PTH (73), binds to PTH receptors (80), and shares similar biological activity to PTH (81). Specifically, it stimulates adenylate cyclase in renal and bone systems (71, 72, 81–83), increases renal tubular reabsorption of calcium and osteoclastic bone resorption (82, 83), decreases renal phosphate uptake (81, 82, 84), and stimulates 1α-hydroxylase (81). PTHrP has been found in a variety of tumor types associated with hypercalcemia including squamous, breast, and renal carcinoma (85, 86). Although the majority of squamous cell carcinomas produce PTHrP (87), the capacity to cause hypercalcemia may depend on the level of PTHrP gene expression, which in turn may be determined by differential transcription of the PTHrP gene promoters (88). The regulation of PTHrP is complex, and factors such as PRL (89), epidermal growth factor (EGF) (90–93), insulin (91), IGF-I (91, 94) and II (91), TGFα (95), TGFβ (93, 96–98), angiotensin II (99), stretch (100), and the *src* protooncogene (101) have been shown to increase expression, while glucocorticoids (91, 94, 102, 103) and 1,25-$(OH)_2D_3$ (90, 91) decrease it. Estrogen has been shown to increase PTHrP expression in uterine tissue, and *in vitro* studies suggest that an estrogen response element is present in the PTHrP gene (104, 105). Mutations in codons 248 and 273 of the p53 tumor suppressor gene repress PTHrP gene expression in some squamous cell carcinomas (106). The cell death inhibitor, Bcl-2, is downstream in a signaling pathway that is required for normal skeletal development (107).

The human PTHrP gene is much larger and more complex than the human PTH gene. It spans approximately 15 kb of genomic DNA and has nine exons and three promoters. Three PTHrP isoforms of 139, 141, or 173 amino acids as well as multiple PTHrP mRNA species exist (93). There is considerable sequence homology across species up to amino acid 111 (108). Cell line-specific utilization of the promoters and of the 3′-alternative splicing pathways among bone, breast, kidney, and lung cell lines have been demonstrated (93). In this study, dexamethasone decreased while EGF and TGFβ increased abundance of each of the alternative mRNA species. Furthermore, EGF treatment increased transcription from promoters 1 and 2 and stabilized exon VII- and IX-containing transcripts in various cell lines (93).

Like PTH and other endocrine peptides, PTHrP undergoes

endoproteolytic posttranslational processing that results in several secretory forms: 1) an amino-terminal PTHrP-(1–36); 2) a midregion species that begins at amino acid 38 that has an undefined carboxyl terminus (109, 110); and 3) a carboxyl-terminal species that is recognized by an antibody directed against the 109–138 region (110–112). The preponderance and arrangement of basic residues in the protein sequence suggest that members of the subtilisin family of endoproteases, such as furin (113), PC 1/3, PC-2, PACE-4, and PC8 (114), are responsible for such processing (109, 115, 116). Posttranslational modification of PTHrP also occurs as glycosylation of an amino-terminal PTHrP species produced by keratinocytes has been reported (117). The subject of posttranslational processing of PTHrP, as well as the receptor and signal transduction pathways employed by the mature secretory forms of PTHrP, has been extensively reviewed by Orloff *et al.* (115). Regulation of PTHrP secretion may be cell specific as PTHrP expressed in neuroendocrine cells is secreted in a regulated fashion as compared with a constitutive secretion when expressed in nonneuroendocrine cell types such as squamous cell carcinoma (116). Although PTHrP mediates its calcemic effects through the classic PTH/PTHrP receptor, there is evidence for a separate PTHrP receptor (118). However, the function of such a receptor remains unclear.

PTHrP has been detected in a variety of tumor types as well as in normal tissue (85, 86). The widespread expression of PTHrP in normal tissue was the first evidence that the hormone had a role in normal physiology. In addition to the PTH-like effects, emerging work testifies to the fact that PTHrP has an important role in normal physiology. Such a topic is beyond the scope of this review, but suffice it to say that PTHrP appears to be important in 1) the regulation of cartilage differentiation and bone formation through endochondral ossification (119–121); 2) growth and differentiation of skin (122), mammary gland (123, 124), and pancreatic islets (125); 3) cardiovascular function (126); 4) transepithelial calcium transport in the distal nephron, mammary epithelia, and the placenta (124, 127); 5) relaxation of smooth muscle in uterus, bladder, arteries, stomach, and ileum (99, 128–130); and 6) host immune function (131–133). Bone perichondrial cell production of PTHrP regulates cartilage cell differentiation and has been linked to expression of Indian Hedgehog gene (134). Indian Hedgehog protein expressed by prehypertrophic cartilage cells inhibited cartilage differentiation, and this inhibitory effect was mediated by PTHrP. The normal physiological functions of PTHrP have been extensively reviewed elsewhere (135, 136).

The role of PTHrP in normal breast physiology (137, 138) sheds light on its potential importance in the pathophysiology of hypercalcemia and bone metastasis associated with breast cancer, which is discussed in later portions of this review. PTHrP is expressed in lactating mammary tissue (139) and secreted into milk at concentrations 10,000–100,000 times greater than plasma concentrations of humans with malignancy-associated hypercalcemia (140–144). Increased plasma PTHrP concentrations have been documented in at least two patients with the rare syndrome of lactational hypercalcemia (145–147) in addition to some breast-feeding mothers (148). Thus, PTHrP may be responsible for mobilizing calcium from maternal bone for use in milk production, and it may mediate lactation-associated bone loss.

In addition to the diverse and accumulating normal physiological functions of PTHrP, it likely has a multifunctional role in cancer as well. Such identified functions include 1) mediating hypercalcemia; 2) aiding in the development and progression of osteolytic bone metastasis in breast cancer; 3) regulating growth of cancer cells (149–151); and 4) acting as a cell survival factor (107).

The hypercalcemia of malignancy syndrome was the first identified consequence of the PTHrP effects in cancer. In this syndrome, tumor-produced PTHrP interacts with PTH receptors in bone and kidney to cause hypercalcemia, osteoclast-mediated bone resorption, and increased nephrogenous cAMP and phosphate excretion. The PTH-like properties of PTHrP, and specifically increasing osteoclastic bone resorption and renal tubular calcium reabsorption, appear to be responsible for the hypercalcemia. Approximately 80% of hypercalcemic patients with solid tumors have detectable or increased plasma PTHrP concentrations (111). Plasma PTHrP concentrations, as measured by a sensitive two-site immunoradiometric assay, are low or undetectable in the plasma of normals (152), but as is the situation with PTH, the C-terminal fragment is increased in patients with chronic renal failure (111). In fact, the plasma C-terminal PTHrP concentration increases as the glomerular filtration rate decreases (112).

i. Humoral hypercalcemia of malignancy (HHM) vs. 1°HPT. Despite similarities between HHM and 1°HPT and the similar biological actions of PTHrP and PTH, respectively, unexplained differences between these syndromes exist. First, patients with PTHrP-mediated HHM have low serum concentrations of 1,25-$(OH)_2D_3$ compared with patients with 1°HPT (153), even though both proteins stimulate renal 1α-hydroxylase activity. Clinical studies in which normal humans received short-term infusions of PTHrP-(1–34) (154) or PTHrP-(1–36) (155) revealed increased serum 1,25-dihydroxyvitamin D concentrations comparable to those who had received a similar infusion of PTH-(1–34). Animal studies have revealed similar findings (156, 157). Female nude mice infused with synthetic PTHrP-(1–40) for 7 days developed hypercalcemia, hypophosphatemia, and increased serum 1,25-dihydroxyvitamin D concentrations (157). Likewise, male nude mice bearing Chinese hamster ovarian (CHO) cell tumors transfected with the cDNA for human prepro-PTHrP or prepro-PTH developed similar hypercalcemia and increased plasma concentrations of 1,25-dihydroxyvitamin D when compared with control animals bearing untransfected CHO tumors (158). Additionally, similar increases in blood ionized calcium and 1,25-dihydroxyvitamin D concentrations were observed in nude mice bearing CHO tumors that were engineered to secrete PTHrP mutants truncated at the carboxyl terminus (156).

Second, human studies using either quantitative bone histomorphometry (159) or biochemical markers of bone turnover (160) have demonstrated that although patients with either HHM or 1°HPT have increased osteoclastic bone resorption, many patients with HHM do not have the coupled increase in osteoblastic bone formation that those with 1°HPT have. Serum osteocalcin concentrations, a marker for

bone formation, were significantly increased in patients with 1°HPT compared with normals (161). In the same study, serum osteocalcin concentrations in hypercalcemic patients with bone metastasis were significantly lower compared with those of normal controls, while normocalcemic patients with bone metastases had values similar to normal humans. These osteocalcin concentrations correlated with histomorphometric parameters of bone formation but not bone resorption (161). In the studies by Fraher *et al*. (154) and Everhart-Caye *et al*. (155), in which normal humans received infusions of PTH or PTHrP, bone histomorphometry or biochemical markers of bone turnover were not measured. Such studies done with PTHrP infusions in rodents have revealed increased osteoclastic bone resorption, as well as increased bone formation, as assessed by dynamic bone histomorphometry (157). In contrast, nude mice bearing a PTHrP-secreting human squamous cell carcinoma demonstrated increased bone resorption and decreased bone formation as assessed by dynamic bone histomorphometry (162). Thus, whether PTHrP alone is responsible for the uncoupling of bone formation from bone resorption is an issue that remains controversial.

Unlike the metabolic acidosis seen in patients with primary hyperparathyroidism, patients with malignancy-associated hypercalcemia often have a metabolic alkalosis with a low plasma chloride and high plasma bicarbonate concentration. Although many explanations have been postulated for the discrepancies between HHM and 1°HPT, such as differences between the pulsatile secretion of PTH and the presumed continuous secretion of PTHrP, suppression of bone formation and 1α-hydroxylase activity by other tumor-associated factors, biologically active PTHrP fragments, or hypercalcemia *per se* (163), the reasons for these differences have not been adequately elucidated.

ii. Modulation of PTHrP effects by other tumor-associated factors. Regardless of the reasons for the clinical differences between HHM and 1°HPT, there is clear evidence that other tumor-produced factors can modulate the end-organ effects of PTHrP as well as its secretion from tumors. Using an *in vivo* model of PTH and PTHrP-mediated hypercalcemia, Uy *et al*. (164) demonstrated that both proteins, when produced by tumors in which the corresponding genes were transfected and then inoculated into nude mice, caused similar hypercalcemia as well as increases in osteoclastic bone resorption, more committed marrow mononuclear osteoclast precursors, and mature osteoclasts. No stimulatory effects were seen on the multipotent osteoclast precursors, the granulocyte/macrophage colony-forming unit. In a similar model system, IL-6 potentiated the hypercalcemia and bone resorption mediated by PTHrP *in vivo* by stimulating production of early osteoclast precursors (165). Likewise, TGFα has been shown to enhance the hypercalcemic effects of PTHrP in an animal model of malignancy-associated hypercalcemia (166) as well as to modulate the renal and bone effects of PTHrP (167, 168). Sato *et al*. (169) demonstrated that IL-1α and PTHrP may have synergistic effects *in vivo*, and others have shown that IL-1 may modulate the renal effects of PTHrP (170). Finally, Uy *et al.* have demonstrated that tumor necrosis factor-α (TNFα) enhanced the hypercalcemic effect of PTHrP by increasing the pool of committed osteoclast progenitors with a subsequent increase in osteoclastic bone resorption. Bone formation parameters in these nude mice indicate that TNFα did not inhibit the new bone formation stimulated by PTHrP (171).

Such tumor-associated factors also appear to be important regulators of PTHrP expression and secretion by tumors. EGF has been shown to stimulate PTHrP expression in a keratinocyte cell line (172) as well as a mammary epithelial line (91) while TGFα enhanced PTHrP expression in a human squamous cell carcinoma of the lung (173). Interleukin-6 (IL-6), TNF, IGF-I, and IGF-II increased the production of PTHrP *in vitro* by a human squamous cell carcinoma (94). TGFβ, which is abundant in bone, released in active form by resorbing bone and expressed by some breast cancers (174, 175) and cancer-associated stromal cells (176), has been shown to enhance secretion of and stabilize the message for PTHrP in a renal cell carcinoma (96) as well as in a squamous cell carcinoma (97, 98). Other data (177, 178) demonstrate that this relationship also exists in a human breast adenocarcinoma cell line, MDA-MB-231.

iii. PTHrP in hypercalcemia associated with breast cancer. Hypercalcemia in breast cancer represents a special situation. Although it is clear that the predominant way in which breast cancer affects bone is through metastatic mechanisms, there is sufficient evidence to support the notion that breast cancers may secrete factors that act systemically to stimulate osteoclastic bone resorption and to increase renal tubular reabsorption of calcium (179–181). Hypercalcemia is associated with breast cancer, occurring in approximately 10% of afflicted women during the course of their disease (182). It is likely more common in those with advanced breast cancer. Osteoclast-mediated skeletal destruction by metastatic tumor is a major mechanism responsible for hypercalcemia, as increased osteoclastic bone resorption has been documented histologically in areas surrounding breast cancer metastases (183–186). However, humoral mechanisms may contribute in 10–60% of cases of breast cancer-associated hypercalcemia as evidenced by increased nephrogenous cAMP and plasma PTHrP in some patients (186–190).

PTHrP is clearly a significant factor in mediating hypercalcemia in breast cancer (191). Since PTHrP is expressed in normal breast tissue and appears to play an important role in normal breast physiology, its overproduction in breast cancer is not surprising. One of the three tumors from which PTHrP was originally purified was a breast cancer from a patient with humoral hypercalcemia of malignancy (70). PTHrP was detected by immunohistochemical staining in 60% of 102 invasive breast tumors removed from normocalcemic women, but not in normal breast tissue (192). At least four other studies have confirmed these percentages (193–196), and one of these has demonstrated immunoreactive PTHrP within the cytoplasm of lobular and ductal epithelial cells in normal and fibrocystic breast tissues (193). Furthermore, 65–92% of hypercalcemic breast cancer patients (with and without bone metastasis) had detectable plasma PTHrP concentrations by RIA similar to those documented in patients with humoral hypercalcemia of malignancy due to nonbreast tumors (180, 195). Not only is PTHrP an important mediator of hypercalcemia in breast cancer, it may have a significant role in the pathophysiology of breast cancer me-

tastasis to bone as evidenced by the clinical studies indicating that PTHrP expression by the primary breast cancer is more commonly associated with the development of bone metastasis and hypercalcemia (195). This topic will be discussed in a later section.

iv. PTHrP in hypercalcemia associated with hematological malignancies. The mechanisms responsible for hypercalcemia associated with hematological malignancies are multifactorial and include secretion of local bone-active cytokines, such as IL-6, IL-1, and lymphotoxin or TNFβ, from tumor in bone or from systemic effects of tumor-produced factors such as 1,25-$(OH)_2D_3$ (discussed below). Recent data from a clinical study of 76 patients with various hematological malignancies demonstrate that PTHrP also may be an important pathogenetic factor in the development of hypercalcemia in some patients (197). In this study, eight of the 14 hypercalcemic patients had non-Hodgkin's lymphoma and, of these, 62% had significant increases in plasma PTHrP concentrations. The serum 1,25-$(OH)_2D_3$ concentrations, when measured, were low in the hypercalcemic non-Hodgkin's lymphoma patients who had increased plasma PTHrP concentrations (197). Additionally, one of two hypercalcemic patients with Hodgkin's disease and one of four hypercalcemic patients with multiple myeloma had increased plasma PTHrP concentrations. Also of interest in this study is the fact that several normocalcemic patients with non-Hodgkin's lymphoma, Hodgkin's lymphoma, multiple myeloma, and Waldenstrom's macroglobulinemia had increased plasma PTHrP concentrations as measured by an amino-terminal PTHrP assay (197). Using a sensitive two-site immunoradiometric assay, other investigators have noted increased plasma PTHrP concentrations in patients with adult T cell leukemia and B cell lymphoma (198). Finally, circulating concentrations of PTHrP, comparable to those in humoral hypercalcemia of malignancy, were present in two of four hypercalcemic patients with non-Hodgkin's lymphoma, in three of nine with myeloma (199), and in a patient with myeloid blast crisis of chronic myeloid leukemia (200). Thus, the humoral mediators in the hypercalcemia associated with hematological malignancies include both 1,25-$(OH)_2D_3$ and PTHrP.

b. 1,25-$(OH)_2D_3$: In the setting of hypercalcemia, serum concentrations of 1,25-$(OH)_2D_3$ are normally suppressed unless an autonomous source of PTH is the cause, such as a parathyroid adenoma. Lack of 1,25-$(OH)_2D_3$ suppression in this situation is evidence of disordered regulation of 1,25-$(OH)_2D_3$ synthesis and indicates extrarenal production such as that observed in the hypercalcemia associated with granulomatous disease. Less commonly, tumors may secrete other humoral factors responsible for hypercalcemia. A major mediator of hypercalcemia in Hodgkin's disease, non-Hodgkin's lymphoma, and other hematological malignancies appears to be extrarenal production of 1,25-$(OH)_2D_3$ (201). The mechanism is similar to that observed in hypercalcemia associated with granulomatous disease in which activated macrophages within the granuloma synthesize 1,25-$(OH)_2D_3$ (52, 202, 203). In this scenario, patients usually have increased plasma 1,25-$(OH)_2D_3$ concentrations in addition to low or normal plasma PTH and urinary cAMP concentrations (204) in the absence of bone involvement. In similar studies, affected patients have also been shown to have increased fasting urinary calcium excretion (204) as well as increased intestinal calcium (^{47}Ca) absorption (205). Increased 1,25-$(OH)_2D_3$ concentrations were noted in 12 of 22 hypercalcemic patients with non-Hodgkin's lymphoma. In addition, 71% of 22 normocalcemic patients with non-Hodgkin's lymphoma were hypercalciuric, and 18% had increased serum 1,25-$(OH)_2D_3$ concentrations. These findings led the investigators to conclude that dysregulated 1,25-$(OH)_2D_3$ production is common in patients with diffuse large cell lymphoma (201).

Thus, the mechanisms responsible for hypercalcemia in this setting appear to be multifactorial and include increased intestinal calcium absorption as well as increased osteoclastic bone resorption. Additionally, many of the reported patients had altered renal function, a finding that suggests that impaired renal calcium clearance may also be contributing to the hypercalcemia in certain patients. The low serum PTH and urinary cAMP concentrations indicate that neither PTH nor PTHrP mediates the hypercalcemia in this setting. Prostaglandins, when measured, have been low, and selected patients had no calcium-lowering effect from indomethacin therapy (199). It is likely that the lymphoma tissue itself hydroxylates 25-hydroxyvitamin D to the active 1,25-$(OH)_2D_3$ similar to the situation in hypercalcemia associated with granulomatous disease (52, 203). One α-hydroxylase activity has been demonstrated in human T cell lymphotrophic virus type I-transformed lymphocytes (206). None of the reported patients with 1,25-$(OH)_2D_3$-mediated hypercalcemia had concomitant granulomatous disease, and hypercalcemia often improved with medical or surgical therapy that resulted in a decrease in serum 1,25-$(OH)_2D_3$ concentrations. Recurrence of hypercalcemia and increased plasma 1,25-$(OH)_2D_3$ concentrations has been documented with recurrence of disease (207).

c. PTH: After Fuller Albright's observations in 1941, it was postulated that malignancy-associated hypercalcemia was due to tumor production of PTH. This notion was strengthened by the early PTH RIA data in hypercalcemic patients with malignancy, which suggested that tumors produced factors recognized in these PTH RIAs (208–211). Although, for many years, malignancy-associated hypercalcemia was attributed to ectopic tumor-produced PTH, it is now clear that PTHrP is responsible for most cases. Analysis of 13 human and three animal nonparathyroid tumors of diverse origin associated with hypercalcemia did not detect PTH RNA transcripts (212). Since that time, four cases of authentic tumor-produced PTH have been convincingly demonstrated in a small cell carcinoma of the lung (78), an ovarian cancer (77), a widely metastatic primitive neuroectodermal tumor (76), and a thymoma (79). Molecular analysis of the ovarian carcinoma revealed both DNA amplification and rearrangement in the upstream regulatory region of the PTH gene (77). Interestingly, the primitive neuroectodermal tumor produced both PTH and PTHrP that resulted in severe hypercalcemia (76). These reported patients did not have coexisting primary hyperparathyroidism since the parathyroid glands were normal at the time of neck exploration or at autopsy in all cases. However, the fact remains that ectopic production of PTH is a rare event, and it is clearly docu-

mented that most patients with malignancy-associated hypercalcemia have suppressed plasma PTH concentrations (69). It should be emphasized that the most likely cause of hypercalcemia in the setting of malignancy that is associated with a normal or increased serum PTH concentration is coexisting primary hyperparathyroidism.

d. Other tumor-associated factors: There is accumulating evidence that solid tumors may produce other factors, alone or in combination with PTHrP, that have the capacity to stimulate osteoclastic bone resorption and cause hypercalcemia (213). These factors include IL-1, IL-6, TGFα, and tumor necrosis factor (TNF). Administration of IL-1 injections to mice caused mild hypercalcemia (214, 215), and this IL-1-induced hypercalcemia has been effectively blocked by the IL-1 receptor antagonist (216). Mice bearing CHO tumors transfected with the cDNA for IL-6 developed mild hypercalcemia (217) as did mice bearing a renal carcinoma that cosecreted IL-6 and PTHrP (218). Human TGFα and TNFα have been demonstrated to stimulate osteoclastic bone resorption in vitro and cause hypercalcemia *in vivo* (219–224). TNFα also caused hypercalciuria, without an increase in nephrogenous cAMP, and increased osteoclastic bone resorption *in vivo* in a mouse model (225). In addition, as noted in the previous section, some of these factors have been shown to modulate the end-organ effects of PTHrP on bone and kidney. In some instances, factors such as TGFα, IL-1, IL-6, and TNF enhance the hypercalcemic effects of PTHrP. The ability of IL-6 to enhance PTHrP-mediated hypercalcemia appears to be due to increased production of the early osteoclast precursor, granulocyte macrophage colony forming units, by IL-6 in combination with increased production of the more committed osteoclast precursors stimulated by PTHrP (165). Figure 3 summarizes the known effect of various tumor-produced factors on stages of the osteoclast lineage as determined in bone marrow cultures from mice treated with respective factors (164, 165, 171, 226).

Prostaglandins of the E series are powerful stimulators of bone resorption (227) although their role in bone destruction associated with malignancy remains unclear (228). Some of the effects of cytokines on bone may be mediated in part through prostaglandins as indomethacin, a prostaglandin synthesis inhibitor, has been shown to block part of the osteoclast-stimulatory effects of IL-1 *in vivo* (214, 215). Although prostaglandins have been demonstrated to be produced by cultured tumor cells *in vitro*, indomethacin treatment of malignancy-associated hypercalcemia is only occasionally effective (229). Thus, it is unlikely that prostaglandins have a major causal role in hypercalcemia associated with malignancy.

3. Treatment of hypercalcemia associated with malignancy. Treatment of hypercalcemia due to malignancy should always involve treating the underlying tumor. Unfortunately, since this is often not effective or cannot be accomplished with the rapidity needed when the patient is faced with life-threatening hypercalcemia, therapy should also be directed against the mechanisms responsible for the hypercalcemia. In essentially all patients with hypercalcemia of malignancy, there is an increase in osteoclastic bone resorption, and in many there is also an increase in renal tubular calcium reabsorption, even in malignancies that are not associated with PTHrP production (230). Medical therapy is therefore aimed at inhibiting bone resorption and promoting renal calcium excretion. Because hypercalcemia associated with cancer is often accompanied by dehydration, volume expansion with isotonic saline is essential. This serves to increase the glomerular filtration rate and reduces the fractional reabsorption of both sodium and calcium. Since hydration alone will normalize serum calcium concentrations only transiently (231), inhibitors of bone resorption such as the bisphosphonates or calcitonin should be administered as well. When possible, mechanism-specific treatment should be attempted (232). Glucocorticoids, for example, are more effective in reducing the serum calcium concentration in hematological malignancies and 1,25-$(OH)_2D_3$-mediated hyper-calcemia than in solid tumors. Dietary calcium restriction is ineffective in reducing serum calcium concentrations except in cases of vitamin D-mediated hypercalcemia. In these cases, dietary calcium should be restricted to 400 mg daily until the underlying disorder is corrected. It is not desirable or advantageous to reduce calcium intake in hypercalcemia due to malignancy.

Bisphosphonates, analogs of pyrophosphate, have become the most useful antiresorptive agents among the currently available armamentarium for the treatment of hypercalcemia. They have a high affinity for hydroxyapatite in bone and concentrate in areas of high bone turnover. The mechanisms by which bisphosphonates inhibit bone resorption are not clearly understood, but potentially include induction of osteoclast apoptosis, inhibition of osteoclast formation and recruitment, or stimulation of osteoblasts to produce an inhibitor of osteoclast formation (233, 234). Another mechanism by

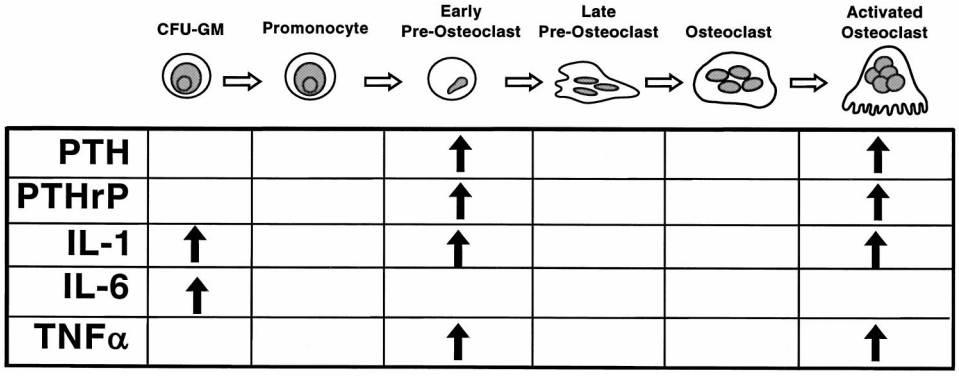

FIG. 3. Effect of tumor-produced factors on cells of the osteoclast lineage *in vivo* (161, 162, 168, 219). Factors were administered to mice via tumor production or injection.

which bisphosphonates might affect bone resorption is by decreasing the function of the osteoclast with respect to attachment and ruffled border formation (233). Recent *in vitro* findings using rodent marrow cultures suggest that tyrosine phosphatase activity is important in osteoclast formation and function and is a potential molecular target of bisphosphonate action (235). Bisphosphonates also inhibit axenic growth of amoebe of the slime mold *Dictyostelium discoideum*, and this property of growth inhibition paralleled the potency of inhibition of bone resorption (236). These findings indicate that bisphophonates may have a mechanism of action that is similar in both the osteoclast and *Dictyostelium discoideum*.

Bisphosphonates vary in potency but, in general, are poorly absorbed and are most effective in treating hypercalcemia when given intravenously. Bisphosphonates are concentrated in bone and remain there until the bone is resorbed. Etidronate, the first available bisphosphonate in the United States, is the least potent. Intravenous etidronate, given in doses of 7.5 mg/kg iv over 3 consecutive days normalized calcium concentration in 30%–40% of patients (237–239). Oral etidronate is generally ineffective in treating hypercalcemia (229), and at sustained dosages of 25 mg/kg per day for more than 6 months, it can cause bone mineralization defects (234). Etidronate can also cause hyperphosphatemia which, in addition to hypercalcemia, may lead to a high calcium-phosphate solubility product.

Pamidronate is a potent aminobisphosphonate available for the treatment of hypercalcemia of malignancy. The drug combines high efficacy with low toxicity profile and thus has become the current bisphosphonate of choice for the treatment of hypercalcemia of malignancy. It is highly effective in normalizing serum calcium concentrations and, when used in dosages recommended for hypercalcemia of malignancy, is not associated with bone mineralization defects. Pamidronate, administered as a single 24-h infusion, normalized serum calcium concentrations in 30% of patients who received 30 mg, 61% of patients who received 60 mg, and 100% of patients who received 90 mg (66). Successful therapy with bisphosphonates is associated with an increase in the plasma PTH and 1,25-$(OH)_2D_3$ concentrations as well as a decrease in the biochemical markers of bone resorption (66, 153, 190). Clinical studies of pamidronate treatment in patients with hypercalcemia of malignancy indicate that the calcium-lowering response to bisphosphonates correlates positively with the presence of bone metastases (240–242) and correlates negatively with plasma PTHrP concentrations (65, 240, 241). Such a relationship has also been demonstrated with clodronate, an oral bisphosphonate (243). This is presumably due to the effects of PTHrP to increase renal tubular reabsorption of calcium, which are not blocked by bisphosphonates. Nonetheless, pamidronate compares favorably to other inhibitors of bone resorption such as plicamycin, calcitonin, and gallium nitrate and is well tolerated. Pamidronate should be delivered as an intravenous infusion over 4–24 h. Clinical studies using 90 mg infusion of pamidronate over 4 h indicate that the mean time to achieve normocalcemia is approximately 4 days while the mean duration of normocalcemia is 28 days (244). Similarly, intravenous pamidronate, 60 mg every 2 weeks, maintained normocalcemia in a majority of patients with malignancy-associated hypercalcemia (245). An effective method for achieving more rapid reduction of the serum calcium is to use the combination of calcitonin and pamidronate (246). Calcitonin acts rapidly to lower the serum calcium, although usually its effects are only transient. Although escape from calcitonin therapy may occur within 48 h, by that time pamidronate is beginning to exert its maximal effects. Calcitonin can be administered either intramuscularly or subcutaneously every 12 h in doses of 200–400 MRC units. Reported side effects of pamidronate include transient low-grade fever and asymptomatic mild hypocalcemia (66). Bone mineralization defects have been reported only in patients receiving high-dose pamidronate at weekly intervals for the treatment of Paget's disease (247). It is possible that other bisphosphonates, such as alendronate, risedronate, and tiludronate, will be effective oral therapy for hypercalcemia of malignancy. Due to its propensity to cause mouth ulcers, oral pamidronate, although effective, is not likely to be approved for such use in the United States.

Calcitonin inhibits osteoclastic bone resorption and renal tubular reabsorption of calcium. The main advantages of calcitonin are its rapid onset of action and its relative lack of serious side effects. Unfortunately, calcitonin alone only transiently normalizes the calcium concentration in patients with hypercalcemia of malignancy. Tachyphylaxis, probably due to down-regulation of calcitonin receptors, frequently develops with calcitonin administration, although this can be delayed with concomitant glucocorticoid treatment (248). However, calcitonin use can be particularly effective in the setting of severe hypercalcemia while waiting for the more sustained hypocalcemic effect of administered bisphosphonates to occur. Calcitonin use with bisphosphonates lowers calcium concentrations more quickly and effectively than either alone (249). Although human calcitonin is available, salmon calcitonin is generally used. If salmon calcitonin is used, a test dose of 1 MRC unit should be administered first, since rare anaphylactic reactions have been reported (250).

Plicamycin, or mithramycin, an antineoplastic agent used in the treatment of certain embryonal cancers (251), is also a potent inhibitor of bone resorption. Plicamycin inhibits DNA-dependent RNA synthesis (252) in tumor cells by binding to the promoter regions on DNA, thus preventing transcription (253). Presumably, osteoclastic bone resorption is inhibited by this mechanism as well. The dosage used to treat hypercalcemia (25 μg/kg) is one-tenth of the usual chemotherapeutic dose and should be infused over 4 h. Although plicamycin is almost invariably effective in lowering serum calcium concentrations, its considerable toxicity has limited its use in more recent years as more potent bisphosphonates have become available. Plicamycin has serious hepato- and nephrotoxicity in addition to local irritation and thrombocytopenic effects, which can limit its use in cancer patients as well as in those with renal impairment.

Gallium nitrate is another antineoplastic agent that, like plicamycin, was found to induce hypocalcemia in normocalcemic cancer patients receiving it (254). It inhibits osteoclastic bone resorption and appears to be more effective in lowering serum calcium concentration than calcitonin (255) and etidronate. Gallium is administered as a continuous infusion over 5 days, making it somewhat less convenient than some other antihypercalcemic agents. Gallium is ex-

creted unchanged by the kidneys and has significant nephrotoxicity. Thus, it should not be administered to patients with renal impairment or to those receiving other nephrotoxic drugs (256).

About 30% of patients treated with glucocorticoids for hypercalcemia associated with nonparathyroid malignancy respond with a fall in calcium concentration (229). However, the response is often not complete and the responsiveness to glucocorticoids is unpredictable (229). Glucocorticoids are most effective in hypercalcemic patients with hematological malignancies, multiple myeloma in particular, as well as in vitamin D-associated disorders such as lymphomas. In hematological malignancies, glucocorticoids inhibit osteoclastic bone resorption by decreasing tumor production of locally active cytokines in addition to having direct tumorolytic effects (257). Glucocorticoids in dosage equivalents of prednisone, 40 to 60 mg daily, should be given for 10 days. If the calcium has not decreased in this period, glucocorticoids should be discontinued. Long-term adverse effects of glucocorticoids, such as osteopenia and Cushing's syndrome, occur with continued administration over several months. This is usually not a consideration in patients with widespread malignancy, who have a very limited prognosis.

B. Oncogenic osteomalacia

Oncogenic osteomalacia is a rare tumor-associated disorder, first recognized in 1947 (258, 259), that is characterized by hypophosphatemia, phosphaturia, normocalcemia, and osteomalacia in the absence of a family history of rickets, heavy metal poisoning, or Fanconi's syndrome. The serum alkaline phosphatase concentration is increased and the serum 1,25-$(OH)_2D_3$ concentration is decreased. Affected patients typically present with bone pain, proximal muscle weakness, and fractures. The disorder may manifest as rickets if it occurs before fusion of the growth plate. Tumors associated with this disorder are generally of mesenchymal origin, small, and benign, although it has occasionally been associated with malignant tumors. Reported tumor types include sclerosing hemangioma (260, 261), paraganglioma (262), prostate cancer (263, 264), oat cell carcinoma of the lung (265), fibrous dysplasia, hemangiopericytoma, osteosarcoma, chondroblastoma, chondromyxoid fibroma, malignant fibrous histiocytoma, giant cell tumor (266), and a metaphyseal fibrous defect (267). Oncogenic osteomalacia associated with metastatic prostate cancer comprises about 10% of all reported cases (264). Regardless of the origin, tumors causing oncogenic osteomalacia are often small and difficult to locate. Some reported locations include the groin, nasopharynx, and the popliteal region. Biochemical abnormalities resolve after complete tumor resection and recur with tumor regrowth. Serum phosphate concentrations increase immediately in the postoperative period while alkaline phosphatase concentrations may take more than 1 yr to normalize with healing of the osteomalacia (260, 262). In one patient, the bone mineral density measurement increased from a preoperative value of 0.627 g/cm^2 to 1.097 g/cm^2 198 days postoperative (262).

Phosphaturia may contribute to the osteomalacia in affected patients as does the apparent decrease in plasma 1,25-$(OH)_2D_3$ concentrations, both of which presumably lower the concentrations of available phosphate ions at the mineralizing bone site. The responsible phosphaturic factor has not been identified, but does not appear to be PTH or PTHrP since calcium and nephrogenous cAMP concentrations are normal in affected patients. Conditioned medium from cell culture of oncogenic osteomalacia tumors has been shown to inhibit phosphate uptake in cultured epithelial opossum kidney cells (260, 262). In one report, there was no measurable immunoactive PTH or PTHrP in the conditioned media from a paraganglioma (262). Another report found that conditioned media from a hemangioma inhibited phosphate uptake in opossum kidney cells without increasing cellular concentrations of cAMP. The media contained PTH-like immunoreactivity without PTHrP immunoreactivity, and the inhibition of phosphate transport was not blocked by a PTH antagonist (260). This putative factor appeared to be heat sensitive and of a molecular mass between 8 and 25 kDa (260).

Serum 1,25-$(OH)_2D_3$ concentrations are low in patients with oncogenic osteomalacia, despite the presence of hypophosphatemia, which normally increases 1,25-$(OH)_2D_3$ production by stimulating renal 1α-hydroxylase activity independent of PTH. Additionally, most reported cases of oncogenic osteomalacia have normal serum 25-hydroxyvitamin D concentrations. Deficient production of 1,25-$(OH)_2D_3$ could be a contributing factor to the pathogenesis of oncogenic osteomalacia in these patients as the clinical and biochemical abnormalities improve during calcitriol therapy in some patients. The pathophysiology of the vitamin D derangement is not well understood, but the clinical features have led investigators to hypothesize that tumor-produced factors inhibit 1α-hydroxylase activity. In one study, tumor extracts from a hemangiopericytoma inhibited the formation of 1,25-$(OH)_2D_3$, and transplantation of this tumor into athymic mice resulted in renal phosphate wasting and decreased 1,25-$(OH)_2D_3$ concentrations (268).

C. Tumor lysis syndrome

Another disorder of calcium homeostasis associated with malignancy is the tumor lysis syndrome, which may occur as a consequence of successful therapy of neoplastic disease (269). The syndrome often occurs during therapy of hematological malignancies, particularly high-grade lymphomas, in which a large number of tumor cells are lysed in a short period of time. It has also been reported during therapy of small cell carcinoma of the lung (270), breast cancer, and medulloblastoma as well as during immunotherapy for sarcoma (271) and therapy with TNFα and monoclonal antibody against GD3 ganglioside in metastatic melanoma (272). Rare reports of spontaneous tumor lysis have also been reported (273). The release of tumoral intracellular ions, such as phosphate and potassium, into the extracellular fluid result in hyperphosphatemia. Hypocalcemia, hyperuricema (due to uric acid release from lysed cells), and renal failure are a consequence.

IV. Local Mechanisms by Which Tumors Affect the Skeleton

It is clear that hypercalcemia associated with cancer conveys a poor prognosis, with survival of less than 3 months (64, 65). However, the majority of patients with metastatic bone disease are not hypercalcemic and, in the case of breast cancer, patients may survive up to 90 months after the detection of the first bone metastases (268, 274). Autopsy studies reveal bone metastasis in 70% of women who died of breast cancer (275–277). Thus, it behooves us to understand the mechanisms responsible for this complication of cancer to effectively decrease the associated morbidity or to prevent it altogether.

A. Clinical manifestations

Both solid tumors and hematological malignancies frequently affect the most vascular areas of the skeleton, specifically in the red bone marrow of the axial skeleton, the proximal ends of the long bones, ribs, and the vertebral bodies. The most common way in which cancer affects the skeleton is directly through local tumor-mediated stimulation of osteoclastic bone resorption. Such osteolytic bone lesions are typical of breast and lung carcinoma as well as hematological malignancies such as multiple myeloma. Although breast cancer cells have been shown to resorb bone directly *in vitro* (278), most evidence [scanning electron microscopic examination of adjacent bone surfaces (184) and response to osteoclast inhibitors] is consistent with the notion that factors secreted by cancer cells can activate osteoclasts locally. This is illustrated in Fig. 4, a photomicrograph of an osteolytic lesion due to the human breast cancer cell line MDA-MB-231. Thus, local and systemic effects of cancer on bone are mediated through one common final pathway, the osteoclast. The resulting osteolytic bone destruction can lead to pain, pathological fractures, nerve compression syndromes, and hypercalcemia.

Tumor in bone may stimulate new bone formation to result in osteoblastic bone metastasis. This is most often associated with prostate cancer, although it less frequently occurs in breast cancer and rarely in a sclerotic variant of myeloma (279) as well as in other malignancies. Osteoblastic metastases are also associated with bone pain and nerve compression syndromes, but unlike osteolytic metastases, this type of bone involvement can cause hypocalcemia (280, 281). Pathological fractures can occur with osteoblastic metastases as well as due to the intrinsically low strength of the new woven bone and/or concomitant osteolysis. In general, sites of pathological fractures commonly include the vertebral bodies and the proximal ends of the long bones. Spinal cord compression is a catastrophic event often associated with metastatic bone disease and can be due to tumor directly impinging on the spinal cord, fracture of vertebral body consumed by destructive osteolytic lesions, or bony overgrowth of osteoblastic lesions.

Hypercalcemia associated with metastatic bone disease or hematological malignancies has been referred to as local osteolytic hypercalcemia, as clinical studies have demonstrated that many patients with bone involvement and hypercalcemia did not have the increase in plasma PTHrP or nephrogeneous cAMP concentration observed in patients with humoral hypercalcemia of malignancy (69, 111). The mechanism of hypercalcemia in this situation was postulated to be local tumor production of factors that stimulate osteoclastic bone resorption, such as TNFβ, IL-6, and IL-1 as in the case of myeloma. However, it is now clear that humoral mediators of hypercalcemia, such as PTHrP, may mediate local osteolysis, even in the absence of hypercalcemia and increased plasma PTHrP concentrations (282). Additionally, if tumor burden in bone is great enough, tumor-produced

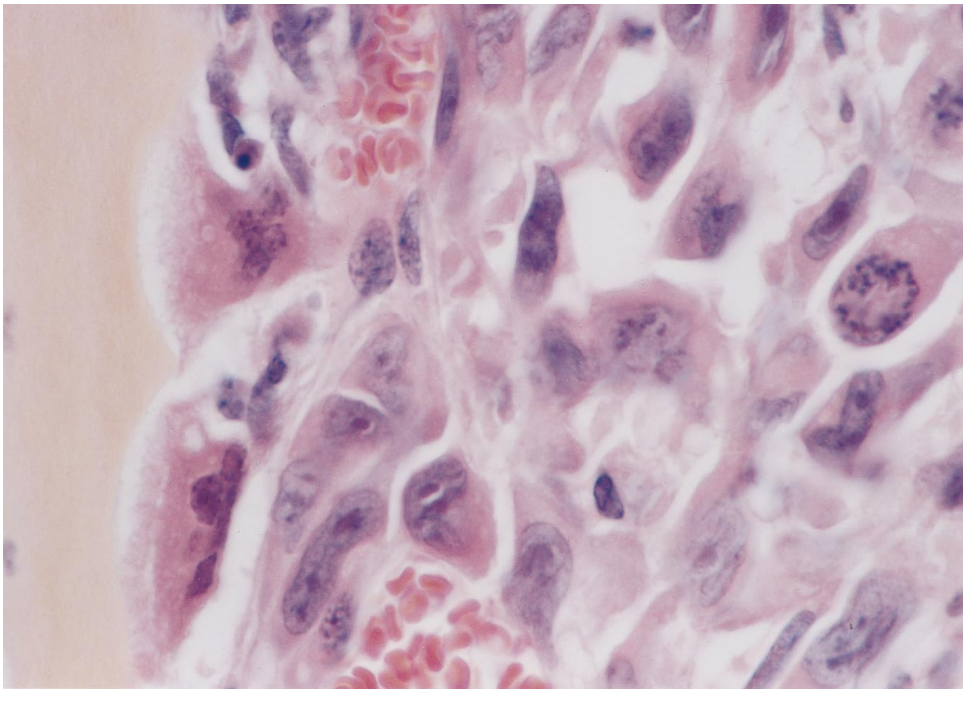

FIG. 4. Photomicrograph of an osteolytic bone lesion. The section was taken from an affected femur of a mouse inoculated into the left cardiac ventricle with the human breast cancer cell line MDA-MB-231. Radiographic appearance of the lesion appears in Fig. 6. Magnification 100×.

factors in bone may be produced in enough quantity locally to reach the systemic circulation and have effects on sites distant from affected bone.

B. Pathophysiology of the metastatic process to bone

Breast and prostate cancer are the most common malignancies in which bone metastases occur. Breast cancer is most often associated with osteolytic metastasis while osteoblastic metastases are more often manifest in prostate cancer. Mixed osteolytic and osteoblastic lesions are often evident in both breast and prostate cancer. The remainder of the review will focus on general principles of metastasis to bone, followed by mechanisms specific to osteolytic and osteoblastic metastasis, citing examples from current research in breast and prostate cancer, respectively. This will be followed by a review of bone involvement in myeloma. The reader should understand that breast and prostate cancer, although predominantly lytic and blastic, respectively, often have components of both osteolysis and osteosclerosis. In this review, delineation of osteolytic and osteoblastic mechanisms of bone metastasis to breast and prostate cancer, respectively, is by no means meant to be exclusive. It is likely that both mechanisms are often operative in the same patient.

1. Anatomical. Tumor metastasis to bone is not a random event, but rather a result of anatomical factors, tumor cell phenotype, and suitability of the metastatic site for tumor growth. Blood flow from the primary site is a significant determinant of the site of metastasis. Studies by Batson (283) describe in detail a low-pressure, high-volume system of valveless vertebral veins that communicate between the spine and intercostal veins independently of the pulmonary, caval, or portal systems. Batson accessed this plexus in cadavers via dye injection into the dorsal vein of the penis, an integral part of the prostatic venous plexus. Through these extensive injection studies of the prostatic plexus and venules of the breast in male and female cadavers, as well as in animals, Batson described this vertebral vein system as 1) consisting of the epidural veins, the perivertebral veins, the veins of the thoraco-abdominal wall, the veins of the head and neck, and the veins of the walls of blood vessels of the extremities; 2) valveless vessels that carry blood under low pressure; 3) subject to arrest and reversal of blood flow; 4) parallels, connects with, and provides bypasses for the portal, pulmonary, and caval systems. This plexus may serve as a major channel by which certain malignancies, such as prostate and breast cancer, metastasize to bone.

This concept that the vertebral system of veins acts as a direct conduit in the spread of prostatic carcinoma to the skeletal system was refuted by Dodds *et al.* (284), who analyzed ^{99m}technetium bone scans in patients with skeletal metastases from assorted primary tumors. They found that the distribution of metastases was virtually identical in patients with prostatic and nonprostatic tumors. Of the patients with prostatic carcinoma, 25% had bone scan lesions exclusively outside the region of the sacrum, pelvis, and lumbar spine. The distribution of skeletal metastases from prostatic carcinoma did not support the concept that the vertebral veins have a substantial role in the dissemination of this tumor.

2. Seed and soil. Regardless of whether or not blood flow or anatomic considerations are important determinants of the site of metastasis, they are not the only ones. The distribution of metastases to various organs are not predicted by anatomic considerations alone in approximately 40% of tumors (285). Thus, other determinants of the site of metastasis, such as properties of both the tumor cell and the metastatic site, are important. Metastasis is an extremely complex event that involves a cascade of linked sequential events that must be completed before a tumor cell successfully establishes a secondary tumor in bone (Fig. 5). Specifically, a tumor cell must 1) detach from the primary site; 2) enter tumor vasculature to reach the circulation; 3) survive host immune response and physical forces in the circulation; 4) arrest in distant capillary bed; 5) escape the capillary bed; and 6) proliferate in the metastatic site. The events involved in entering the tumor

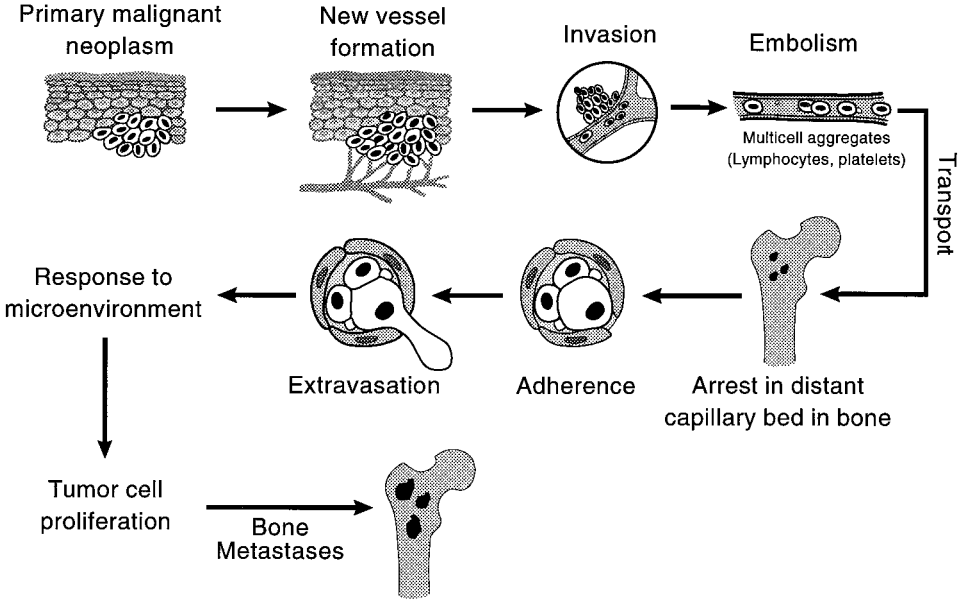

FIG. 5. General mechanisms of tumor cell metastasis to bone. Multiple steps involved in tumor cell metastasis from a primary site to the skeleton. Each of these steps represents a potential therapeutic target for the development of drugs to reverse or prevent metastatic bone disease.

vasculature are similar to those involved with exiting the vasculature in the bone marrow cavity. These include 1) attachment of tumor cells to the basement membrane; 2) tumor cell secretion of proteolytic enzymes to disrupt the basement membrane; and 3) migration of tumor cells through the basement membrane. Attachment of tumor cells to basement membranes and to other cells are mediated through cell adhesion molecules such as laminin and E-cadherin. Tumor cell secretion of substances such as metalloproteinases facilitate disruption of the basement membranes and enhance invasion. Inherent tumor cell motility or motility in response to chemotactic stimuli are also important factors for tumor cell invasion to the secondary site.

The fact that breast cancer is associated with significant morbidity in the skeleton was noted in 1889 when Stephen Paget observed that "in a cancer of the breast the bones suffer in a special way, which cannot be explained by any theory of embolism alone"(1). Indeed, breast cancer is one of a limited number of primary neoplasms that display osteotropism, an extraordinary affinity to grow in bone. Greater than 70% of women dying from breast cancer have bone metastasis (274–277). The mechanisms underlying this osteotropism are complex and involve unique characteristics of both the breast cancer cells and the bone to which these tumors metastasize.

Why is breast cancer one of the limited primary tumors to display osteotropism? Paget, during his observations of breast cancer in 1889, proposed the "seed and soil" hypothesis to explain this phenomenon. "When a plant goes to seed, its seeds are carried in all directions; but they can only grow if they fall on congenial soil" (1). In essence, the microenvironment of the organ to which the cancer cells metastasize may serve as a fertile soil on which the cancer cells (or seeds) may grow. Although this concept was proposed over a century ago, it remains a basic principle in the field of cancer metastasis at the present time. Thus, breast cancer cells possess certain properties that enable them to grow in bone, and the bone microenvironment provides a fertile soil on which to grow.

C. Local tumor syndromes in bone

Understanding the pathophysiology of bone metastasis has been a slow process scientifically, as very few useful animal models of spontaneous bone metastasis exist. Thus, various techniques of experimental bone metastasis have been used throughout the years and include injection of tumor cells directly into 1) the intramedullary cavity (286); 2) abdominal aorta (287); 3) tail vein with inferior vena cava occlusion (288); 4) left upper thigh muscle (289); 5) left thoracic artery with renal artery occlusion; and 6) left cardiac ventricle (290, 291). A complete review of the advantages and disadvantages of these models has been extensively discussed by Orr *et al.* (292).

1. Osteolytic metastases. Cancer metastatic to bone often causes bone destruction or osteolysis. Although several tumor types, such as prostate, lung, renal cell, and thyroid, are associated with osteolytic lesions, breast cancer is the most common. A comprehensive review of more than 500 patients dying of breast cancer revealed that 69% had bone metastasis, and bone was the most common site of first distant relapse (182). In those patients with disease confined to the skeleton, the median survival was 24 months compared with 3 months in those patients whose first relapse occurred in the liver. For these reasons, the following discussion on the pathophysiology of cancer-mediated osteolysis will focus on breast cancer.

a. Breast cancer cells as the seed: A variety of common characteristics are necessary for tumor cells to possess the metastatic phenotype. Such properties include 1) the production of proteolytic enzymes necessary for detachment from the primary site, invasion into surrounding soft tissues, intravasation, extravasation, and bone matrix degradation; 2) expression or loss of cell adhesion molecules essential for detachment from the primary site and arrest at a metastatic site; 3) migratory activity to travel in the circulation; 4) escape from the host immune surveillance to survive; and 5) capacity to respond to a chemoattractant. Although these properties are common to tumor cells metastasizing to any organ, they are insufficient to explain the propensity of breast cancer to metastasize to bone. Therefore, it is likely that breast cancer cells have additional characteristics that are specifically required for causing metastases in bone.

Since bone is mainly composed of a hard mineralized tissue, it is more resistant to destruction than other soft tissues. Thus, in order for cancer cells to grow in bone, they must possess the capacity to cause bone destruction. Histological review of breast cancer metastatic to bone reveals that tumor cells are adjacent to osteoclasts resorbing bones (184–186) and indicate that breast cancer cells possess the capacity to stimulate osteoclastic bone resorption. Breast cancer cells may either induce osteoclastic differentiation of hematopoietic stem cells, activate mature osteoclasts already present in bone, or do both, through releasing soluble mediators or via cell-to-cell contact. Clinical and experimental evidence indicates that tumor-produced PTHrP is a major candidate factor responsible for the osteoclastic bone resorption present at sites of breast cancer metastatic to bone (293–295). PTHrP has been detected by immunohistochemistry (293) and *in situ* hybridization (294) in 92% of breast cancer metastases in bone compared with only 17% of similar metastases to nonbone sites, an observation that prompted speculation that production of PTHrP as a bone-resorbing agent may contribute to the ability of breast cancers to grow as bone metastases. Bundred and colleagues (195) found positive immunohistochemical staining for PTHrP in 56% of 155 primary breast tumors from normocalcemic women, and PTHrP expression was positively correlated to the development of bone metastases and hypercalcemic episodes. PTHrP expression was detected by RT-PCR in 37 of 38 primary breast cancers, and subsequent development of bone metastases was associated with a higher PTHrP expression (295). Finally, PTHrP was detected by immunohistochemistry in 83% of patients who developed bone metastases compared with 38% in those who developed lung metastases and 38% in those without recurrence (196). There have been no consistent correlations between PTHrP expression in the primary breast tumor and standard prognostic factors, recurrence, or survival. The only significant and consistent

correlations have been between PTHrP positivity and the development of bone metastases and hypercalcemia.

These clinical observations have been extended by using a mouse model of bone metastases (290, 296) in which inoculation of a human breast cancer cell line, MDA-MB-231 (297), into the left cardiac ventricle reliably causes osteolytic metastases. MDA-MB-231 cells produce low amounts of PTHrP *in vitro* and when the cells were engineered to overexpress PTHrP, by transfection with the cDNA for human prepro-PTHrP, an increase in the number of osteolytic metastases was observed (178). In contrast, when mice were treated with monoclonal antibodies directed against the 1–34 region of PTHrP, before inoculation with parental MDA-MB-231 cells, the number and size of observed osteolytic lesions were dramatically less than similar animals treated with control (Fig. 6). Mice with established osteolytic metastases due to MDA-MB-231, treated with the antibody, had a decrease in the rate of progression of metastases when compared with mice that received a control injection (282, 298). Similar findings have been demonstrated in this model using a human lung squamous cell carcinoma (299). Taken together, these data strongly suggest that PTHrP expression by breast cancer cells is important for the development and progression of breast cancer metastases in bone. It stands to reason, then, that production of other osteoclast-stimulating factors should potentiate the development of bone metastases as well.

Just as production of bone-resorbing factors by breast cancer cells enhances bone metastases, production of other factors may render the breast cancer cell ineffective as a seed and result in less metastases in bone or other sites. Using the same mouse model of breast cancer metastases to bone, MDA-MB-231 cells transfected to overexpress either the cell adhesion molecule, E-cadherin, or tissue inhibitor of metalloproteinase-2 (TIMP-2) inoculated into nude mice resulted in a decrease in osteolytic metastases compared with nontransfected MDA-MB-231 cells (300, 301).

Cancer cell expression of factors affecting motility are important in the general metastatic process (302) as well in those processes specific to bone metastasis. Once tumor cells arrive in the bone marrow sinusoids, they must possess the capacity to move through those sinusoids to the bone tissue. Autocrine motility factor (303, 304), Thymosin β15, and possibly the small heat shock protein 27 (Hsp27) have emerged as potential factors controlling cell motility. Thymosin β15 increases cell motility, and when its production was decreased by expression of antisense constructs, as recently reported by Bao *et al.* (305), metastases were prevented in the Dunning rat prostate adenocarcinoma model. Similarly, overexpression of Hsp27 in MDA-MB-231 cells decreased cell motility *in vitro* and bone metastasis in mice (306).

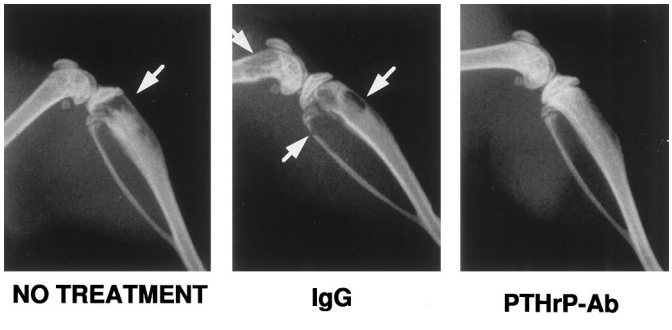

FIG. 6. Radiographs of osteolytic bone lesions in hind limbs from female nude mice inoculated via the left cardiac ventricle from respective treatment groups of nothing, control IgG, and PTHrP antibody. Radiographs were taken 26 days after tumor inoculation with MDA-MB-231 cells. Arrows indicate osteolytic metastases in distal femur, proximal tibia, and fibula. [Reproduced from T. A. Guise *et al.* (282) by copyright permission of The American Society for Clinical Investigation, Inc.]

Another important property of the breast cancer seed that enables it to establish growth in bone resides in the adhesion molecules. Experimental evidence supports the notion that tumor cell surface expression of such molecules mediates targeting to bone and the resultant development of bone metastasis. For example, bone marrow stromal cells express the vascular cell adhesion molecule-1 (VCAM-1), a ligand for $\alpha_4\beta_1$ integrin (307). Tumor cells expressing $\alpha_4\beta_1$ integrin may preferentially adhere to bone marrow stromal cells to establish bone metastasis. CHO cells transfected with $\alpha_4\beta_1$ caused bone and lung metastases when inoculated intravenously into nude mice compared with only lung metastases in mice similarly inoculated with untransfected CHO cells (308). In that report, bone metastases were inhibited by antibodies against $\alpha_4\beta_1$ or VCAM-1. Similar expression of $\alpha_3\beta_1$, $\alpha_6\beta_1$, or $\alpha_v\beta_1$ did not induce bone metastases (308). Although many breast cancer cells express the $\alpha_v\beta_3$ integrin receptor that binds the bone matrix protein, osteopontin, a potential avenue for the development of bone metastasis, MDA-MB-231 cell populations with high-level expression of the $\alpha_v\beta_3$ integrin were less likely to cause bone metastasis than those cells expressing low amounts of $\alpha_v\beta_3$ in the mouse model of bone metastasis (309). Bone sialoprotein peptides containing RGD sequences have been shown to decrease MDA-MB-231 cell adhesion to extracellular bone matrix *in vitro* (310). Finally, tumor cell expression of CD44 may mediate binding to osteopontin via RGD-independent mechanisms (311). Such observations illustrate the complex and multifactorial nature of the mechanisms underlying the metastatic process.

Taken together, tumor cell expression of osteolytic factors, adhesion molecules, and motility factors significantly impact the ability of the tumor cell, or seed, to develop and grow as bone metastases.

b. Bone microenvironment as the soil: Bone is unique among metastatic target tissues since it undergoes continual remodeling under the influence of systemic hormones and local bone-derived growth factors. Mineralized bone matrix is a repository for growth factors, of which TGFβ and IGF-II constitute the majority (3). As described earlier, these growth factors are released from the bone matrix as a result of normal osteoclastic bone resorption (5), a part of the normal remodeling process necessary for maintenance of the structural integrity of bone. The hematopoietic stem cells in the bone marrow can differentiate into bone-resorbing osteoclasts. Other cells in the bone marrow, stromal and immune cells in particular, produce cytokines and growth factors that may potentiate tumor cell growth or expression of osteolytic factors. Thus, once breast cancer cells arrest in bone, the high concentrations of growth factors and cytokines in the bone microenvironment provide a fertile soil on which the cells

can grow. Such host cytokines may also enhance osteoclastic bone resorption stimulated by tumor-produced factors such as PTHrP. Furthermore, when the tumor cells stimulate osteoclastic bone resorption, this bone microenvironment is even more enriched with bone-derived growth factors that enhance survival of the cancer. Finally, bone-derived TGFβ may have an important role as a chemoattractant for breast cancer cells.

A large body of indirect evidence to support the concept that bone is a fertile soil, further enriched by the process of osteoclastic bone resorption, has accumulated in studies using bisphosphonates in the treatment of bone metastases. It is already clear from clinical studies that the use of bisphosphonates, potent inhibitors of bone resorption, significantly reduces skeletal morbidity in advanced breast cancer (312–323). In a recent multicenter trial that consisted of more than 700 patients with stage IV breast cancer with two or more predominantly lytic lesions, with at least one lesion that was 1 cm or greater in diameter, treatment with pamidronate 90 mg iv every 3–4 weeks for 12 months in conjunction with chemotherapy or hormonal therapy resulted in a significant reduction in skeletal complications and bone pain compared with the control group (321). Bisphosphonates have also been shown to decrease the number of bone metastases as well as tumor burden in animal models (296, 324, 325). Thus, by decreasing osteoclastic bone resorption, the bone microenvironment is a less fertile soil for the growth of tumor.

TGFβ, which is present in high concentrations in the bone microenvironment and expressed by some breast cancers (177, 178) and cancer-associated stromal cells (176), has been shown to enhance secretion of and stabilize the mRNA for PTHrP in a renal cell carcinoma (96), a squamous cell carcinoma (97, 98), and a human breast adenocarcinoma, MDA-MB-231 (177, 178). In fact, of the known growth factors present in the mineralized bone matrix other than TGFβ, such as IGF-I and -II, fibroblast growth factors (FGFs) 1 and 2, bone morphogenetic proteins (BMPs) and platelet-derived growth factor, only TGFβ has been shown to significantly stimulate PTHrP secretion from the human breast cancer cell line, MDA-MB-231 (178). The fact that TGFβ is abundant in bone (3) and can enhance PTHrP expression by cancer cells makes it an important candidate factor in the establishment and progression of breast cancer metastases to bone. TGFβ is a member of a large superfamily of proteins that are important regulators of bone cell activity (326). Multiple isoforms of TGFβ exist in mammals and appear to control cell proliferation and differentiation in many human cell types (327). The prototype of these isoforms, TGFβ1, is highly expressed by differentiated osteoblasts and osteoclasts, is stored in bone matrix, and is released in active form during osteoclastic bone resorption (5). The effects of TGFβ include stimulation of cell proliferation of mesenchymal cells, growth inhibition of epithelial cells, synthesis of extracellular matrix proteins, and enhancement of cell adhesion. These effects of TGFβ are mediated through complex receptor interactions (328). TGFβ binds to the type II receptor, and this complex recruits and phosphorylates the type I receptor, which in turn initiates signal transduction mediated by the recently identified Smad protein family (328). The effects of TGFβ on cancer cells are complex and variable (329). In some cancer cells, TGFβ inhibits growth, while in others growth is stimulated (330). It likely has effects on apoptosis as well.

Further evidence for the role of bone-derived TGFβ in the development and progression of breast cancer metastasis to bone has been demonstrated in the same in vivo animal model of osteolysis described above. Since TGFβ increases PTHrP expression by MDA-MB-231 cells *in vitro*, this cell line was transfected with a cDNA encoding a TGFβ type II receptor lacking a cytoplasmic domain (TβRIIΔcyt) (331). This receptor binds TGFβ, but since it cannot phosphorylate the type I receptor, signal transduction is not initiated and it acts in a dominant-negative fashion to block the biological effects of TGFβ (332). Stable clones expressing TβRIIΔcyt did not increase PTHrP secretion in response to TGFβ stimulation compared with controls of untransfected MDA-MB-231 cells or those transfected with the empty vector. Mice inoculated into the left cardiac ventricle with MDA-MB-231 cells expressing TβRIIΔcyt had fewer osteolytic lesions as well as a smaller area of osteolytic lesions by radiography and histomorphometry compared with the controls of parental cells or those transfected with the empty vector (333). These data indicate that TGFβ responsiveness of this human breast cancer cell line is important for the expression of PTHrP in bone and the development of osteolytic bone metastasis *in vivo*.

In the mouse model of bone metastasis in which human tumor cells inoculated into the left cardiac ventricle cause bone metastasis, metastasis to the calvariae rarely occur due to the relatively low rate of bone turnover at this site compared with other bones. To increase the rate of bone turnover in the calvariae, Sasaki *et al.* (334) injected recombinant IL-1α subcutaneously over the calvariae of nude mice for 3 days. Upon completion of these treatments, MDA-MB-231 cells were inoculated into the left cardiac ventricle of female nude mice. Four weeks after tumor cell inoculation, IL-1-treated mice had obvious metastatic tumor deposits in the calvariae compared with none in the control-treated mice. Further experiments demonstrated that pretreatment of the mice injected with IL-1 with the bisphosphonate, risedronate, profoundly diminished the development of metastatic tumor deposits in the calvariae (334). These data suggest that growth factors released from bone matrix may potentiate tumor cell growth in bone metastases. However, these findings do not exclude some other alteration in the bone matrix or microenvironment that may be enhanced due to increased bone turnover.

Growth factors released from resorbing bone likely have significant effects on tumor cell growth as well. Experimental evidence suggest that IGFs may be important in this regard. Culture supernatants from resorbing neonatal mouse calvariae strongly increased the proliferation of MDA-MB-231 breast cancer cells in culture (335). Inhibition of bone resorption by adding risedronate to the calvarial organ cultures blocked the subsequent breast cancer cell proliferation. Additionally, neutralizing antibodies to the IGF-I receptor markedly impaired the growth-stimulating effects of the resorbing bone culture supernatants on the tumor cells (335). These results strongly suggest that IGFs are released from bone during bone resorption and promote breast cancer cell proliferation.

Another property of bone that may explain the predilec-

tion of certain tumor types to grow in bone is chemotactic attraction of circulating cancer cells. Culture supernatants of resorbing bone stimulate chemotactic movement of breast cancer cells in a Boyden chamber assay (336). Bone matrix factors such as TGFβ, type I collagen and its fragments, osteocalcin, and IGFs have been shown to stimulate chemotaxis of breast cancer cells (337). Most recently, IGF-I was shown to stimulate $\alpha_v\beta_5$ integrin-mediated chemotactic migration of human breast cancer cell lines (338).

c. Tumor cell-bone interactions: In addition to the properties of breast cancer as a seed and the bone microenvironment as a soil, there are likely complex interactions between the bone microenvironment and the tumor cell as well as between and within tumor cells that influence osteoclast activation. Both bone-derived and tumor-associated factors have been shown to increase PTHrP expression by tumor cells as well as modulate the end-organ effects of PTHrP. Thus, such factors may enhance the ability of tumor cells to activate osteoclasts and promote bone destruction.

Other tumor-associated factors in addition to bone-derived growth factors may be important regulators of PTHrP expression in breast cancer metastatic to bone. As indicated in the previous section on hypercalcemia, many tumor-associated factors, such as EGF (90, 91), TGFα (166), IL-6 (165), TNF, IGF-I, and IGF-II (91), have the potential not only to enhance tumor production of PTHrP but to modulate its end-organ effects on bone as well.

d. Implications of PTHrP status in breast cancer: These findings have important implications for breast cancer effects on the skeleton. First, breast cancers expressing PTHrP may affect the skeleton through humoral and osteolytic mechanisms. Second, the effects of PTHrP on bone may be enhanced if the breast cancer expresses other bone-active factors, such as TGFα or IL-6, in addition to PTHrP. Finally, growth of breast cancer cells in bone may be enhanced if the tumor cells express PTHrP or other bone-resorbing factors. TGFβ, as well as other bone-derived growth factors, increase PTHrP expression by breast cancer cells in bone, so that TGFβ-responsive tumors may preferentially grow in bone. Thus, enhanced osteoclastic bone resorption causes increased release of TGFβ and other growth factors into the bone microenvironment. The result is 1) greater PTHrP expression by the breast cancer cells; 2) enhanced growth of the cancer cells; and 3) chemoattraction of more tumor cells by bone-derived factors. A cycle is thus established, as illustrated in Fig. 7, which ends in bone destruction and the other consequences of lytic bone metastases. The clinical finding of increased PTHrP expression in bone compared with other sites supports the notion that production of PTHrP as a bone-resorbing agent may contribute to the ability of breast cancers to grow as bone metastases and/or that the bone microenvironment enhances production of PTHrP. These and other reasons may, in part, explain the propensity of breast cancers to metastasize to bone and the alacrity with which breast cancer grows in bone.

If PTHrP expression in the primary breast tumor indicates a propensity to metastasize and destroy bone due to its potent bone-resorbing capability, early treatment with inhibitors of bone resorption or agents that inhibit the production of or biological effects of PTHrP are likely to prevent or delay the development of bone metastases as well as reduce the catastrophic complications of pain, hypercalcemia, fracture, and nerve compression syndromes.

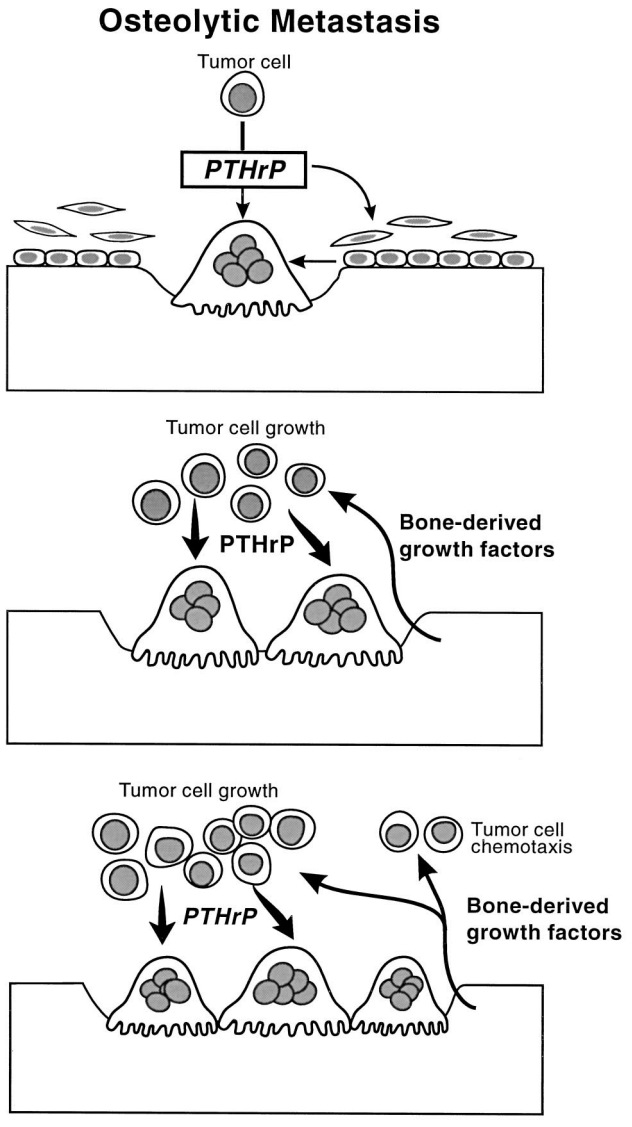

FIG. 7. Schematic illustration of a proposed mechanism of local bone destruction in osteolytic bone metastasis mediated by PTHrP. Other osteolytic factors may mediate this process as well. In the *top panel*, tumor cell arrives in bone and stimulates osteoclastic bone resorption via secretion of PTHrP, an effect that is mediated through the osteoblast and stromal cells. In the *middle panel*, osteoclastic bone resorption results in release and activation of growth factors present in bone matrix, such as TGFβ, IGF-I and -II, etc. Such factors may increase tumor production of PTHrP (in the case of TGFβ) and/or increase tumor cell growth (in the case of IGFs). The *lower panel* illustrates the end result of this cycle in which increased tumor-stimulated osteoclastic bone resorption results in increased local concentration of bone-derived growth factors. Such factors increase PTHrP production, tumor cell growth, and chemotaxis.

2. Osteoblastic metastasis. Osteoblastic metastases occur most commonly in prostate cancer and less so in breast cancer. Rarely, osteoblastic bone lesions have been described in other malignancies such as an osteosclerotic variant of myeloma (279), colon cancer (339), astrocytoma (340), glioblastoma

multiforme (341), thymoma (342), carcinoid (343), nasopharyngeal carcinoma (344), leptomeningial gliomatosis (345), Zollinger-Ellison syndrome (346), and cervical carcinoma (347). Similar to the pathophysiology of breast cancer-mediated osteolysis, the seed and soil hypothesis applies to this situation as well in that tumor cells secrete factors that stimulate bone formation, and the bone microenvironment readily supports the growth of prostate cancer cells (348).

Prostate cancer is relatively unique in its ability to form osteoblastic bone metastases, and there is much speculation on the mechanisms involved. Understanding the pathophysiology of prostate cancer-mediated osteoblastic metastasis has been limited, in part, to the paucity of *in vivo* models that adequately reproduce the spectrum of human disease, including the osteoblastic phenotype. However, over the past few years several models have been reported that may provide insight into the mechanisms responsible for the osteoblastic metastasis. Thalmann *et al.* (349) reported that an androgen-independent clone of the LNCaP human prostate cancer cell line, when inoculated subcutaneously or orthotopically into castrated male nude mice, spontaneously metastasized to bone in 11–50% of mice. Histological evidence of new bone formation was observed at the site of bone metastasis. Greenberg *et al.* (350) developed a transgenic mouse model of spontaneous prostate cancer, in which the simian virus 40 (SV40) large tumor T antigen is driven by the rat probasin promoter to target the dorsolateral epithelium of the prostate (TRAMP mice). In this model, 100% of mice develop distinct pathology in the dorsolateral epithelium of the prostate by 10 weeks of age that range from mild intraepithelial hyperplasia to large multinodular malignant neoplasia (350). Distant metastases occur as early as 12 weeks in common sites of periaortic lymph nodes and lungs and less common sites of kidney, adrenal gland, and bone (351). As reported by Gingrich *et al.* (351), one of these TRAMP mice developed paraplegia and was found to have tumor metastatic to the spinal canal. Bone pathology revealed osteoclastic bone resorption and new bone formation at vertebral sites adjacent to the spinal metastasis. Finally, in a model similar to the one described previously for breast cancer metastasis to bone, described in a later section of this review, rat prostate cancer cells inoculated into the left cardiac ventricle of syngeneic rats caused osteoblastic bone metastasis (352). Further investigation of these animal models should provide significant insight into the pathophysiology of prostate cancer metastasis to bone.

a. Prostate cancer as the seed: As prostate cancer is more frequently associated with osteoblastic metastases, prostate cancer cells must possess properties different from those of other tumor types commonly associated with osteolytic metastases. The histomorphometric studies of Charhon et al. (353) indicate that osteoblastic metastases are likely due to soluble factors that are produced by metastatic prostate cancer cells that stimulate bone formation. Osteoblast-stimulating activity produced by prostate cancer has been described by a number of investigators. Conditioned media from *Xenopus* oocytes injected with total RNA from the human prostate cancer cell line PC3 stimulated both mitogenesis and alkaline phosphatase activity in osteosarcoma cells with the osteoblast phenotype (354). In fetal rat calvarial cells, PC-3-conditioned media stimulated osteoblast proliferation (355). Koutsilieris *et al.* (356) found that extracts of prostate cancer tissue and normal prostate tissue stimulated proliferation of bone cells.

b. Osteoblastic factors: Such data indicate that prostate cancer is a source of osteoblast-stimulating activity. The following tumor products have been proposed to be important in the genesis of the osteoblastic response to tumor cells in bone.

i. TGFβ. TGFβ is secreted by osteoblasts in a latent biologically inactive form that is incorporated into bone extracellular matrix. TGFβ is synthesized as latent high molecular mass complexes, composed of TGFβ, the amino-terminal portion of the TGFβ precursor, and the latent TGFβ-binding protein (LTBP). Osteoblasts not only produce TGFβ but they also possess high-affinity receptors for it (357), providing the opportunity for autocrine stimulation of osteoblast replication. Latent TGFβ can be activated by a number of agents including acid pH, or by proteases such as plasmin or cathepsin D (358, 359). TGFβ1 and -2 are homologous disulfide-linked homodimers of 25 kDa that have powerful effects on bone.

The local function of TGFβ may be very important in contributing to the differentiated activity of osteoblasts. It stimulates collagen synthesis and regulates gene expression of mRNA for pro-αI (I) collagen, osteonectin, alkaline phosphatase, fibronectin (360), osteopontin (361), and osteocalcin (362). TGFβ increases the abundance of matrix proteins by stimulating their synthesis and inhibiting their degradation. It is a potent stimulator of collagen and fibronectin synthesis and secretion in fibroblasts and osteoblasts (363, 364), acting by increasing the mRNA for collagen and fibronectin (365). TGFβ also inhibits degradation of matrix proteins by decreasing the synthesis of matrix-degrading enzymes, as well as increasing the synthesis of protease inhibitors (366). TGFβ promotes the differentiation of cells of the osteoblast lineage toward the mature osteoblast and the formation of new bone.

TGFβ, injected subcutaneously adjacent to bone surfaces, causes a profound increase in new bone formation (367–369). When TGFβ is administered by injection over the calvariae of mice daily for 3 days, bone width is increased 40% over the next month (368). This is initially woven bone, but it is later replaced by lamellar bone. Similar effects are seen when TGFβ is injected or infused directly into the marrow cavity of the femur.

TGFβ may also affect osteoclastic bone resorption as indicated by recent studies using a transgenic mouse model in which active TGFβ2 overexpression was targeted to osteoblasts through the use of an osteocalcin promoter (370). This osteoblast-specific overexpression of TGFβ2 resulted in progressive bone loss associated with increases in osteoblastic matrix deposition and osteoclastic bone resorption.

TGFβ, isoforms 1 and 2 in particular, are produced by prostate cancer. TGFβ2 is produced in abundant amounts in the human prostate cancer cell line PC3 (371). Although most studies in primary prostate indicate that TGFβ is produced by prostate cancer cells, one study demonstrated immunohistochemical localization of TGFβ in peritumoral fibroblasts (372). TGFβ expression in human prostate cancer tissue appears to be greater than in normal prostate or benign prostatic hypertrophy (373). Studies from histopathologically

verified human prostate cancer indicate that TGFβ is produced without associating with the LTBP whereas in normal and benign prostatic hyperplasia tissues, TGFβ may be produced in a complex associated with LTBP (374). Other investigators have found that prostate cancer cells secrete TGFβ1 in the latent form, and the prostate cancer cells themselves further activate approximately 50% to the bioactive form (373). Other studies have shown that overexpression of TGFβ1 in the rat prostate cancer cell line MATLyLu was associated with enhanced growth, viability, and aggressiveness *in vivo* (375). Although there is little evidence in the literature to demonstrate a direct relationship between tumor-produced TGFβ and the development of osteoblastic metastasis, the fact that TGFβ is produced by human prostate cancer coupled with its profound effects on bone formation have obvious implications in the pathophysiology of prostate cancer-mediated osteoblastic metastasis.

ii. IGF-I and -II. The IGF system is a fairly complex one and consists of two ligands, IGF-I and IGF-II, two receptors, and six binding proteins. The topic of IGFs, their binding proteins, and their biological actions has been extensively reviewed by Jones and Clemmons (376). Most of the cellular effects of the IGFs are mediated by binding of the peptides to the IGF-I receptor. The affinity of the IGF-I receptor for IGF-II is 2- to 15-fold lower than for IGF-I. The IGF-II/cation-independent mannose 6-phosphate receptor binds IGF-II with a 500-fold greater affinity than IGF-I. IGFs mediate their biological effects via interaction with their respective receptors, and these receptor interactions are affected by the presence of IGF-binding proteins. Six IGF-binding proteins (IGFBPs) have been identified, and binding of these proteins to IGFs can enhance or inhibit the biological effects of IGFs. IGF-I and -II are weak bone cell mitogens, but have clear and potent stimulatory effects on the differentiated function of the osteoblast, as evidenced by an increase in osteocalcin and type I collagen synthesis in osteoblasts. As a result, IGFs increase bone matrix apposition rates and bone formation. IGFs also decrease collagen degradation and the expression of interstitial collagenase, functions that suggest a role in the preservation of bone matrix. IGFs enhance bone formation *in vivo*, and mice with null mutation of type I IGF receptor have delayed skeletal development and ossification (377). The anabolic properties of IGF-I and -II, their inhibitory actions on matrix degradation, and their abundance in bone tissue suggest that these factors play a central role in the maintenance of bone mass (378).

Regulation of IGF-I in bone is further complicated by the production of IGFBPs by osteoblasts, which express all six IGFBPs. Binding of IGF to one of these binding proteins can inhibit or potentiate the biological effect of IGF. Binding to IGFBP-1, for example, decreases the biological activity of IGF-I. Conversely, IGFBP-5 has been shown to increase bone formation and, thus, appears to enhance the effect of IGF-I. This system is further complicated by the observation that growth factors such as TGFβ, platelet-derived growth factor, FGF, and BMP-2 inhibit synthesis of IGFBP-5 in bone cell cultures (379, 380) while IGF-I and retinoic acid increase it (381). Thus, it appears that the effect of IGFs on bone is anabolic, and that local regulation of IGFs in bone is highly complex.

IGFs are potent mitogens for the growth of human prostate cancer cells, and primary cultures of prostate epithelial cells have been demonstrated to express all aspects of a functional IGF system: IGFs, IGF receptors, and IGFBPs (382). Human seminal fluid contains IGF-I and -II, IGFBP-2 and -4, as well as IGFBP-3 fragments and IGFBP-3 protease activity (383). This IGFBP-3 protease activity in seminal fluid has been attributed to prostate-specific antigen (PSA) (384) while production of other proteases such as urokinase receptor and cathepsin D have been demonstrated in prostate cancer (385, 386). IGFBP-2 appears to be the main binding protein produced by prostate cancer cells and, accordingly, clinical studies have demonstrated serum concentrations of IGFBP-2 to be increased and IGFBP-3 to be decreased in patients with prostate cancer (387, 388). Furthermore, significant positive correlations between serum concentrations of IGFBP-2 and PSA as well as between IGFBP-2 and tumor stage have been observed in men with prostate cancer (387, 388). Although at least one of these studies included patients with bone metastases, neither report comments on whether there was a significant correlation between IGFBP-2, PSA, and the presence of bone metastases. Immunohistochemistry and *in situ* hybridization in prostate tissue containing benign epithelium, high-grade prostate intraepithelial neoplasia, and adenocarcinoma indicate that mRNA and immunostaining intensity for IGFBP-2 progressively increased from benign prostate tissue to malignant adenocarcinoma whereas the immunostaining intensity for IGFBP-3 was increased in prostate intraepithelial neoplasia compared with normal, but decreased in malignant, cells (389). These authors conclude that the decreased expression of IGFBP-3 in malignant prostate tissue may be due to pre- and/or posttranslational mechanisms, including proteolysis, and that these observations correlate with serum changes of IGFBPs described in men with prostate cancer.

There is accumulating evidence that prostate cancers produce a variety of proteases, such as PSA, urokinase type plasminogen activator, and cathepsin D, that may be responsible for dissociating IGF-I and IGF-II from respective binding proteins to result in enhanced effects on not only tumor growth, but also, in the case of prostate cancer metastatic to bone, mitogenic effects on osteoblasts. PAIII cell-conditioned media has been shown to contain a 35-kDa proteinase capable of digesting IGFBPs that may serve to increase the bioavailability of osteoblast-derived IGFs (390). In addition to these proteolytic effects to activate growth factors, these proteases may be mitogenic for tumor cells as well.

Based on the above observations of the presence of an intact IGF system (including IGFBP proteases) in prostate cancer, the mitogenic effect of IGFs on prostate cancer, as well as on osteoblasts, and the positive correlations between serum IGFBP-2 and PSA, it is conceivable that local production of IGFs by prostate cancer in bone may mediate the osteoblastic response so characteristic of prostate cancer metastatic to bone. Unfortunately, the data described above are associations at best, and a direct causal role has yet to be proven.

iii. Proteases.

1. PSA. PSA is a serine protease, single-chain glycoprotein

that has trypsin-like and chymotrypsin-like enzymatic activity (391). As PSA was initially believed to be produced exclusively by prostate epithelial cells, it has been extensively used as a marker for prostate cancer (392). The three clinical diseases associated with an increased serum PSA concentration are prostate cancer, benign prostatic hypertrophy, and acute bacterial prostatitis (393). In patients with prostate cancer, the serum PSA concentration is a valuable biological marker for diagnosis, prognosis, and management. The pretreatment serum PSA concentration has been shown to be a significant predictor of disease outcome after radiation therapy for local and regional prostate cancer (394). Androgenic hormones increase the production of PSA via transcriptional regulation (395). Serum PSA concentrations have been shown to correlate significantly with the presence of bone metastases by radionuclide scanning (396). In a large clinical study of 521 men with newly diagnosed and untreated prostate cancer, only one of 306 patients with a serum PSA concentration of less than 20 ng/ml had a positive bone scan (397). Serum PSA concentration proved to be the best predictor of bone scan findings when compared with tumor grade, local clinical stage, acid phosphatase, and prostatic acid phosphatase (397–399). Thus, in a newly diagnosed patient with prostate cancer, a serum PSA concentration of less than 10 ng/ml, and no skeletal symptoms, a bone scan may not be necessary (396) although others recommend measurement of PSA in conjunction with bone-specific alkaline phosphatase (400). Immunoreactive PSA has recently been demonstrated in 27% of 174 primary breast cancers (401) even though it was once believed to be an exclusive product of prostate epithelium. Breast-derived PSA was identical to PSA derived from prostate (402), and PSA has been shown to be produced at the ovarian metastatic site of a breast cancer (403). Furthermore, in a larger study of breast tumor cytosols from women and men, a positive correlation between immunoreactive PSA and progesterone receptor was observed (404).

The function of PSA in prostate cancer is unclear, but its proteolytic activity may prove to be important in the genesis of osteoblastic response to prostate tumor in bone. PSA has been shown to proteolyze IGFBP-3 into at least seven fragments with molecular masses of 13 kDa to 26 kDa with at least five different proteolytic recognition sites in this binding protein for PSA (405). Three of the five proteolytic sites were consistent with a kallikrein-like enzymatic activity while two of the sites were consistent with a chymotrypticlike enzymatic activity. Furthermore, some of the IGFBP-3 fragments retained the ability to bind IGF (405). Additionally, PSA has been shown to stimulate osteoblast proliferation at concentrations of 2.5 ng/ml possibly through activation of latent TGFβ (406). Thus, it is tempting to speculate that PSA-induced proteolytic cleavage of IGFBP-IGF complex results in locally active IGF at the site of prostate cancer metastatic to bone to stimulate the osteoblastic response. Furthermore, recent evidence demonstrates that PSA also cleaves PTHrP-(1–141) at the carboxyl-terminal phenylalanine 23 and inactivates the biological effects of PTHrP to stimulate cAMP production in an osteoblast cell line (407). This may have important implications for the predominantly osteoblastic phenotype observed in prostate cancer. The fact that breast cancers also express PSA is equally interesting. Metastatic breast cancer to bone is one of the few other carcinomas associated with osteoblastic metastases, albeit at a much lower frequency than observed with prostate cancer.

2. Urokinase type plasminogen activator (uPA). Urokinase-type plasminogen activator is a member of the serine protease family that also includes tissue-type plasminogen activator (tPA). These proteins are expressed in normal cells, and the major function of tPA is related to intravascular thrombolysis while uPA is involved in proteolysis during cell migration and tissue remodeling. Although both tPA and uPA have been identified in malignant tissue, uPA appears to have a more prominent role in malignancy by promoting tumor cell migration and invasion by activating plasminogen to plasmin which, in turn, cleaves extracellular matrix components of laminin, fibronectin, and collagen.

uPA has been isolated from several prostate cancer cell lines that promote new bone formation *in vivo*. The rat prostate PA III tumor line causes new bone formation when inoculated over the scapula of rats and athymic nude mice (408). Conditioned media from PA III cells stimulated proliferation of osteoblasts *in vitro*. uPa expression by human PC-3 prostate cancer cells is increased by EGF and *trans*-retinoic acid and decreased by dexamethasone (409). In an experiment to demonstrate the influence of uPA on the nature of prostate cancer metastasis, Achbarou *et al.* (352) used gene transfer techniques to overexpress uPA in the rat prostate cancer cell line, Mat LyLu, by 5-fold compared with the same cells expressing empty vector. A separate Mat LyLu cell line that expressed uPA mRNA in the antisense orientation had 3-fold reduction in uPA mRNA compared with the empty vector cells. The uPA-overexpressing, underexpressing, and parental cell lines were compared in a rat model of bone metastases in which tumor cells inoculated into the left cardiac ventricle of inbred male Copenhagen rats cause bone metastasis. Rats inoculated with the uPA-overexpressing cell line developed hind limb paralysis sooner than rats inoculated with empty vector Mat LyLu cells. Similarly, rats inoculated with the uPA antisense-expressing Mat LyLu cells developed hind limb paralysis later that rats inoculated with parental or uPA-overexpressing Mat LyLu (352). Histological assessment of the sites of tumor metastasis indicated that more metastatic tumor was present sooner in both skeletal and nonskeletal sites of the rats inoculated with the uPA-overexpressing Mat LyLu cell line, compared with those inoculated with the empty vector or antisense cell line. Furthermore, histological analysis of bone indicated that although both osteolytic and osteoblastic lesions were present in both control and experimental rats, the osteoblastic response was the predominant feature in rats bearing the uPA-overexpressing Mat LyLu cells.

iv. FGFs. Both acidic and basic FGFs, now known as FGF-1 and -2, respectively, are present in mineralized bone matrix and stimulate the replication of cells in the skeletal system, but do not increase the differentiated function of the osteoblast. Therefore, they may play an important role in bone repair where bone cell mitogenesis may be necessary (378). FGFs enhance TGFβ expression in cells with the osteoblast phenotype and have powerful stimulatory effects on bone formation *in vivo*. When injected locally over the calvariae of mice, FGF causes a 50% increase in bone thickness. When administered to ovariectomized rats, FGF blocked the asso-

ciated bone loss and also increased trabecular connectivity and bone microarchitecture (410).

Prostate cancer cells express large amounts of both FGF-1 and -2 (411, 412). Not only have various prostate cancer cell lines been demonstrated to produce FGF-1 and -2 (413–415) as well as FGF receptor, but other tumor-produced FGF-like polypeptides have been demonstrated as well (415). An extended amino-terminal form of FGF-2 was purified from a human amnion tumor by its ability to stimulate proliferation of the osteoblast cell line MG-63 (416). This tumor has been reported to cause bone formation *in vivo* when inoculated into nude mice. Other data suggest that FGF-2 inhibits osteoclast formation via stromal cells and osteoblasts (417). Although this evidence supports the notion that FGFs may mediate the predominantly osteoblastic phenotype of metastasis in patients with prostate cancer, like TGFβ, there are presently no direct associations between tumor-produced FGFs and osteoblastic metastasis.

v. BMPs. BMPs are bone-derived polypeptides and, with the exception of BMP-1, are members of the extended TGFβ superfamily. At least 15 members are currently recognized, and the list is growing. BMP-2 through BMP-8 share some TGFβ-related gene sequences. BMPs are synthesized by bone cells locally and stimulate the formation of ectopic bone when injected intraperitoneally or subcutaneously into rodents (418). BMPs stimulate the replication and differentiation of normal cells of the osteoblast lineage and, in contrast to TGFβ, enhance the expression of the differentiated osteoblastic phenotype (378, 419). BMP-1, -2, -3, -4, and -6 are temporally expressed in primary cultures of fetal rat calvarial cells (420). BMP-2, -4, and -7 have been shown to induce differentiation of primitive mesenchymal cells into bone when implanted into subcutaneous tissue (421). BMP-2 accelerates differentiation in primary cultures of fetal rat calvarial cells as demonstrated by an increase in expression of alkaline phosphatase and osteocalcin (421). BMP-3 decreases osteoclastic bone resorption and is chemotactic for monocytes. BMP-7 (osteogenic protein-1) suppresses cell proliferation and stimulates the expression of markers characteristic of the osteoblast phenotype in rat osteosarcoma cells but stimulates growth and differentiation in rat calvarial cultures (422). *In vivo*, human BMP-7 was capable of inducing new bone formation in the rat subcutaneous bone induction model (423). Recently, overexpression of BMP-4 in lymphocytes was described in association with the disabling ectopic osteogenesis of fibrodysplasia ossificans progressiva (424).

Normal and neoplastic prostate tissue express BMP-2, -3, -4, and -6 mRNA. The predominant form in normal human prostate tissue was shown to be BMP-4. While this pattern was observed in human prostate cancer cell lines, PC-3 and DU-145, PC-3 also expressed BMP-2 and -3 in large amounts. The rat prostate cancer PAIII expressed predominantly BMP-3 mRNA (425). PAIII is a cell line derived from a strain of rats, Lobund-Wistar, that has a 10% frequency of spontaneous prostate adenocarcinoma (426). PAIII stimulates new bone formation in this strain of rats, as well as in nude mice, when inoculated over the scapula. Rat BMP-3 was isolated from PAIII cells (427), and transfection of the PAIII tumor cells with a BMP-3 antisense construct somewhat reduced the osteoblastic response (428). Thus, biologically active BMPs expressed by prostate tumor in bone may contribute to the new bone formation at metastatic tumor sites in bone.

vi. Endothelin-1 (ET-1). ET-1 is the most recent factor implicated in the genesis of osteoblastic metastases. It is a potent vasoconstrictor and was originally purified from endothelial cells (429). Prostatic epithelium produces ET-1, and high-affinity ET-1 receptors are present throughout the prostate gland (430). ET-1 concentrations in seminal fluid are 500 times greater than those in plasma. ET-1 stimulates mitogenesis in osteoblasts, and osteoblasts have high-affinity receptors for ET-1 (431, 432). Osteoclastic bone resorption and osteoclast motility are decreased by ET-1 as well (433). Moreover, mean plasma endothelin concentrations in men with advanced, hormone-refractory prostate cancer with bone metastases were significantly higher than those concentrations in men with organ-confined prostate cancer or normal controls (417). However, these endothelin measurements were not correlated to tumor burden in bone and did not correlate with serum PSA concentrations. Human prostate cancer cell lines, DU-145, LNCaP, PC3, PPC-1, and TSU, have been shown to express ET-1 by RT-PCR. Finally, *in vivo*, ET-1 stimulated BMP-induced bone formation as assessed by alkaline phosphatase activity in a rat model of matrix-induced bone formation (434). Additionally, IL-6, but not estrogen, tamoxifen, TGFβ, TNF, γ-interferon, or IL-1, stimulated ET-1 production from human breast cancer cells MCF-7 and ZR-75–1 (435). This is of interest since breast cancer is occasionally associated with osteoblastic metastasis.

Thus, the mechanisms responsible for the predominantly osteoblastic phenotype of prostate cancer metastatic to bone is complex and likely is the result of multiple tumor-produced factors on normal bone remodeling. Figure 8 is a schematic model based on available data from the literature that identify potential tumor-bone interactions.

c. Bone microenvironment as the soil for prostate cancer: As bone matrix is an abundant source of growth factors, some of which are released as a consequence of osteoclastic bone resorption, it is likely a fertile soil for prostate cancer cells as well as breast cancer cells. For example, human prostate cancer cell lines proliferate in response to conditioned media from human, rat, or bovine bone marrow (436). Conditioned media from osteoblast-like cells enhance growth of LNCaP, PC-3, and DU-145 (437). Other in vitro studies indicate that TGFβ stimulated adhesion of the human prostate cancer cell line PC3 to bone matrix and that this adhesion appears to be mediated via $\alpha_2\beta_1$ integrins (438). TGFβ has also been shown to stimulate cell motility of the MATLyLu, an *in vitro* observation that suggests that bone-derived TGFβ may be an important chemotactic factor in prostate cancer (439). EGF, secreted by MG-63 bone cells stimulated chemomigration of the TSU-pr1 prostate cancer line in Boyden chambers (440).

3. Hematogenous. Multiple myeloma is a plasma cell malignancy that is almost invariably associated with destructive bone lesions, either in the form of diffuse osteopenia or localized osteolysis throughout the skeleton (441). Eighty percent of patients with myeloma first present with pain, which can be related to the bone disease, and bone complications are the most obvious clinical feature in most patients

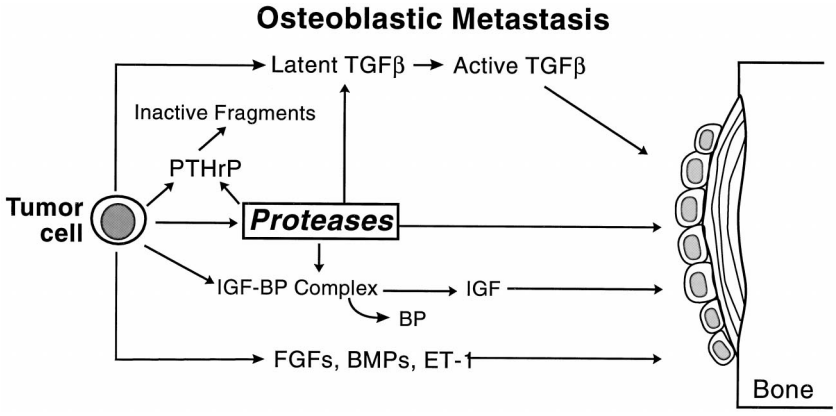

FIG. 8. Model for the formation of osteoblastic bone metastases from prostate cancer. Tumor production of factors such as TGFβ, FGFs, BMPs, and ET-1 may directly stimulate osteoblastic activity and subsequent bone formation. Proteases such as PSA, uPA, and cathepsin D may activate latent TGFβ, release IGFs from inhibitory binding proteins, and inactivate PTHrP.

(441). The bone lesions of myeloma may be diffuse or localized and comprise three types. In the majority of patients (more than 95%), they are osteolytic. These lesions occur predominantly in those bones that are rich in red marrow, *e.g.*, the axial skeleton, and are associated with increased osteoclast activity adjacent to sites of myeloma cell accumulation. This suggests that myeloma cells produce locally active soluble factors that stimulate the remaining osteoclasts to resorb bone (442). In some patients, the bone loss is more generalized and its appearance more closely resembles that of osteoporosis. In these patients the myeloma cells tend to be more diffusely spread throughout the axial skeleton. Some patients may have a combination of these two pictures, *i.e.*, osteopenia of vertebral bodies but discrete osteolytic bone lesions of the skull. Myeloma bone disease is always an important differential diagnosis in the patient who presents with apparent osteoporosis. The bone disease of myeloma tends to be steadily progressive in most patients and can be used as one of the parameters to monitor the course of the disease. For example, obvious progression of the bone lesions or the appearance of new discrete lesions indicates that the disease is active. On the other hand, vertebral body collapse can occur in patients in remission because of the weakened state of the skeleton, and bone pain itself is not a reliable indicator of the state of the disease activity.

The type of osteolytic disease that occurs in myeloma may be quite different from the bone disease associated with other types of malignancy, such as carcinoma of the breast. In myeloma bone disease there is often no increase in bone formation or osteoblast activity. The reason for this complete uncoupling of bone formation and bone resorption is unknown. It is paralleled clinically by the absence of an increase in the markers of bone formation, which are frequently present in other types of osteolytic bone disease due to malignancy, such as serum osteocalcin and alkaline phosphatase (161). In addition, the radionuclide bone scan shows no evidence of increased isotope uptake at the site of bone lesions.

A small number of patients with myeloma bone disease present with an entirely different picture of diffuse osteosclerosis (279, 443). Osteosclerotic myeloma often occurs as a part of a syndrome of polyneuropathy and is associated with the cutaneous and endocrine features that comprise POEM's syndrome (polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes) (444).

Thus, the majority of patients with myeloma have a crippling form of bone disease associated with intractable pain, susceptibility to fracture upon trivial injury, and nerve compression syndromes (most frequently spinal cord compression), associated with vertebral body collapse. About 30% of patients develop hypercalcemia at some stage during the course of the disease, usually in association with impaired renal function (441). The pathophysiology of bone destruction that is so characteristic of myeloma bone disease remains unclear. Cultures of myeloma cells *in vitro* produce several osteoclast activating factors such as IL-6, IL-1β, and TNFβ or lymphotoxin (445–447), which have been implicated. Lymphotoxin (or TNFβ) is a powerful bone-resorbing cytokine that is produced by lymphoid cell lines *in vivo*. It is expressed by many B cell lines in culture, including cell lines derived from patients with myeloma (447). It is capable of causing hypercalcemia *in vivo* and has effects on bone resorption that are indistinguishable from those of TNFα, namely an increase in osteoclast formation and activity, associated with impaired osteoblast differentiation and increased osteoblast precursor proliferation (224). Although produced by many cell lines derived from patients with myeloma, it has not been detected in freshly isolated marrow cells from these patients.

The second cytokine that has been implicated in the bone lesions in myeloma is IL-1β (445, 446). IL-1β is also a powerful stimulator of osteoclastic bone resorption, which increases osteoclast formation and osteoblast proliferation and can cause hypercalcemia *in vivo*. Freshly isolated marrow cells from patients with myeloma, which contain myeloma cells plus other marrow cells, have been shown to contain IL-1β in the conditioned medium harvested from these cells. Moreover, bone-resorbing activity present in these conditioned media can be neutralized by antibodies to IL-1β.

Finally, IL-6 is a cytokine that has an important growth-regulatory role in many patients with myeloma (448). IL-6 concentrations in bone marrow and in plasma correlate with the stage of disease (449–451). It may be produced by the myeloma cells or other stromal cells in the marrow microenvironment. Endogenous IL-6 production is 2–30 times greater in patients with myeloma than in normals (450). Administration of a murine-human chimeric anti-IL-6 antibody to patients with multiple myeloma resistant to second-line chemotherapy suppressed this IL-6 production, but did not prevent infection-induced IL-6 production (450). Such

results suggest that this IL-6 antibody inhibited a positive feedback IL-6-dependent loop.

IL-6 is not a powerful bone-resorbing factor in its own right, but is capable of enhancing the effects of other factors on bone resorption, presumably by increasing generation of precursors for cells in the osteoclast lineage (165). Myeloma cells occasionally produce PTHrP and since IL-6 may potentiate the osteoclastic bone resorption mediated by PTHrP, such cytokine interactions have important implications in the genesis of the bone destruction associated with myeloma.

Just as there may be a vicious cycle in the bone microenvironment between the tumor cells and bone-derived growth factors in breast cancer, there may be a similar relationship between the process of bone resorption and myeloma cell behavior (Fig. 9). In this latter case, the responsible mediator may be IL-6, which is the major growth factor for myeloma cells. In myeloma, stimulation of bone resorption may lead to IL-6 generation (452), which in turn may be responsible for maintaining aggressive growth of the malignant cells. Primary tumor cells from myeloma patients induced IL-6 secretion by adherent cells from long-term bone marrow cultures (451). Interestingly, this IL-6 production did not occur when tissue-culture inserts, which prevented direct contact between the adherent cells from bone marrow and the myeloma cells, were used in the wells. Such findings imply an important role for cell-cell contact in mediating the bone marrow cell induction of IL-6 by myeloma cells. Binding of myeloma cells to adherent cells from long-term bone marrow cultures was partly inhibited by antibodies against the adhesion molecules, very late antigen-4 (VLA-4), CD44, and lymphocyte function-associated antigen 1 (LFA-1), as was IL-6 production (451). These data indicate that one source of IL-6 may be the normal cells in the bone marrow and that adhesion molecules expressed on myeloma cells may mediate the induction of IL-6.

Other properties of myeloma cells contribute to its predilection to grow in the bone microenvironment. Cell surface adhesion molecules expressed by myeloma cells may bind to bone marrow stromal or endothelial cells. A variety of adhesion molecules, such as the VLAs, intercellular adhesion molecules, and LFAs (453–458), are expressed on myeloma cells. VLA-5 and MPC-1 were reported to be involved in the adhesion of mature myeloma cells to bone marrow stromal cells (459). Additionally, expression of the cell surface adhesion molecule CD21 was demonstrated on both mature and immature myeloma cells. CD23 present on bone marrow stromal cells bind CD21 and, thus, may mediate myeloma cell adhesion in the bone microenvironment (460). VLA-4 is the principal integrin present on the U266, ARH-77, IM-9, and HS-Sultan human myeloma cell lines, and work by Uchiyama *et al.* (454) reveals that such cells bind fibronectin through VLA-4 as well as through RGD-dependent mechanisms. This binding was down-regulated by IL-6. Studies from human subjects with myeloma and monoclonal gammopathy of unknown significance indicate that expression of plasma cell adhesion molecules LFA-1, VLA-4, and CD44 was highest in myeloma patients and was associated with increased evidence of angiogenesis (461). Finally, CD44 expression by myeloma cells may mediate colonization in bone by binding osteopontin (311).

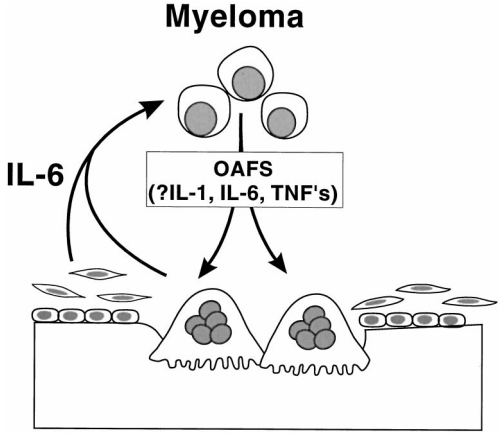

FIG. 9. Model for the bone destruction associated with myeloma. Myeloma cells produce osteoclast activating factors (OAFs) which stimulate osteoclastic bone resorption. IL-6 derived from the osteoblast, osteoclast, stromal cell, and myeloma cell itself stimulate growth of the myeloma cells.

At this point in time, it is not possible to say which is the most important cytokine involved in myeloma. The bone disease may be due to the combination and interaction of a number of cytokines and other molecules working in parallel, derived from both myeloma as well as from the normal host cells. Several animal models of myeloma bone disease have been developed recently that should provide significant insight into the pathophysiology of this disease (462, 463).

Until recently, the management of myeloma bone disease comprised treatment of the underlying malignancy, management of bone pain with analgesics, radiation therapy if the bone disease was localized, and cautious treatment of hypercalcemia when it occurred because it is likely accompanied by impaired glomerular filtration. Recently, this has changed with the availability of more potent bisphosphonates. The Food and Drug Administration approved the use of pamidronate for myeloma bone disease in 1995. Pamidronate has been shown to reduce skeletal events associated with myeloma, including the need for radiation therapy and pathological fractures. It also probably reduces the number of episodes of hypercalcemia. When given in doses of 90 mg by intravenous infusion over 4 h monthly for 9 months, it has been shown to be very effective in patients with advanced disease, and the number of skeletal events is almost halved in patients taking pamidronate. Moreover, an objective assessment of quality of life in treated patients has shown a beneficial effect of pamidronate (464). Similar studies in Europe have also suggested that potent bisphosphonates such as clodronate are effective in improving performance status, reducing bone pain, vertebral fractures, and progression of osteolytic bone lesions, as well as preventing hypercalcemic episodes. Animal studies using a mouse model of myeloma with the bisphosphonate, risedronate, reveal similar findings (465). Previous clinical studies with less potent bisphosphonates in patients with myeloma have been less successful as oral etidronate was ineffective (466) and oral clodronate inhibited the progression of osteolytic bone lesions but did not reduce bone pain or fracture rate (467). Clinical studies in patients with myeloma indicate that adjuvant treatment with

low-dose gallium nitrate attenuates the rate of bone loss as well as the associated bone pain (468). There is no definitive evidence as yet for a beneficial effect of inhibition of bone resorption on tumor burden. Bisphosphonates seem to be most effective in patients with minimal disease, but are appropriate for almost all patients.

As with solid tumors that cause destructive bone lesions and hypercalcemia, hypercalcemia is also frequent in myeloma, occurring in about 30% of all patients sometime during the course of the disease. Hypercalcemia is always associated with bone destruction and often with impaired renal function. When hypercalcemia occurs in patients with myeloma, it is usually accompanied by impaired renal function, which limits the availability of specific therapies for treatment of the hypercalcemia. Under these circumstances, the most appropriate medical agent to use is a new generation bisphosphonate, although experience with these drugs is limited in patients with markedly impaired glomerular filtration. An alternative is the use of a combination of calcitonin and glucocorticoids, which has been shown to be very effective in reducing hypercalcemia in patients with myeloma (67, 248). However, since calcitonin must be given by injection, this combination is not as convenient as the new generation bisphosphonates.

In myeloma, bone destruction occurs as a consequence of osteoclastic bone resorption, as osteoclasts accumulate on bone-resorbing surfaces adjacent to myeloma cells. In an early clinical study of patients with myeloma, biopsy samples indicate that active osteoclastic bone resorption correlated with the presence of greater than 20% myeloma cells in the adjacent marrow cell population (442, 469). The striking feature in myeloma is that there is a marked increase in osteoclastic bone resorption, usually without manifestations of increased bone formation (469). These abnormalities in bone formation were confirmed by transiliac bone biopsies from 118 patients with myeloma in which quantitative bone histomorphometry demonstrated that osteoid seams were reduced in thickness and had a lowered calcification rate (470). This is in contrast to breast cancer, where although the bone lesions are mainly destructive, there is usually a slight increase in bone formation and an increase in serum alkaline phosphatase and radionuclide uptake at sites of osteolytic deposits associated with increased osteoblast activity. The mechanism of this uncoupling of bone formation from bone resorption is not known but is the subject of intense study.

D. Therapy of tumor in bone

Most patients with bone metastasis are normocalcemic. In a majority of breast cancer patients with bone metastases, local osteolysis occurs without hypercalcemia (268), increases in nephrogenous cAMP (69), or increases in PTHrP (111). Osteolytic bone lesions are most frequent in patients with carcinoma of the breast, carcinoma of the lung, and myeloma, the same malignancies that are associated with hypercalcemia. However, there are also other solid tumors in which hypercalcemia is rare but osteolytic bone lesions are relatively frequent. These include patients with carcinoma of the thyroid. These patients suffer considerably because of their bone lesions, which cause intractable pain, pathological fracture after trivial injury, nerve compression syndrome such as spinal cord compression, and propensity to develop hypercalcemia. Until recently, therapy of tumor in bone was directed against tumor cells for breast and prostate cancer as well as myeloma and other malignancies. This usually involved chemo- or hormonal therapy, local field irradiation, radionuclide therapy, or surgery (274, 471, 472). The advent of bisphosphonates has changed this perspective somewhat in that it has added therapy directed against the osteoclast to our current armamentarium of anticancer drugs.

As metastatic bone disease is mediated by osteoclastic bone resorption, and factors that stimulate osteoclastic bone resorption, such as PTHrP, enhance bone destruction by tumor, it is logical to consider therapy with inhibitors of bone resorption to prevent the development of bone metastasis or to delay their progression. Other mechanisms by which bisphosphonates exert their effects to decrease bone metastases may involve tumor cell adhesion to bone. *In vitro* studies demonstrate that a number of bisphosphonates decrease attachment of MDA-MB-231 breast cancer cells to extracellular bone matrix. Of interest is the fact that the effect of these bisphosphonates to decrease tumor adhesion positively correlated to the antiresorptive potency (473).

Several prospective, double-blind placebo-controlled trials have been published documenting the efficacy of the bisphosphonate, pamidronate, in decreasing the skeletal complications associated with breast cancer (321) and myeloma (464). For patients with myeloma, the Food and Drug Administration (FDA) has recently approved pamidronate for use in patients with osteolytic lesions who are not hypercalcemic. This is based on a recent study (464), which shows that intravenous pamidronate given every 4 weeks for nine cycles in almost 400 patients with myeloma caused a significant reduction in skeletal complications (defined as pathological fracture, requirement for radiation to bone or surgery, or spinal cord compression), decreased the occurrence of new pathological fractures, and prevented development of hypercalcemia. In addition, this treatment alleviated bone pain and improved quality of life. There was a suggestion in these patients that there may have also been a beneficial effect on overall survival. Pamidronate is therefore now being widely used early in the course of myeloma since it is a relatively nontoxic drug and may have a beneficial effect not only on bone complications. There is no definitive evidence as yet for a beneficial effect on tumor burden or survival, and this will require careful controlled studies in more extended numbers of patients.

Clinical studies have been ongoing for 20 yr in normocalcemic patients with solid tumors and osteolytic bone metastases. All of the available evidence from these studies suggests that drugs that decrease bone resorption, such as the potent bisphosphonates, have a beneficial effect on skeletal complications, including pain and pathological fracture, prevention of hypercalcemia, and improved quality of life. In a recent multicenter trial which consisted of more than 700 patients with stage IV breast cancer with two or more predominantly lytic lesions, with at least one lesion that was 1 cm or greater in diameter, treatment with pamidronate 90 mg iv every 3 to 4 weeks for 12 months in conjunction with chemotherapy or hormonal therapy resulted in a significant

reduction in skeletal complications and bone pain compared with the control group (321). However, there may be an added beneficial effect of the bisphosphonates that is even more important. In experimental studies in which human breast cancer cells are inoculated into the left ventricle of the nude mouse, Sasaki *et al.* (296) have shown that bisphosphonates such as risedronate and ibandronate not only prevent the development of skeletal complications and bone metastases, but they also reduce tumor burden in bone. This likely occurs because the bisphosphonates make bone a less favorable environment for the growth of tumor cells by reducing bone turnover and decreasing the supply of local bone-derived growth factors that also act as tumor growth factors in the bone microenvironment. It is apparent from clinical studies that the use of bisphosphonates reduces significant skeletal morbidity in advanced breast cancer (312–323). These data suggest that drugs that inhibit bone resorption may be useful adjuvant therapy in patients with malignant disease by preventing the growth of tumor cells in the skeleton.

Studies using bisphosphonates in the treatment of prostate cancer metastatic to bone are fewer and less impressive. Clodronate treatment of men with advanced prostate cancer not only resulted in decreased osteoclastic bone resorption, as assessed histomorphometrically, but also in osteomalacia. The authors explained the transient relief of bone pain in the clodronate group to this resultant osteomalacia (474). In a small study of breast and prostate cancer patients with osteosclerotic lesions, treatment with pamidronate resulted in a decrease in bone pain. This response was mostly predicted by a decrease in the urinary marker of bone resorption, deoxypyridinoline (475).

Despite the encouraging results presented in these and other studies, several important questions remain regarding the use of bisphosphonates for treatment of tumor in bone. 1) Will bisphosphonates be useful as adjuvant therapy in tumor types other than breast cancer and myeloma? 2) Will bisphosphonate treatment in cancer improve survival? 3) Will bisphosphonates be beneficial in prevention of bone metastasis if therapy is initiated before the development of bone metastasis in patients with limited disease? 4) Will bisphosphonate therapy in cancer prove to be cost effective? 5) Is there a role for bisphosphonate therapy in osteoblastic metastasis? Although animal studies suggest that the answers to these questions may already be obvious, only prospective trials in humans will provide us with the definitive answers.

References

1. **Paget S** 1889 The distribution of secondary growths in cancer of the breast. Lancet 1:571–572
2. **Parker SL, Tong T, Bolden S, Wingo PA** 1996 Cancer statistics, 1996. CA Cancer J Clin 46:5–27
3. **Hauschka PV, Mavrakos AE, Iafrati MD, Doleman SE, Klagsbrun M** 1986 Growth factors in bone matrix. Isolation of multiple types by affinity chromatography on heparin-sepharose. J Biol Chem 261:12665–12674
4. **Mohan S, Baylink DJ** 1991 Bone growth factors. Clin Orthop 263: 30–48
5. **Pfeilschifter J, Mundy GR** 1987 Modulation of type β transforming growth factor activity in bone cultures by osteotropic hormones. Proc Natl Acad Sci USA 84:2024–2028
6. **Yoneda T** 1996 Mechanisms of preferential metastasis of breast cancer to bone. Int J Oncol 9:103–109
7. **Chattopadhyay N, Mithal A, Brown EM** 1996 The calcium-sensing receptor: a window into the physiology and pathophysiology of mineral ion metabolism. Endocr Rev 17:289–307 [Published erratum appears in Endocr Rev 1996; 17:517]
8. **Parfitt AM** 1987 Bone and plasma calcium homeostasis. Bone 8[Suppl 1]:S1–S8
9. **Parfitt AM** 1976 The actions of parathyroid hormone on bone: relation to bone remodeling and turnover, calcium homeostasis, and metabolic bone diseases. II. PTH and bone cells: bone turnover and plasma calcium regulation. Metabolism 25:909–955
10. **Brown EM** 1982 PTH secretion *in vivo* and *in vitro*. Regulation by calcium and other secretagogues. Miner Electrolyte Metab 8:130–150
11. **Mayer GP, Hurst JG** 1978 Sigmoidal relationship between parathyroid hormone secretion rate and plasma calcium concentration in calves. Endocrinology 102:1036–1042
12. **Samuels MH, Veldhuis J, Cawley C, Urban RJ, Luther M, Bauer R, Mundy G** 1993 Pulsatile secretion of parathyroid hormone in normal young subjects: assessment by deconvolution analysis. J Clin Endocrinol Metab 77:399–403
13. **Brown EM, Gamba G, Riccardi O, Lombardi M, Butters R, Kifor O, Sun A, Hediger M, Lytton J, Hebert SC** 1993 Cloning and characterization of an extracellular Ca^{2+} sensing receptor from bovine parathyroid. Nature 366:575–580
14. **Pollak MR, Brown EM, Yah-Huei WC, Hebert SC, Marx SJ, Steinmann B, Levi T, Seidman CE, Seidman JG** 1994 Mutations in the human Ca^{2+}-sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Cell 75: 1297–1303
15. **Strewler GJ** 1994 Familial benign hypocalciuric hypercalcemia-from the clinic to the calcium sensor. West J Med 160:579–580
16. **Janicic N, Pausova Z, Cole DEC, Hendy GN** 1995 Insertion of an Alu sequence in the Ca^{2+}-sensing receptor gene in familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Am J Hum Genet 56:880–886
17. **Nemeth EF, Heath H** 1995 The calcium receptor and familial benign hypocalciuric hypercalcemia. Curr Opin Endocrinol Diabetes 2:556–561
18. **Pearce SHS, Trump D, Wooding C, Besser GM, Chew SL, Grant DB, Heath DA, Hughes IA, Paterson CR, Whyte MP, Thakker RV** 1995 Calcium-sensing receptor mutations in familial benign hypercalcemia and neonatal hyperparathyroidism. J Clin Invest 96: 2683–2692
19. **Brown EM, Pollak M, Seidman CE, Seidman JG, Chou YW, Riccardi D, Hebert SC** 1995 Calcium-ion-sensing cell-surface receptors. N Engl J Med 333:234–240
20. **Finegold DN, Armitage MM, Galiani M, Matise TC, Pandian MR, Perry YM, Deka R, Ferrell RE** 1994 Preliminary localization of a gene for autosomal dominant hypoparathyroidism to chromosome 3q13. Pediatr Res 36:414–417
21. **Pollak MR, Brown EM, Estep HL, McLaine PN, Kifor O, Park J, Hebert SC, Seidman CE, Seidman JG** 1994 Autosomal dominant hypocalcaemia caused by a Ca^{2+}-sensing receptor gene mutation. Nat Genet 8:303–307
22. **Silver J, Russell J, Sherwood LM** 1985 Regulation by vitamin D metabolites of messenger RNA for preproparathyroid hormone in isolated bovine parathyroid cells. Proc Natl Acad Sci USA 82:4279–4273
23. **Silver J, Naveh-Many T, Mayer H, Schmelzer HJ, Popovtzer MM** 1986 Regulation by vitamin D metabolites of parathyroid hormone gene transcription *in vivo* in the rat. J Clin Invest 78:1296–1301
24. **Jüppner H, Abou-Samra AB, Freeman M, Kong XF, Schipani E, Richards J, Kolakowski LF, Kronenberg HM, Segre GV** 1991 A G protein-linked receptor for parathyroid hormone and parathyroid hormone-related peptide. Science 254:1024–1026
25. **Schipani E, Krese K, Jüppner H** 1995 A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. Science 268:98–100
26. **Schipani E, Langman CB, Parfitt AM, Jensen GS, Kikuchi S, Kooh**

SW, Cole WG, Jüppner H 1996 Constitutively activated receptors for parathyroid hormone and parathyroid hormone-related peptide in Jansen's metaphyseal chondrodysplasia. N Engl J Med 335: 708–714
27. **Usdin TB, Gruber C, Bonner TI** 1995 Identification and functional expression of a receptor selectively recognizing parathyroid hormone, the PTH2 receptor. J Biol Chem 270:15455–15458
28. **Inomata N, Akiyama M, Kubota N, Juppner H** 1995 Characterization of a novel parathyroid hormone (PTH) receptor with specificity for the carboxyl-terminal region of PTH-(1–84). Endocrinology 136:4732–4740
29. **Segre GV, Niall HD, Habener JF, Potts Jr JT** 1974 Metabolism of parathyroid hormone: physiologic and clinical significance. Am J Med 56:774–784
30. **Martin KJ, Hruska KA, Greenwalt A, Klahr S, Slatopolsky E** 1976 Selective uptake of intact parathyroid hormone by the liver: differences between hepatic and renal uptake. J Clin Invest 58:781–788
31. **Hruska KA, Korkor A, Martin K, Slatopolsky E** 1981 Peripheral metabolism of intact parathyroid hormone. Role of liver and kidney and the effect of chronic renal failure. J Clin Invest 67:885–892
32. **D'Amour P, Huet PM, Segre GV, Rosenblatt M** 1981 Characteristics of bovine parathyroid hormone extraction by dog liver *in vivo*. Am J Physiol 241:E208–E214
33. **Segre GV, Perkins AS, Witters LA, Potts Jr JT** 1981 Metabolism of parathyroid hormone by isolated rat Kupffer cells and hepatocytes. J Clin Invest 67:449–457
34. **Segre GV, D'Amour P, Potts Jr JT** 1976 Metabolism of radiodinated bovine parathyroid hormone in the rat. Endocrinology 99:1645–1652
35. **Segre GV, D'Amour P, Hultman A, Potts Jr JT** 1981 Effects of hepatectomy, nephrectomy, and nephrectomy/uremia on the metabolism of parathyroid hormone in the metabolism of parathyroid hormone in the rat. J Clin Invest 67:439–448
36. **Hruska KA, Kopelman R, Rutherford WE, Klahr S, Slatopolsky E, Greenwalt A, Bascom T, Markham J** 1975 Metabolism of immunoreactive parathyroid hormone in the dog. The role of the kidney and the effects of chronic renal disease. J Clin Invest 56: 39–48
37. **Nussbaum SR, Zahradnik RJ, Lavigne JR, Brennan GL, Noxawa-Ung K, Kim LY, Keutmann HT, Wang CA, Potts Jr JT, Segre GV** 1987 Highly sensitive two-site immunoradiometric assay of parathyrin and its clinical utility in evaluating patients with hypercalcemia. Clin Chem 33:1364–1367
38. **Deftos LJ** 1996 Calcitonin. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, ed 3. Raven Press, New York, pp 83–87
39. **Garrett JE, Tamir H, Kifor O, Simin RT, Rogers KV, Mithal A, Gagel RF, Brown EM** 1995 Calcitonin-secreting cells of the thyroid express an extracellular calcium receptor gene. Endocrinology 136: 5202–5211
40. **Barbot N, Calmettes C, Schuffenecker I, Saint-Andre JP, Franc B, Rohmer V, Jallet P, Bigorgne JC** 1994 Pentagastrin stimulation test and early diagnosis of medullary thyroid carcinoma using an immunoradiometric assay of calcitonin: comparison with genetic screening in hereditary medullary thyroid carcinoma. J Clin Endocrinol Metab 78:114–120
41. **Friedman J, Au WY, Raisz LG** 1968 Responses of fetal rat bone to thyrocalcitonin in tissue culture. Endocrinology 82:149–156
42. **Heersche JNM, Marcus R, Aurbach GD** 1974 Calcitonin and the formation of 3′,5′-AMP in bone and kidney. Endocrinology 94: 241–247
43. **Moonga BS, Alam AS, Bevis PJ, Avaldi F, Soncini R, Huang CL, Zaidi M** 1992 Regulation of cystosolic free calcium in isolated osteoclasts by calcitonin. J Endocrinol 132:241–249
44. **Chambers TJ, Magnus CJ** 1982 Calcitonin alters behavior of isolated osteoclasts. J Pathol 136:27–40
45. **Lin HY, Harris TL, Flannery MS, Aruffo A, Kaji EH, Gorn A, Kolakowski Jr LF, Lodish HF, Goldring SR** 1991 Expression cloning of an adenylate cyclase-coupled calcitonin receptor. Science 254:1022–1024
46. **Webb AR, Holick MF** 1988 The role of sunlight in the cutaneous production of vitamin D_3. Annu Rev Nutr 8:375–399
47. **Holick MF** 1996 Vitamin D: photobiology, metabolism, mechanism of action, and clinical applications. In: Favus MJ (ed) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, ed 3. Lippincott-Raven Publishers, Philadelphia, pp 74–81
48. **Kawashima H, Kraut JA, Kurokawa K** 1982 Metabolic acidosis suppresses 25-hydroxyvitamin D_3-1alpha-hydroxylase in the rat kidney. Distinct site and mechanism of action. J Clin Invest 70: 135–140
49. **Bell NH** 1985 Vitamin D-endocrine system. J Clin Invest 76:1–6
50. **DeLuca HR** 1983 Metabolism and mechanism of action of vitamin D-1982. In: Peck WA (ed) Bone and Mineral Research Annual 1. Excerpta Medica, Princeton, NJ, pp 7–73
51. **Norman AW, Roth J, Orci L** 1982 The vitamin D endocrine system: steroid metabolism, hormone receptors, and biological response (calcium binding proteins). Endocr Rev 3:331–366
52. **Gkonos PJ, London R, Hendler ED** 1984 Hypercalcemia and elevated 1,25-dihydroxyvitamin D levels in a patient with end-stage renal disease and active tuberculosis. N Engl J Med 311:1683–1685
53. **Barbour GL, Coburn JW, Slatopolsky E, Norman AW, Horast RL** 1981 Hypercalcemia in an anephric patient with sarcoidosis: evidence for extrarenal generation of 1,25-dihydroxyvitamin D. N Engl J Med 305:440–443
54. **Zerwekh JE, Breslau NA** 1986 Human placental production of 1 alpha,25-dihydroxyvitamin D3: biochemical characterization and production in normal subjects and patients with pseudohypoparathyroidism. J Clin Endocrinol Metab 62:192–196
55. **Holtrop ME, Cox KA, Clark MB, Holick MF, Anast CS** 1981 1,25-dihydroxycholecalciferol stimulates osteoclasts in rat bones in the absence of parathyroid hormone. Endocrinology 108:2293–2301
56. **Takahashi N, Yamana H, Yoshiki S, Roodman GD, Mundy GR, Jones SJ, Boyde A, Suda T** 1988 Osteoclast-like cell formation and its regulation by osteotropic hormones in mouse bone marrow cultures. Endocrinology 122:1373–1382
57. **Suda T, Takahashi N, Martin TJ** 1992 Modulation of osteoclast differentiation. Endocr Rev 13:66–80 [Published erratum appears in Endocr Rev 1992; 13:191]
58. **Liberman UA, Marx SJ** 1996 Vitamin D dependent rickets. In: Favus MJ (ed) Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, ed 3. Lippincott-Raven Publishers, Philadelphia, pp 311–316
59. **Sone T, Marx SJ, Liberman UA, Pike JW** 1990 A unique point mutation in the human vitamin D receptor chromosomal gene confers hereditary resistance to 1,25-dihydroxyvitamin D_3. Mol Endocrinol 4:623–631
60. **Ritchie HH, Hughes MR, Thompson ET, Hochberg Z, Feldman D, Pike JW, O'Malley BW** 1989 An ochre mutation in the vitamin D receptor gene causes hereditary 1,25-dihydroxyvitamin-D3 resistant rickets in three families. Proc Natl Acad Sci USA 86:9783–9787
61. **Hughes MR, Malloy PJ, Kieback DG, Kesterson RA, Pike JW, Feldman D, O'Malley BW** 1988 Point mutations in the human vitamin D receptor gene associated with hypocalcemic rickets. Science 242:1702–1705
62. **St-Arnaud R, Arabian A, Glorieux FH** 1996 Abnormal bone development in mice deficient for the vitamin D 24-hydroxylase gene. J Bone Miner Res 11[Suppl 1]:S126 (Abstract 127)
63. **Favus MJ (ed)** 1996 Laboratory values of importance for calcium metabolism, metabolic bone disease. In: Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, ed 3. Lippincott-Raven Publishers, Philadelphia, p 451
64. **Ralston SH, Gallacher SJ, Patel U, Campbell J, Boyle IT** 1990 Cancer-associated hypercalcemia: morbidity and mortality. Clinical experience in 126 treated patients. Ann Intern Med 112:499–504
65. **Wimalawansa SJ** 1994 Significance of plasma PTH-rp in patients with hypercalcemia of malignancy treated with bisphosphonate. Cancer 73:2223–2230
66. **Nussbaum SR, Younger J, Vandepol CJ, Gagel RF, Zubler MA, Chapman R, Henderson IC, Mallette LE** 1993 Single-dose intravenous therapy with pamidronate for the treatment of hypercalcemia of malignancy: comparison of 30-, 60-, and 90-mg dosages. Am J Med 95:297–304
67. **Mundy GR, Martin TJ** 1982 The hypercalcemia of malignancy: pathogenesis and management. Metabolism 31:1247–1277

68. **Case Records of the Massachusetts General Hospital (case 27461)** 1941 N Engl J Med 22:789–791
69. **Stewart AF, Horst R, Deftos LJ, Cadman EC, Lang R, Broadus AE** 1980 Biochemical evaluation of patients with cancer-associated hypercalcemia: evidence for humoral and nonhumoral groups. N Engl J Med 303:1377–1383
70. **Moseley JM, Kubota M, Diefenbach-Jagger H, Wettenhall REH, Kemp BE, Suva LJ, Rodda CP, Ebeling PR, Hudson PJ, Zajac JD, Martin TJ** 1987 Parathyroid hormone-related protein purified from a human lung cancer cell line. Proc Natl Acad Sci USA 84:5048–5052
71. **Burtis WJ, Wu T, Bunch C, Wysolmerski JJ, Insogna KL, Weir EC, Broadus AE, Stewart AF** 1987 Identification of a novel 17,000-dalton parathyroid hormone-like adenylate cyclase-stimulating protein from a tumor associated with humoral hypercalcemia of malignancy. J Biol Chem 262:7151–7156
72. **Strewler GJ, Stern PH, Jacobs JW, Eveloff J, Klein RF, Leung SC, Rosenblatt M, Nissenson R** 1987 Parathyroid hormone-like protein from human renal carcinoma cells. Structural and functional homology with parathyroid hormone. J Clin Invest 80:1803–1807
73. **Suva LJ, Winslow GA, Wettenhall REH, Hammonds RG, Moseley JM, Dieffenbach-Jagger H, Rodda C, Kemp BE, Rodriguez H, Chen EY, Hudson PJ, Martin TJ, Wood W** 1987 A parathyroid hormone-related protein implicated in malignant hypercalcemia: cloning and expression. Science 237:893–896
74. **Wysolmerski JJ, Broadus AE** 1994 Hypercalcemia of malignancy: the central role of parathyroid hormone-related protein. Annu Rev Med 45:189–200
75. **Strewler GJ, Nissenson RA** 1990 Hypercalcemia in malignancy. West J Med 153:635–640
76. **Strewler GJ, Budayr AA, Clark OH, Nissenson RA** 1993 Production of parathyroid hormone by a malignant nonparathyroid tumor in a hypercalcemic patient. J Clin Endocrinol Metab 76:1373–1375
77. **Nussbaum SR, Gaz RD, Arnold A** 1990 Hypercalcemia and ectopic secretion of parathyroid hormone by an ovarian carcinoma with rearrangement of the gene for parathyroid hormone. N Engl J Med 323:1324–1328
78. **Yoshimoto K, Yamasaki R, Sakai H, Tezuka U, Takahashi M, Iizuka M, Sekiya T, Saito S** 1989 Ectopic production of parathyroid hormone by small cell lung cancer in a patient with hypercalcemia. J Clin Endocrinol Metab 68:976–981
79. **Rizzoli R, Pache JC, Didierjean L, Burger A, Bonjour JP** 1994 A thymoma as a cause of true ectopic hyperparathyroidism. J Clin Endocrinol Metab 79:912–915
80. **Abou-Samra AB, Jüppner H, Force T, Freeman MW, Kong XF, Schipani E, Urena P, Richards J, Bonventre JV, Potts Jr JT, Kronenberg HM, Segre GV** 1992 Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells: a single receptor stimulates intracellular accumulation of both cAMP and inositol triphosphates and increases intracellular free calcium. Proc Natl Acad Sci USA 89:2732–2736
81. **Horiuchi N, Caulfield MP, Fisher JE, Goldman ME, McKee RL, Reagan JE, Levy JJ, Nutt RF, Rodan SB, Schoefield TL, Clemens T, Rosenblatt M** 1987 Similarity of synthetic peptide from human tumor to parathyroid hormone *in vivo* and *in vitro*. Science 238: 1566–1568 [Published erratum appears in Science 1988; 239:128]
82. **Kemp BE, Moseley JM, Rodda CP, Ebeling PR, Wettenhall REH, Stapleton D, Dieffenbach-Jagger H, Ure F, Michelangeli VP, Simmons HA, Raisz LG, Martin TJ** 1987 Parathyroid hormone-related protein of malignancy: active synthetic fragments. Science 238: 1568–1570
83. **Yates AJP, Gutierrez GE, Smolens P, Travis PS, Katz MS, Aufdemorte TB, Boyce BF, Hymer TK, Poser JW, Mundy GR** 1988 Effects of a synthetic peptide of a parathyroid hormone-related protein on calcium homeostasis, renal tubular calcium reabsorption, and bone metabolism *in vivo* and *in vitro* in rodents. J Clin Invest 81:932–938
84. **Sartori L, Weir EC, Stewart AF, Broadus AE, Mangin M, Barrett PQ, Insogna KL** 1988 Synthetic and partially-purified adenylate cyclase-stimulating proteins from tumors associated with humoral hypercalcemia of malignancy inhibit phosphate transport in a PTH-responsive renal cell line. J Clin Endocrinol Metab 66:459–461
85. **Asa SL, Henderson J, Goltzman D, Drucker DJ** 1990 Parathyroid hormone-like peptide in normal and neoplastic human endocrine tissues. J Clin Endocrinol Metab 71:1112–1118
86. **Danks JA, Ebeling PR, Hayman J, Chou ST, Moseley JM, Dunlop J, Kemp BE, Martin TJ** 1989 Parathyroid hormone-related protein: immunohistochemical localization in cancers and in normal skin. J Bone Miner Res 4:273–278
87. **Dunne FP, Lee S, Ratcliffe WA, Hutchesson AC, Bundred NJ, Heath DA** 1993 Parathyroid hormone-related protein (PTHrP) gene expression in solid tumors associated with normocalcemia and hypercalcemia. J Pathol 171:215–221
88. **Wysolmerski JJ, Vasavada R, Foley J, Weir EC, Burtis WJ, Kukreja SC, Guise TA, Broadus AE, Philbrick WM** 1996 Transactivation of the PTHrP gene in squamous carcinomas predicts the occurrence of hypercalcemia in athymic mice. Cancer Res 56:1043–1049
89. **Thiede MA** 1989 The mRNA encoding a parathyroid hormone-like peptide is produced in mammary tissue in response to elevations in serum prolactin. Mol Endocrinol 3:1443–1447
90. **Kremer R, Karaplis AC, Henderson J, Gulliver W, Banville D, Hendy GN, Goltzman D** 1991 Regulation of parathyroid hormone-like peptide in cultured normal human keratinocytes. Effect of growth factors and 1,25 dihydroxyvitamin D_3 on gene expression and secretion. J Clin Invest 87:884–893
91. **Sebag M, Henderson J, Goltzman D, Kremer R** 1994 Regulation of parathyroid hormone-related peptide production in normal human mammary epithelial cells *in vitro*. Am J Physiol 267:C723–C730
92. **Ferrari SL, Rizzoli R, Bonjour J-P** 1994 Effects of epidermal growth factor on parathyroid hormone-related protein production by mammary epithelial cells. J Bone Miner Res 9:639–644
93. **Southby J, Murphy LM, Martin TJ, Gillespie MT** 1996 Cell-specific and regulator-induced promoter usage and messenger ribonucleic acid splicing for parathyroid hormone-related protein. Endocrinology 137:1349–1357
94. **Rizzoli R, Feyen JHM, Grau G, Wohlwend A, Sappino AP, Bonjour J-P** 1994 Regulation of parathyroid hormone-related protein production in a human lung squamous cell carcinoma line. J Endocrinol 143:333–341
95. **Burton PBJ, Knight DE** 1992 Parathyroid hormone-related peptide can regulate growth of human lung cancer cells. FEBS Lett 305: 228–232
96. **Zakalik D, Diep D, Hooks MA, Nissenson RA, Strewler GJ** 1992 Transforming growth factor β increases stability of parathyroid hormone-related protein messenger RNA. J Bone Miner Res 7[Suppl 1]:S118 (Abstract 104A)
97. **Merryman JI, DeWille JW, Werkmeister JR, Capen CC, Rosol TJ** 1994 Effects of transforming growth factor-β on parathyroid hormone-related protein production and ribonucleic acid expression by a squamous carcinoma cell line *in vitro*. Endocrinology 134: 2424–2430
98. **Kiriyama T, Gillespie MT, Glatz JA, Fukumoto S, Moseley JM, Martin TJ** 1993 Transforming growth factor β stimulation of parathyroid hormone-related protein (PTHrP): a paracrine regulator? Mol Cell Endocrinol 92:55–62 [Published erratum appears in Mol Cell Endocrinol 1993; 94:145]
99. **Pirola CJ, Wang H-M, Kamyar A, Wu S, Enomoto H, Sharifi B, Forrester JS, Clemens TL, Fagin JA** 1993 Angiotensin II regulates parathyroid hormone-related protein expression in cultured rat aortic smooth muscle cells through both transcriptional and post-transcriptional mechanisms. J Biol Chem 268:1987–1994
100. **Daifotis AG, Weir EC, Dreyer BE, Broadus AE** 1992 Stretch-induced parathyroid hormone-related protein gene expression in the rat uterus. J Biol Chem 267:23455–23458
101. **Li X, Drucker DJ** 1994 Parathyroid hormone-related peptide is a downstream target for ras and src activation. J Biol Chem 269: 6263–6266
102. **Glatz JA, Heath JK, Southby J, O'Keeffe LM, Kiriyama T, Moseley JM, Martin TJ, Gillespie MT** 1994 Dexamethasone regulation of parathyroid hormone-related protein (PTHrP) expression in a squamous cancer cell line. Mol Cell Endocrinol 101:295–306
103. **Lu C, Ikeda K, Deftos LJ, Gazdar AF, Mangin M, Broadus AE** 1989 Glucocorticoid regulation of parathyroid hormone-related peptide gene transcription in a human neuroendocrine cell line. Mol Endocrinol 3:2034–2040
104. **Thiede MA, Harm SC, Hasson DM, Gardner RM** 1991 *In vivo*

regulation of parathyroid hormone-related peptide messenger ribonucleic acid in the rat uterus by 17 β-estradiol. Endocrinology 128:2317–2323
105. **Paspaliaris V, Petersen DN, Thiede MA** 1995 Steroid regulation of parathyroid hormone-related protein expression and action in the rat uterus. J Steroid Biochem Mol Biol 53:259–265
106. **Foley J, Wysolmerski JJ, Broadus AE, Philbrick WM** 1996 Parathyroid hormone-related protein gene expression in human squamous carcinoma cells is repressed by mutant isoforms of p53. Cancer Res 56:4056–4062
107. **Amling M, Neff L, Tanaka S, Inoue D, Kuida K, Weir E, Philbrick WM, Broadus AE, Baron R** 1997 Bcl-2 lies downstream of parathyroid hormone-related peptide in a signaling pathway that regulates chondrocyte maturation during skeletal development. J Cell Biol 136:205–213
108. **Martin TJ, Moseley JM, Gillespie MT** 1991 Parathyroid hormone-related protein: biochemistry and molecular biology. Crit Rev Biochem Mol Biol 26:377–395
109. **Soifer NE, Dee KE, Insogna KL, Burtis WJ, Matovcik LM, Wu TL, Milstone LM, Broadus AE, Philbrick WM, Stewart AF** 1992 Parathyroid hormone-related protein. Evidence for secretion of a novel mid-region fragment by three different cell types. J Biol Chem 267:18236–18243
110. **Burtis WJ, Fodero JP, Gaich G, Debeyssey M, Stewart AF** 1992 Preliminary characterization of circulating amino- and carboxyl-terminal fragments of parathyroid hormone-related peptide in humoral hypercalcemia of malignancy. J Clin Endocrinol Metab 75: 1110–1114
111. **Burtis WJ, Brady TG, Orloff JJ, Ersbak JB, Warrell Jr RP, Olson BR, Wu TL, Mitnick ME, Broadus AE, Stewart AF** 1990 Immunochemical characterization of circulating parathyroid hormone-related protein in patients with humoral hypercalcemia of cancer. N Engl J Med 322:1106–1112
112. **Orloff JJ, Soifer NE, Fodero JP, Dann P, Burtis WJ** 1993 Accumulation of carboxyl-terminal fragments of parathyroid hormone-related protein in renal failure. Kidney Int 43:1371–1376
113. **Liu B, Amizuka N, Goltzman D, Rabbani SA** 1995 Inhibition of processing of parathyroid hormone-related peptide by antisense furin: effect *in vitro* and *in vivo* on rat Leydig (H-500) tumor cells. Int J Cancer 63:276–281
114. **Bruzzaniti A, Goodge K, Jay P, Taviaux SA, Lam MH, Berta P, Martin TJ, Moseley JM, Gillespie MT** 1996 PC8, a new member of the convertase family Biochem J 314:727–731 [Published erratum appears in Biochem J 1996;316:1007]
115. **Orloff JJ, Reddy D, de Papp AE, Yang KH, Soifer NE, Stewart AF** 1994 Parathyroid hormone-related protein as a prohormone: post-translational processing and receptor interactions. Endocr Rev 15: 40–60
116. **Plawner LL, Philbrick WM, Burtis WJ, Broadus AE, Stewart AF** 1995 Cell type-specific secretion of parathyroid hormone-related protein via the regulated *vs.* the constitutive secretory pathway. J Biol Chem 270:14078–14084
117. **Wu TL, Soifer NE, Burtis WJ, Milstone LM, Stewart AF** 1991 Glycosylation of parathyroid hormone-related peptide secreted by human epidermal keratinocytes. J Clin Endocrinol Metab 73:1002–1007
118. **Orloff JJ, Kats Y, Urena P, Schipani E, Vasavada RC, Philbrick WM, Behal A, Abou-Samra A-B, Segre GV, Jüppner H** 1995 Further evidence for a novel receptor for amino-terminal parathyroid hormone-related protein on keratinocytes and squamous carcinoma cell lines. Endocrinology 136:3016–3023
119. **Karaplis AC, Luz A, Glowacki J, Bronson RT, Tybulewicz VL, Kronenberg HM, Mulligan RC** 1994 Lethal skeletal dysplasia from targeted disruption of the parathyroid hormone-related peptide gene. Genes Dev 8:277–289
120. **Lanske B, Karaplis AC, Lee K, Luz A, Vortkamp A, Pirro A, Karperien M, Defize LHK, Ho C, Mulligan RC, Abou-Samra AB, Jüppner H, Segre GV, Kronenberg HM** 1996 PTH/PTHrP receptor in early development and Indian hedgehog-regulated bone growth. Science 273:663–666
121. **Weir EC, Philbrick WM, Amling M, Neff LA, Baron R, Broadus AE** 1996 Targeted overexpression of parathyroid hormone-related peptide in chondrocytes causes chondrodysplasia and delayed endochondral bone formation. Proc Natl Acad Sci USA 93:10240–10245
122. **Wysolmerski JJ, Broadus AE, Zhou J, Fuchs E, Milstone LM, Philbrick WM** 1994 Overexpression of parathyroid hormone-related protein in the skin of transgenic mice interferes with hair follicle development. Proc Natl Acad Sci USA 91:1133–1137
123. **Wysolmerski JJ, McCaughern-Carucci JF, Daifotis AG, Broadus AE, Philbrick WM** 1995 Overexpression of parathyroid hormone-related protein or parathyroid hormone in transgenic mice impairs branching morphogenesis during mammary gland development. Development 121:3539–3547
124. **Wysolmerski JJ, Carucci JM, Broadus AE, Philbrick WM** 1994 PTH and PTHrP antagonize mammary gland growth and development in transgenic mice. J Bone Miner Res 9[Suppl 1]:S121 (Abstract 4)
125. **Vasavada RC, Cavaliere C, D'Ercole AJ, Dann P, Burtis WJ, Madlener AL, Zawalich K, Zawalich W, Philbrick W, Stewart AF** 1996 Overexpression of parathyroid hormone-related protein in the pancreatic islets of transgenic mice causes islet hyperplasia, hyperinsulinemia, and hypoglycemia. J Biol Chem 271:1200–1208
126. **Massfelder T, Helwig JJ, Stewart AF** 1996 Parathyroid hormone-related protein as a cardiovascular regulatory peptide. Endocrinology 137:3151–3153
127. **Kovacs CS, Lanske B, Hunzelman JL, Guo J, Karaplis AC, Kronenberg HM** 1996 Parathyroid hormone-related peptide (PTHrP) regulates fetal-placental calcium transport through a receptor distinct from the PTH/PTHrP receptor. Proc Natl Acad Sci USA 93: 15233–15238
128. **Nakayama T, Ohtsuru A, Enomoto H, Namba H, Ozeki S, Shibata Y, Yokota T, Nobutoshi M, Ito M, Sekine I, Yamashita S** 1994 Coronary atherosclerotic smooth muscle cells overexpress human parathyroid hormone-related peptides. Biochem Biophys Res Commun 200:1028–1035
129. **Hongo T, Kupfer J, Enomoto H, Sharifi B, Giannella-Neto D, Forrester JS, Singer FR, Goltzman D, Hendy GN, Pirola C, Fagin JA, Clemens TL** 1991 Abundant expression of parathyroid hormone-related protein in primary rat aortic smooth muscle cells accompanies serum-induced proliferation. J Clin Invest 88:1841–1847
130. **Pirola CJ, Wang H-M, Strgacich MI, Kamyar A, Cercek B, Forrester JS, Clemens TL, Fagin JA** 1994 Mechanical stimuli induce vascular parathyroid hormone-related protein gene expression *in vivo* and *in vitro*. Endocrinology 134:2230–2236
131. **Funk JL, Lausier J, Moser AH, Shigenaga JK, Huling S, Nissenson RA, Strewler GJ, Grunfeld C, Feingold KR** 1995 Endotoxin induces parathyroid hormone-related protein gene expression in splenic stromal and smooth muscle cells, not in splenic lymphocytes. Endocrinology 136:3412–3421
132. **Funk JL, Shigenaga JK, Moser AH, Krul EJT, Strewler GJ, Feingold K, Grunfeld C** 1994 Cytokine regulation of parathyroid hormone-related protein messenger ribonucleic acid levels in mouse spleen: paradoxical effects of interferon-γ and interleukin-4. Endocrinology 135:351–358
133. **Funk JL, Krul EJ, Moser AH, Shigenaga JK, Strewler GJ, Grundeld C, Feingold KR** 1992 Endotoxin increases PTHrP mRNA levels in mouse spleen: mediation by tumor necrosis factor. J Clin Invest 92:2546–2552
134. **Vortkamp A, Lee K, Lanske B, Segre GV, Kronenberg HM, Tabin CJ** 1996 Regulation of rate of cartilage differentiation by Indian hedgehog and PTH-related protein. Science 273:613–622
135. **Philbrick WM, Wysolmerski JJ, Galbraith S, Holt E, Orloff JJ, Yang KH, Vasavada RC, Weir EC, Broadus AE, Stewart AF** 1996 Defining the roles of parathyroid hormone-related protein in normal physiology. Physiol Rev 76:127–173
136. **Guise TA, Mundy GR** 1996 Physiological and pathological roles of parathyroid hormone-related peptide. Curr Opin Nephrol Hypertension 5:307–315
137. **Strewler GJ, Nissenson RA** 1994 The parathyroid hormone-related protein as a regulator of normal tissue functions. Curr Opin Endocrinol Diabetes 1:286–292
138. **Ratcliffe WA** 1992 Role of parathyroid hormone-related protein in lactation. Clin Endocrinol (Oxf) 37:402–404
139. **Thiede MA, Rodan GA** 1988 Expression of a calcium-mobilizing

parathyroid hormone-like peptide in lactating mammary tissue. Science 242:278–280
140. **Budayr AA, Halloran BP, King JC, Diep D, Nissenson RA, Strewler GJ** 1989 High levels of parathyroid hormone-like protein in milk. Proc Natl Acad Sci USA 86:7183–7185
141. **Yamamoto M, Fisher JE, Thiede MA, Caulfield MP, Rosenblatt M, Duong LT** 1992 Concentrations of parathyroid hormone-related protein in rat milk change with duration of lactation and interval from previous suckling, but not with milk calcium. Endocrinology 130:741–747
142. **Khosla S, Johansen KL, Ory SJ, O'Brien PC, Kao PC** 1990 Parathyroid hormone-related peptide in lactation and in umbilical cord blood. Mayo Clin Proc 65:1408–1414
143. **Stewart AF, Wu TL, Insogna KL, Milstone LM, Burtis WJ** 1991 Immunoaffinity purification of parathyroid hormone-related protein from bovine milk and human keratinocyte-conditioned medium. J Bone Miner Res 6:305–311
144. **Ratcliffe WA, Thompson GE, Care AD, Peaker M** 1992 Production of parathyroid hormone-related protein by the mammary gland of the goat. J Endocrinol 133:87–93
145. **Lepre F, Grill V, Danks JA** 1990 Hypercalcemia in pregnancy and lactation due to parathyroid hormone-related protein production. Bone Miner 10:S317
146. **Khosla S, van Heerden JA, Garib H, Jackson IT, Danks J, Hayman JA, Martin TJ** 1990 Parathyroid hormone-related protein and hypercalcemia secondary to massive mammary hyperplasia [letter]. N Engl J Med 322:1157
147. **Lepre F, Grill V, Ho PWM, Martin TJ** 1993 Hypercalcemia in pregnancy and lactation associated with parathyroid hormone-related protein. N Engl J Med 328:666–667
148. **Grill V, Hillary J, Ho PM, Law FM, MacIsaac RJ, MacIsaac IA, Moseley JM, Martin TJ** 1992 Parathyroid hormone-related protein: a possible endocrine function in lactation. Clin Endocrinol (Oxf) 37:405–410
149. **Luparello C, Burtis WJ, Raue F, Birch MA, Gallagher JA** 1995 Parathyroid hormone-related peptide and 8701-BC breast cancer cell growth and invasion *in vitro*: evidence from growth-inhibiting and invasion-promoting effects. Mol Cell Endocrinol 111:225–232
150. **Luparello C, Ginty AF, Gallagher JA, Pucci-Minafra I, Minafra S** 1993 Transforming growth factor-β1, β2, β3, urokinase and parathyroid hormone-related peptide expression in 8701-BC breast cancer cells and clones. Differentiation 55:73–80
151. **Li H, Seitz PK, Selvanayagam P, Rajaraman S, Cooper CW** 1996 Effect of endogenously produced parathyroid hormone-related peptide on growth of a human hepatoma cell line (Hep G2). Endocrinology 137:2367–2374
152. **Pandian MR, Morgan CH, Carlton E, Segre GV** 1992 Modified immunoradiometric assay of parathyroid hormone-related protein: clinical application in the differential diagnosis of hypercalcemia. Clin Chem 38:282–288
153. **Schilling T, Pecherstorfer M, Blind E, Leidig G, Ziegler R, Raue F** 1993 Parathyroid hormone-related protein (PTHrP) does not regulate 1,25-dihydroxyvitamin D serum levels in hypercalcemia of malignancy. J Clin Endocrinol Metab 76:801–803
154. **Fraher LJ, Hodsman AB, Jonas K, Saunders D, Rose CI, Henderson JE, Hendy GN, Goltzman D** 1992 A comparison of the *in vivo* biochemical responses to exogenous parathyroid hormone-(1–34)[PTH-(1–34)] and PTH-related peptide-(1–34) in man. J Clin Endocrinol Metab 75:417–423
155. **Everhart-Caye M, Inzucchi SE, Guinness-Henry J, Mitnick MA, Stewart AF** 1996 Parathyroid hormone (PTH)-related protein(1–36) is equipotent to PTH(1–34) in humans. J Clin Endocrinol Metab 81:199–208
156. **Guise TA, Chirgwin JM, Taylor S, Boyce BF, Dunstan CR, Mundy GR** 1993 Deletions of C-terminal end of parathyroid hormone-related protein (PTHrP) have equivalent effects on bone and calcium homeostasis *in vivo*. J Bone Miner Res 8[Suppl 1]:229A (Abstract)
157. **Rosol TJ, Capen CC, Horst RL** 1988 Effects of infusion of human parathyroid hormone-related protein-(1–40) in nude mice: histomorphometric and biochemical investigations. J Bone Miner Res 3:699–706
158. **Guise TA, Chirgwin JM, Boyce B, Favarato G, Mundy GR** 1992 Comparison of similar models of parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHrP) excess in nude mice. J Bone Miner Res 7[Suppl 1]:S241 (Abstract 593)
159. **Stewart AF, Vignery A, Silverglate A, Ravin ND, LiVolsi V, Broadus AE, Baron R** 1982 Quantitative bone histomorphometry in humoral hypercalcemia of malignancy: uncoupling of bone cell activity. J Clin Endocrinol Metab 55:219–227
160. **Nakayama K, Fukumoto S, Takeda S, Takeuchi Y, Ishikawa T, Miura M, Hata K, Hane M, Tamura Y, Tanaka Y, Kitaoka M, Obara T, Ogata E, Matsumoto T** 1996 Differences in bone and vitamin D metabolism between primary hyperparathyroidism and malignancy-associated hypercalcemia. J Clin Endocrinol Metab 81: 607–611
161. **Delmas PD, Demiaux B, Malaval L, Chapuy MC, Edouard C, Meunier PJ** 1986 Serum bone gamma carboxyglutamic acid-containing protein in primary hyperparathyroidism and in malignant hypercalcemia. Comparison with bone histomorphometry. J Clin Invest 77:985–991
162. **Kukreja SC, Rosol TJ, Shevrin DH, York PA** 1988 Quantitative bone histomorphometry in nude mice bearing a human squamous cell lung cancer. J Bone Miner Res 3:341–346
163. **Hulter HN, Halloran BP, Toto RD, Peterson JC** 1985 Long-term control of plasma calcitriol concentration in dogs and humans. Dominant role of plasma calcium concentration in experimental hyperparathyroidism. J Clin Invest 76:695–702
164. **Uy HL, Guise TA, De La Mata J, Taylor SD, Story BM, Dallas MR, Boyce BF, Mundy GR, Roodman GD** 1995 Effects of parathyroid hormone (PTH)-related protein and PTH on osteoclasts and osteoclast precursors *in vivo*. Endocrinology 136:3207–3212
165. **de la Mata J, Uy HL, Guise TA, Story B, Boyce BF, Mundy GR, Roodman GD** 1995 IL-6 enhances hypercalcemia and bone resorption mediated by PTHrP *in vivo*. J Clin Invest 95:2846–2852
166. **Guise TA, Yoneda T, Yates AJP, Mundy GR** 1993 The combined effect of tumor-produced parathyroid hormone-related peptide and transforming growth factor-α enhance hypercalcemia *in vivo* and bone resorption *in vitro*. J Clin Endocrinol Metab 77:40–45
167. **Pizurki L, Rizzoli R, Caverzasio J, Bonjour JP** 1990 Effect of transforming growth factor α and parathyroid hormone-related protein on phosphate transport in renal cells. Am J Physiol 259:F929–F935
168. **Pizurki L, Rizzoli R, Caverzasio J, Bonjour JP** 1991 Stimulation by parathyroid hormone-related protein and transforming growth factor α of phosphate transport in osteoblast-like cells. J Bone Miner Res 6:1235–1241
169. **Sato K, Fujii Y, Kasono K, Ozawa M, Imamura H, Kanaji Y, Kurosawa H, Tsushima T, Shizume** 1989 Parathyroid hormone-related protein and interleukin-1 alpha synergistically stimulate bone resorption *in vitro* and increase the serum calcium concentration in mice *in vivo*. Endocrinology 124:2172–2178
170. **Torring O, Turner RT, Carter WB, Firek AF, Jacobs CA, Heath H** 1992 Inhibition by human interleukin-1 of parathyroid hormone-related peptide effects on renal calcium and phosphorous metabolism in the rat. Endocrinology 131:5–13
171. **Uy HL, Mundy GR, Boyce BF, Story BM, Dunstan CR, Yin JJ, Roodman GD, Guise TA** 1997 Tumor necrosis factor enhances parathyroid hormone-related protein (PTHrP)-induced hypercalcemia and bone resorption without inhibiting bone formation *in vivo*. Cancer Res 57:3194–3199
172. **Allinson ET, Drucker DJ** 1992 Parathyroid hormone-like peptide shares features with members of early response gene family: rapid induction by serum growth factors and cyclohexamide. Cancer Res 52:3103–3109
173. **Burton PB, Moniz C, Knight DE** 1990 Parathyroid hormone-related peptide can function as an autocrine growth factor in human renal cell carcinoma. Biochem Biophys Res Commun 167:1134–1138
174. **Dublin EA, Barnes DM, Wang DY, King RJ, Levison DA** 1993 TGF alpha and TGF beta expression in mammary carcinoma. J Pathol 170:15–22
175. **Herman ME, Katzenellenbogen BS** 1994 Alterations in transforming growth factor-alpha and -beta production and cell responsiveness during the progression of MCF-7 human breast cancer cells to estrogen-autonomous growth. Cancer Res 54:5867–5874
176. **van Roozendaal CEP, Klijn JGM, van Ooijen B, Claassen C,**

Eggermont AMM, Henzen-Logmans SC, Foekens JA 1995 Transforming growth factor beta secretion from primary breast cancer fibroblasts. Mol Cell Endocrinol 111:1–6
177. **Firek A, Jennings J, Tabuenca A, Caulfield M, Garberoglio C, Linkhart T**, TGF-β stimulates PTHrP release from human mammary cancer. Program of the 76th Annual Meeting of The Endocrine Society, Anaheim, CA, 1994, p 395, (Abstract 769)
178. **Guise TA, Taylor SD, Yoneda T, Sasaki A, Wright K, Boyce BF, Chirgwin JM, Mundy GR** 1994 PTHrP expression by breast cancer cells enhance osteolytic bone metastases *in vivo*. J Bone Miner Res 9[Suppl 1]:S128 (Abstract 33)
179. **Percival RC, Yates AJP, Gray RE, Galloway J, Rogers K, Neal FE, Kanis JA** 1985 Mechanisms of malignant hypercalcemia in carcinoma of the breast. Br Med J 291:776–779
180. **Grill V, Ho P, Body JJ, Johanson N, Lee SC, Kukreja SC, Mosely JM, Martin TJ** 1991 Parathyroid hormone-related protein: elevated levels in both humoral hypercalcemia and hypercalcemia complicating metastatic breast cancer. J Clin Endocrinol Metab 73:1309–1315
181. **Isales C, Carcangiu ML, Stewart AF** 1987 Hypercalcemia in breast cancer: reassessment of the mechanism. Am J Med 82:1143–1146
182. **Coleman RE, Rubens RD** 1987 The clinical course of bone metastases from breast cancer. Br J Cancer 55:61–66
183. **Galasko CSB** 1976 Mechanisms of bone destruction in the development of skeletal metastasis. Nature 263:517–518
184. **Boyde A, Maconnachie E, Reid SA, Delling G, Mundy GR** 1986 Scanning electron microscopy in bone pathology: review of methods. Potential and applications. Scanning Electron Microsc 4:1537–1554
185. **Taube T, Elomaa I, Blomqvist C, Beneton MNC, Kanis JA** 1994 Histomorphometric evidence for osteoclast-mediated bone resorption in metastatic breast cancer. Bone 15:161–166
186. **Francini G, Petrioloi R, Maioli E, Gonnelli S, Marsili S, Aquino A, Bruni S** 1993 Hypercalcemia in breast cancer. Clin Exp Metastasis 11:359–367
187. **Kimura S, Adachi I, Yamaguchi K, Suzuki M, Shimada A, Sato Y, Nagaoka K, Abe K** 1985 Stimulation of calcium reabsorption observed in advanced breast cancer patients with hypercalcemia and multiple bone metastasis. Jpn J Cancer Res 76:308–314
188. **Gallacher SJ, Fraser WD, Patel U, Logue FC, Soukop M, Boyle IT, Ralston SH** 1990 Breast cancer-associated hypercalcemia: a reassessment of renal calcium and phosphate handling. Ann Clin Biochem 27:551–556
189. **Coleman RE, Fogelman I, Rubens RD** 1988 Hypercalcemia and breast cancer - an increased humoral component in patients with liver metastases. Eur J Surg Oncol 14:423–428
190. **Budayr AA, Zysset E, Jenzer A, Thiebaud D, Ammann P, Rizzoli R, Jaquet-Muller F, Bonjour JP, Gertz B, Burckhardt P, Halloran BP, Nissenson RA, Strewler GJ** 1994 Effects of treatment of malignancy-associated hypercalcemia on serum parathyroid hormone-related protein. J Bone Miner Res 9:521–526
191. **Heath DA** 1993 Parathyroid hormone related protein. Clin Endocrinol (Oxf) 38:135–136
192. **Southby J, Kissin MW, Danks JA, Hayman JA, Moseley JM, Henderson MA, Bennett RC, Martin TJ** 1990 Immunohistochemical localization of parathyroid hormone-related protein in breast cancer. Cancer Res 51:7710–7716
193. **Liapis H, Crouch EC, Grosso LE, Kitazawa S, Wick MR** 1993 Expression of parathyroid like protein in normal, proliferative, and neoplastic human breast tissues. Am J Pathol 143:1169–1178
194. **Bundred NJ, Walker RA, Ratcliffe WA, Warwick J, Morrison JM, Ratcliffe JG** 1992 Parathyroid hormone related protein and skeletal morbidity in breast cancer. Eur J Cancer 28:690–692
195. **Bundred NJ, Ratcliffe WA, Walker RA, Coley S, Morrison JM, Ratcliffe JG** 1991 Parathyroid hormone related protein and hypercalcaemia in breast cancer. Br Med J 303:1506–1509
196. **Kohno N, Kitazawa S, Fukase M, Sakoda Y, Kanabara Y, Furuya Y, Ohashi O, Ishikawa Y, Saitoh Y** 1994 The expression of parathyroid hormone-related protein in human breast cancer with skeletal metastases. Surg Today 24:215–220
197. **Kremer R, Shustik C, Tabak T, Papavasiliou V, Goltzman D** 1996 Parathyroid hormone-related peptide in hematologic malignancies. Am J Med 100:406–411
198. **Ikeda K, Ohno H, Hane M, Yokoi H, Okada M, Honma T, Yamada A, Tatsumi Y, Tanaka T, Saitoh T, Hirose S, Mori S, Takeuchi Y, Fukumoto S, Terukina S, Iguchi H, Kiriyama T, Ogata E, Matsumoto T** 1994 Development of a sensitive two-site immunoradiometric assay for parathyroid hormone-related peptide: evidence for elevated levels in plasma from patients with adult T-cell leukemia/lymphoma and B-cell lymphoma. J Clin Endocrinol Metab 79:1322–1327
199. **Firkin F, Seymour JF, Watson AM, Grill V, Martin TJ** 1996 Parathyroid hormone-related protein in hypercalcaemia associated with haematological malignancy. Br J Haematol 94:486–492
200. **Seymour JF, Grill V, Martin TJ, Lee N, Firkin F** 1993 Hypercalcemia in the blastic phase of chronic myeloid leukemia associated with elevated parathyroid hormone-related protein. Leukemia 7:1672–1675
201. **Seymour JF, Gagel RF, Hagemeister FB, Dimopoulos MA, Cabanillas F** 1994 Calcitriol production in hypercalcemic and normocalcemic patients with non-Hodgkin lymphoma. Ann Intern Med 121:633–640
202. **Mason RS, Frankel T, Chan YL, Lissner D, Posen S** 1984 Vitamin D conversion by sarcoid lymph node homogenate. Ann Intern Med 100:59–61
203. **Adams JS, Sharma OP, Gacad MA, Singer FR** 1983 Metabolism of 25-hydroxyvitamin D3 by cultured pulmonary alveolar macrophages in sarcoidosis. J Clin Invest 72:1856–1860
204. **Rosenthal N, Insogna KL, Godsall JW, Smaldone L, Waldron JA, Stewart AF** 1985 Elevations in circulating 1,25-dihydroxyvitamin D in three patients with lymphoma-associated hypercalcemia. J Clin Endocrinol Metab 60:29–33
205. **Breslau NA, McGuire JL, Zerwekh JE, Frenkel EP, Pak CYC** 1984 Hypercalcemia associated with increased serum calcitriol levels in three patients with lymphoma. Ann Intern Med 100:1–7
206. **Fetchik DA, Bertolini DR, Sarin PS, Weintraub ST, Mundy GR, Dunn JF** 1986 Production of 1,25-dihydroxyvitamin D_3 by human T-cell lymphotrophic virus-1 transformed lymphocytes. J Clin Invest 78:592–596
207. **Mercier RJ, Thompson JM, Harman GS, Messerschmidt GL** 1988 Recurrent hypercalcemia and elevated 1,25-dihydroxyvitamin D levels in Hodgkin's disease. Am J Med 84:165–168
208. **Munson PL, Tashjian Jr AH, Levine L** 1965 Evidence for parathyroid hormone in nonparathyroid tumors associated with hypercalcemia. Cancer Res 25:1062–1067
209. **Benson Jr RC, Riggs BL, Pickard BM, Arnaud CD** 1974 Radioimmunoassay of parathyroid hormone in hypercalcemic patients with malignant disease. Am J Med 56:821–826
210. **Benson Jr RC, Riggs BL, Pickard BM, Arnaud CD** 1974 Immunoreactive forms of circulating parathyroid hormone in primary and ectopic hyperparathyroidism. J Clin Invest 54:175–181
211. **Sherwood LM, O'Riordan JL, Aurbach GD, Potts Jr JT** 1967 Production of parathyroid hormone by nonparathyroid tumors. J Clin Endocrinol Metab 27:140–146
212. **Simpson EL, Mundy GR, D'Souza SM, Ibbotson KJ, Bockman R, Jacobs JW** 1983 Absence of parathyroid hormone messenger RNA in nonparathyroid tumors associated with hypercalcemia. N Engl J Med 309:325–330
213. **Mori H, Aoki K, Katayama I, Nishioka K, Umeda T** 1996 Humoral hypercalcemia with elevated plasma PTHrP, TNF alpha and IL-6 in cutaneous squamous cell carcinoma. J Dermatol 23:460–462
214. **Sabatini M, Boyce B, Aufdemorte T, Bonewald L, Mundy GR** 1988 Infusions of recombinant human interleukins 1α and 1β cause hypercalcemia in normal mice. Proc Natl Acad Sci USA 85:5235–5239
215. **Boyce BF, Aufdemorte TB, Garrett IR, Yates AJ, Mundy GR** 1989 Effects of interleukin-1 on bone turnover in normal mice. Endocrinology 125:1142–1151
216. **Guise TA, Garrett IR, Bonewald LF, Mundy GR** 1993 The interleukin-1 receptor antagonist inhibits hypercalcemia mediated by interleukin-1. J Bone Miner Res 8:583–587
217. **Black K, Garrett IR, Mundy GR** 1991 Chinese hamster ovarian cells transfected with the murine interleukin-6 gene cause hypercalcemia as well as cachexia, leukocytosis and thrombocytosis in tumor-bearing mice. Endocrinology 128:2657–2659
218. **Weissglas M, Schamhart D, Lowik C, Papapoulos S, Vos P, Kurth**

KH 1995 Hypercalcemia and cosecretion of interleukin-6 and parathyroid hormone related peptide by a human renal cell carcinoma implanted into nude mice. J Urol 153:854–857
219. **Stern PH, Krieger NS, Nissenson RA, Williams RD, Winkler ME, Derynck R, Strewler GJ** 1985 Human transforming growth factor-alpha stimulates bone resorption *in vitro*. J Clin Invest 76:2016–2019
220. **Ibbotson KJ, Harrod J, Gowen M, D'Souza S, Smith D, Winkler ME, Derynk R, Mundy GR** 1986 Human recombinant transforming growth factor α stimulates bone resorption and inhibits formation *in vitro*. Proc Natl Acad Sci USA 83:2228–2232
221. **Ibbotson KJ, Twardzik DR, D'Souza SM, Hargreaves WR, Todaro GJ, Mundy GR** 1985 Stimulation of bone resorption *in vitro* by synthetic transforming growth factor-alpha. Science 228:1007–1009
222. **Tashjian Jr AH, Voelkel EF, Lloyd W, Derynck R, Winkler ME, Levine L** 1986 Actions of growth factors on plasma calcium. Epidermal growth factor and human transforming growth factor-alpha cause elevation of plasma calcium in mice. J Clin Invest 78: 1405–1409
223. **Yates AJ, Boyce BF, Favarato G, Aufdemorte TB, Marcelli C, Kester MB, Walker R, Langton BC, Bonewald LF, Mundy GR** 1992 Expression of human transforming growth factor alpha by Chinese hamster ovarian tumors in nude mice causes hypercalcemia and increased osteoclastic bone resorption. J Bone Miner Res 7:847–853
224. **Bertolini DR, Nedwin GE, Bringman TS, Smith DD, Mundy GR** 1986 Stimulation of bone resorption and inhibition of bone formation *in vitro* by human tumour necrosis factors. Nature 319:516–518
225. **Johnson RA, Boyce B, Mundy GR, Roodman GD** 1989 Tumors producing human tumor necrosis factor induce hypercalcemia and osteoclastic bone resorption in nude mice. Endocrinology 124:1424–1427
226. **Uy HL, Dallas M, Calland JW, Boyce BF, Mundy GR, Roodman GD** 1995 Use of an *in vivo* model to determine the effects of interleukin-1 on cells at different stages in the osteoclast lineage. J Bone Miner Res 10:295–301
227. **Klein DC, Raisz LG** 1970 Prostaglandins: stimulation of bone resorption in tissue culture. Endocrinology 86:1436–1440
228. **Mundy GR** 1995 Hypercalcemia. In: Mundy GR (ed) Bone Remodeling and Its Disorders. Murtin Dunitz, London, pp 88–103
229. **Mundy GR, Wilkinson RB, Heath DA** 1983 Comparative study of available medical therapy for hypercalcemia of malignancy. Am J Med 74:421–432
230. **Tuttle KR, Kunau RT, Loveridge N, Mundy GR** 1991 Altered renal calcium handling in hypercalcemia of malignancy. J Am Soc Nephrol 2:191–199
231. **Hosking DJ, Cowley A, Bucknall CA** 1981 Rehydration in the treatment of severe hypercalcemia. Q J Med 51:473–481
232. **Nussbaum SR** 1993 Pathophysiology and management of severe hypercalcemia. Endocrinol Metab Clin North Am 22:343–362
233. **Rodan GA, Fleisch HA** 1996 Bisphosphonates: mechanisms of action. J Clin Invest 97:2692–2696
234. **Fleisch H** 1991 Bisphosphonates: pharmacology and use in the treatment of tumor-induced hypercalcemia and metastatic bone disease. Drugs 42:919–944
235. **Schmidt A, Rutledge SJ, Endo N, Opas EE, Tanaka H, Wesolowski G, Leu CT, Huang Z, Ramachandaran C, Rodan SB, Rodan GA** 1996 Protein-tyrosine phosphatase activity regulates osteoclast formation and function: inhibition by alendronate. Proc Natl Acad Sci USA 93:3068–3073
236. **Rogers MJ, Watts DJ, Russell RGG, Ji X, Xiong X, Blackburn GM, Bayless AV, Ebetino FH** 1994 Inhibitory effects of bisphosphonates on growth of amoebae of the cellular slime mold *Dictyostelium discoideum*. J Bone Miner Res 9:1029–1039
237. **Singer FR, Ritch PS, Lad TE, Ringenberg QS, Schiller JH, Recker RR, Ryzen E** 1991 Treatment of hypercalcemia of malignancy with intravenous etidronate-a controlled, multicenter study. Arch Intern Med 151:471–476
238. **Gucalp R, Ritch P, Wiernik PH, Sarma PR, Keller A, Richman SP, Tauer K, Neidhart J, Mallette LE, Siegel R, VandePol CJ** 1992 Comparative study of pamidronate disodium and etidronate disodium in the treatment of cancer-related hypercalcemia. J Clin Oncol 10:134–142
239. **Kanis JA, Urwin GH, Gray RE, Beneton MN, McCloskey EV, Hamdy NA, Murray SA** 1987 Effects of intravenous etidronate disodium on skeletal and calcium metabolism. Am J Med 82[Suppl 2A]:55–70
240. **Walls J, Ratcliffe WA, Howell A, Bundred NJ** 1994 Response to intravenous bisphosphonate therapy in hypercalcaemic patients with and without bone metastases: the role of parathyroid hormone-related protein. Br J Cancer 70:169–172
241. **Pecherstorfer M, Schilling T, Blind E, Zimmer-Roth I, Baumgartner G, Ziegler R, Raue F** 1994 Parathyroid hormone-related protein and life expectancy in hypercalcemic cancer patients. J Clin Endocrinol Metab 78:1268–1270
242. **Dodwell DJ, Abbas SK, Morton AR, Howell A** 1991 Parathyroid hormone-related protein$^{(50-69)}$ and response to pamidronate therapy for tumour-induced hypercalcaemia. Eur J Cancer 27:1629–1633
243. **Kanis JA** 1995 Bone and cancer: pathophysiology and treatment of metastases. Bone 17[Suppl 2]:101S–105S
244. **Purohit OP, Radstone CR, Anthony C, Kanis JA, Coleman RE** 1995 A randomised double-blind comparison of intravenous pamidronate and clodronate in the hypercalcaemia of malignancy. Br J Cancer 72:1289–1293
245. **Wimalawansa SJ** 1994 Optimal frequency of administration of pamidronate in patients with hypercalcaemia of malignancy. Clin Endocrinol (Oxf) 41:591–595
246. **Ralston SH, Alzaid AA, Gardner MD, Boyle IT** 1986 Treatment of cancer associated hypercalcemia with combined aminohydroxypropylidene diphosphonate and calcitonin. Br Med J 292: 1549–1551
247. **Adamson BB, Gallacher SJ, Byars J, Ralston SH, Boyle IT, Boyce BF** 1993 Mineralisation defects with pamidronate therapy for Paget's disease. Lancet 342:1459–1460
248. **Binstock MD, Mundy GR** 1980 Effect of calcitonin and glucocorticoids in combination of the hypercalcemia of malignancy. Ann Intern Med 93:269–272
249. **Thiebaud D, Jacquet AF, Burckhardt P** 1990 Fast and effective treatment of malignant hypercalcemia. Arch Intern Med 151:2125–2128
250. **Physicians Desk Reference, ed 51** 1997 Medical Economics Data, Montvale NJ, pp 2176–2178
251. **Brown JH, Kennedy BJ** 1965 Mithramycin in the treatment of disseminated testicular neoplasms. N Engl J Med 272:111–118
252. **Yarbro JW, Kennedy BJ, Barnum CP** 1966 Mithramycin inhibition of ribonucleic acid synthesis. Cancer Res 26:36–39
253. **Ray R, Snyder RC, Thomas S, Koller CA, Miller DM** 1989 Mithramycin blocks protein binding and function of the SV40 early promoter. J Clin Invest 83:2003–2007
254. **Warrell Jr RP, Bockman RS, Coonley CJ, Isaacs M, Staszewski H** 1984 Gallium nitrate inhibits calcium resorption from bone and is effective treatment for cancer-related hypercalcemia. J Clin Invest 73:1487–1490
255. **Warrell Jr RP, Israel R, Frisone M, Snyder T, Gaynor JJ, Bockman RS** 1988 Gallium nitrate for acute treatment of cancer-related hypercalcemia. A randomized, double-blind comparison to calcitonin. Ann Intern Med 108:669–674
256. **Physicians Desk Reference, ed 51** 1997 Medical Economics Data, Montvale NJ, pp 2711–2712
257. **Mundy GR, Rick ME, Turcotte R, Kowalski MA** 1978 Pathogenesis of hypercalcemia in lymphosarcoma cell leukemia. Role of an osteoclast activating factor-like substance and a mechanism of action for glucocorticoid therapy. Am J Med 65:600–606
258. **McCance RA** 1947 Osteomalacia with Looser's nodes (Milkman's syndrome) due to raised resistance to vitamin D acquired about the age of fifteen years. Q J Med 16:33–47
259. **Salassa RM, Jowsey J, Arnaud CD** 1970 Hypophosphatemic osteomalacia associated with "nonendocrine" tumors. N Engl J Med 283:65–70
260. **Cai Q, Hodgson SF, Kao PC, Lennon V, Klee GG, Zinsmiester AR, Kumar R** 1994 Brief report: inhibition of renal phosphate transport by a tumor product in a patient with oncogenic osteomalacia. N Engl J Med 330:1645–1649
261. **Econs MJ, Drezner MK** 1994 Tumor-induced osteomalacia — unveiling a new hormone. N Engl J Med 330:1679–1681
262. **Wilkins GE, Granleese S, Hegele RG, Holden J, Anderson DW,**

Bondy GP 1995 Oncogenic osteomalacia: evidence for a humoral phosphaturic factor. J Clin Endocrinol Metab 80:1628–1634
263. **Lyles KW, Loubaugh B, Paulsen DF, Drezner MK** 1982 Heterotransplantation of prostate cancer from an affected patient creates an animal model for tumor-induced osteomalacia in the athymic nude mouse. Calcif Tissue Int 34:S339
264. **McMurtry CT, Godschalk M, Malluche HH, Geng Z, Adler RA** 1993 Oncogenic osteomalacia associated with metastatic prostate carcinoma: case report and review of the literature. J Am Geriatr Soc 41:983–985
265. **Taylor HC, Fallen MD, Velasco ME** 1984 Oncogenic osteomalacia and inappropriate hormone secretion due to an oat cell carcinoma. Ann Intern Med 101:786–790
266. **Drezner MK, Feinglos MN** 1977 Osteomalacia due to a 1α25-dihydroxycholecalciferol deficiency: association with a giant cell tumor of bone. J Clin Invest 60:1046–1053
267. **Park YK, Unni KK, Beabout JW, Hodgson SF** 1994 Oncogenic osteomalacia: a clinicopathologic study of 17 bone lesions. J Korean Med Sci 9:289–298
268. **Miyauchi A, Fukase M, Tsutsumi M, Fujita T** 1988 Hemangiopericytoma-induced osteomalacia: tumor transplantation in nude mice causes hypophosphatemia and tumor extracts inhibit renal 25-hydroxyvitamin D1-hydroxylase activity. J Clin Endocrinol Metab 67:46–53
269. **Zusman J, Brown DM, Nesbit ME** 1973 Hyperphosphatemia, hyperphosphaturia and hypocalcemia in acute lymphoblastic leukemia. N Engl J Med 289:1335–1340
270. **Hussein AM, Feun LG** 1990 Tumor lysis syndrome after induction chemotherapy in small-cell lung carcinoma. Am J Clin Oncol 13: 10–13
271. **Gold JE, Malamud SC, LaRosa F, Osband ME** 1993 Adoptive chemoimmunotherapy using ex vivo activated memory T-cells and cyclophophamaide: tumor lysis syndrome of a metastatic soft tissue sarcoma. Am J Hematol 44:42–47
272. **Minasian LM, Szatrowski TP, Rosenblum M, Steffens T, Morrison ME, Chapman PB, Williams L, Nathan CF, Houghton AN** 1994 Hemorrhagic tumor necrosis during a pilot trial of tumor necrosis factor-alpha and anti-GD3 ganglioside monoclonal antibody in patients with metastatic melanoma. Blood 83:56–63
273. **Jasek AM, Day HJ** 1994 Acute spontaneous tumor lysis syndrome. Am J Hematol 47:129–131
274. **Aaron AD** 1994 The management of cancer metastatic to bone. J Am Med Assoc 272:1206–1209
275. **Walther HE** 1948 Krebsmetastasen. Bens Schwabe Verlag, Basel, Switzerland
276. **Weiss L** 1992 Comments on hematogenous metastastic patterns in humans as revealed by autopsy. Clin Exp Metastasis 10:191–199
277. **Cifuentes N, Pickren JW** 1979 Metastases from carcinoma of mammary gland: an autopsy study. J Surg Oncol 11:193–205
278. **Eilon G, Mundy GR** 1978 Direct resorption of bone by human breast cancer cells *in vitro*. Nature 276:726–728
279. **Case Records of the Massachusetts General Hospital (case 29–1972)** 1972 N Engl J Med 287:138–143
280. **Szentirmai M, Constantinou C, Rainey JM, Loewenstein JE** 1995 Hypocalcemia due to avid calcium uptake by osteoblastic metastases of prostate cancer. West J Med 163:577–578
281. **Weigand MC, Burshell A, Jaspan J, Odugbesan AO** 1994 Case report: clinical hypocalcemia: the endocrine conference of the Alton Ochsner Medical Institutions and Tulane University Medical Center. Am J Med Sci 19:194–196
282. **Guise TA, Yin JJ, Taylor SD, Kumagai Y, Dallas M, Boyce BF, Yoneda T, Mundy GR** 1996 Evidence for a causal role of parathyroid hormone-related protein in breast cancer-mediated osteolysis. J Clin Invest 98:1544–1548
283. **Batson OV** 1940 The function of the vertebral veins and their role in the spread of metastases. Ann Surg 112:138–149
284. **Dodds PR, Caride VJ, Lytton B** 1981 The role of vertebral veins in the dissemination of prostatic carcinoma. J Urol 126:753–755
285. **Liotta LA, Kohn E** 1990 Cancer invasion and metastases. J Am Med Assoc 263:1123–1126
286. **Galasko CS, Bennett A** 1976 Relationship of bone destruction in skeletal metastases to osteoclast activation and prostaglandins. Nature 263:508–510
287. **Powles TJ, Clark SA, Easty DM, Easty GC, Neville AM** 1973 The inhibition by aspirin and indomethacin of osteolytic tumor deposits and hypercalcaemia in rats with Walker tumour, and its possible application to human breast cancer. Br J Cancer 28:316–321
288. **Shevrin D, Kukreja SC, Ghosh L, Lad TE** 1988 Development of skeletal metastasis by human prostate cancer in athymic nude mice. Clin Exp Metastasis 6:401–409
289. **Kostenuik PJ, Singh G, Suyama KL, Orr FW** 1992 A quantitative model for spontaneous bone metastasis: evidence for a mitogenic effect of bone on Walker 256 cancer cells. Clin Exp Metastasis 10:403–410
290. **Arguello F, Baggs RB, Frantz CN** 1988 A murine model of experimental metastasis to bone and bone marrow. Cancer Res 48:6876–6881
291. **Nakai M, Mundy GR, Williams PJ, Boyce B, Yoneda T** 1992 A synthetic antagonist to laminin inhibits the formation of osteolytic metastases by human melanoma cells in nude mice. Cancer Res 52:5395–5399
292. **Orr FW, Sanchez-Seatman OH, Kostenuik P, Singh G** 1995 Tumor-bone interactions in skeletal metastasis. Clin Orthop 312:19–33
293. **Powell GJ, Southby J, Danks JA, Stillwell RG, Hayman JA, Henderson MA, Bennett RC, Martin TJ** 1991 Localization of parathyroid hormone-related protein in breast cancer metastases: increased incidence in bone compared with other sites. Cancer Res 51:3059–3061
294. **Vargas SJ, Gillespie MT, Powell GJ, Southby J, Danks JA, Moseley JM, Martin TJ** 1992 Localization of parathyroid hormone-related protein mRNA expression and metastatic lesions by *in situ* hybridization. J Bone Miner Res 7:971–980
295. **Bouizar Z, Spyratos F, Deytieux S, de Vernejoul CM, Jullienne A** 1993 Polymerase chain reaction analysis of parathyroid hormone-related protein gene expression in breast cancer patients and occurrence of bone metastases. Cancer Res 53:5076–5078
296. **Sasaki A, Boyce BF, Story B, Wright KR, Chapman M, Boyce R, Mundy GR, Yoneda T** 1995 The bisphosphonate risedronate reduces metastatic human breast cancer burden in bone in nude mice. Cancer Res 55:3551–3557
297. **Cailleau R, Yong R, Olive M, Reeves WJ** 1974 Breast tumor cell lines from pleural effusions. J Natl Cancer Inst 53:661–674
298. **Yin JJ, Taylor SD, Yoneda T, Dallas M, Boyce BF, Kumagai Y, Mundy GR, Guise TA** 1995 Evidence that parathyroid hormone-related protein (PTHrP) causes osteolytic metastases without hypercalcemia. J Bone Miner Res 10[Suppl 1]:S169 (Abstract 122)
299. **Iguchi H, Tanaka S, Ozawa Y, Kashiwakuma T, Kimura T, Hiraga T, Ozawa H, Kono A** 1996 An experimental model of bone metastasis by human lung cancer cells: the role of parathyroid hormone-related protein in bone metastasis. Cancer Res 56:4040–4043
300. **Mbalaviele G, Dunstan CR, Sasaki A, Williams PJ, Mundy GR, Yoneda T** 1996 E-cadherin expression in human breast cancer cells suppresses the development of osteolytic bone metastases in an experimental metastasis model. Cancer Res 56:4063–4070
301. **Williams P, Mbalaviele G, Sasaki A, Dunstan C, Adams R, Bauss F, Mundy GR, Yoneda T** 1995 Multi-step inhibition of breast cancer metastasis to bone. J Bone Miner Res 10[Suppl 1]:S169 (Abstract 121)
302. **Coffey DS** 1996 Prostate cancer metastasis: talking the walk. Nature Med 2:1305–1306
303. **Watanabe H, Carmi P, Hogan V, Raz T, Silletti S, Nabi IR, Raz A** 1991 Purification of human tumor cell autocrine motility factor and molecular cloning of its receptor. J Biol Chem 266:13442–13448
304. **Wantanabe H, Takehana K, Date M, Shinozaki T, Raz A** 1996 Tumor cell autocrine motility factor is the neuroleukin/phosphohexose isomerase polypeptide. Cancer Res 56:2960–2963
305. **Bao L, Loda M, Janmey PA, Stewart R, Anand-Apte B, Zetter BR** 1996 Thymosin β15: a novel regulator of cell tumor motility upregulated in metastatic prostate cancer. Nature Med 2:1322–1328
306. **Lemieux PM, Oesterreich S, Guise TA, Fuqua SAW** 1996 Transfection of Hsp27 in MDA-MB-231 breast cancer cells decreases osteolytic metastases *in vivo*. Breast Cancer Res Treat 41:282 (Abstract 440)
307. **Kikuchi T, Takeuchi Y, Matsumoto T, Fujita T, Ogata E** 1996 Direct interaction of melanoma cells with bone marrow stromal

cells via $\alpha_4\beta_1$ integrin/VCAM-1 inhibits osteoblastic differentiation. J Bone Miner Res 11[Suppl 1]:S117 (Abstract 90)
308. **Matsuura N, Puzon-McLaughlin W, Irie A, Morikawa Y, Kakudo K, Takada Y** 1996 Induction of experimental bone metastasis in mice by transfection of integrin $\alpha4\beta1$ into tumor cells. Am J Pathol 148:55–61
309. **Tondravi M, Quiroz M, Wang M, Teitelbaum SL** 1996 Increased rate of skeletal metastases by human breast cancer cells expressing low levels of $\alpha_v\beta_3$ integrin. J Bone Miner Res 11[Suppl 1]:S98 (Abstract 15)
310. **van der Pluijm G, Vloedgraven HJM, Ivanov B, Robey FA, Grzesik WJ, Robey PG, Papapoulos SE, Löwik CWGM** 1996 Bone sialoprotein peptides are potent inhibitors of breast cancer cell adhesion to bone. Cancer Res 56:1948–1955
311. **Weber GF, Ashkar S, Glimcher MJ, Cantor H** 1996 Receptor-ligand interaction between CD44 and osteopontin (Eta-1). Science 271:509–512
312. **Thurlimann B, Morant R, Jungi WF, Radziwill A** 1994 Pamidronate for pain control in patients with malignant osteolytic bone disease: a prospective dose-effect study. Support Care Cancer 2:61–65
313. **Glover D, Lipton A, Keller A, Miller AA, Browning S, Fram RJ, George S, Zelenakas K, Macerata RS, Seaman JJ** 1994 Intravenous pamidronate disodium treatment of bone metastases in patients with breast cancer. A dose-seeking study. Cancer 74:2949–2955
314. **van Holten-Verzantvoort AT, Bijvoet OL, Cleton FJ, Hermans J, Kroon HM, Harinck HIJ, Vermes P, Elte JW, Neijt JP, Beex LV, Blijham G** 1987 Reduced morbidity from skeletal metastases in breast cancer patients during long-term bisphosphonate (APD) treatment. Lancet 2:983–985
315. **Cleton FJ, van Holten-Verzantvoort AT, Bijvoet OL** 1989 Effect of long-term bisphosphonate treatment on morbidity due to bone metastases in breast cancer patients. Recent Results Cancer Res 116:73–78
316. **Burckhardt P, Thiebaud D, Peres L, von Fliedner V** 1989 Treatment of tumor-induced osteolysis by APD. Recent Results Cancer Res 116:54–66
317. **Grutters JC, Hermus AR, de Mulder PH, Beex LV** 1993 Long-term follow up of breast cancer patients treated for hypercalcaemia with aminohydroxypropylidene bisphosphonate (APD). Breast Cancer Res Treat 25:277–281
318. **Paterson AHG, Ernst DS, Powles TJ, Ashley S, McMloskey EV, Kanis J** 1991 Treatment of skeletal disease in breast cancer with clodronate. Bone 2[Suppl 1]:S25–S30
319. **Morton AR, Cantrill JA, Pilliai GV, McMahon A, Anderson DC, Howell A** 1988 Sclerosis of lytic bone metastases after disodium aminohydroxypropylidene bisphosphonate (APD) in patients with breast carcinoma. Br Med J 297:772–773
320. **Paterson AH, Powles TJ, Kanis JA, McCloskey E, Hanson J, Ashley S** 1993 Double-blind controlled trial of oral clodronate in patients with bone metastases from breast cancer. J Clin Oncol 11: 59–65
321. **Hortobagyi GN, Theriault RL, Porter L, Blayney D, Lipton A, Sinoff C, Wheeler H, Simeone JF, Seaman J, Knight RD** 1996 Efficacy of pamidronate in reducing skeletal complications in patients with breast cancer and lytic bone metastases. Protocol 19 Aredia Breast Cancer Study Group. N Engl J Med 335:1785–1791
322. **Delmas PD** 1996 Bisphosphonates in the treatment of bone diseases. N Engl J Med 335:1836–1837
323. **Delmas PD, Balena R, Confravreaux E, Hardouin C, Hardy P, Bremond A** 1997 Bisphosphonate risedronate prevents bone loss in women with artificial menopause due to chemotherapy of breast cancer: a double blind placebo-controlled trial. J Clin Oncol 15: 955–962
324. **Bassani D, Sabatini M, Scanziani E, De Francesco L, Coccioli G, Guaitani A, Bartosek I** 1990 Bone invasion by Walker 256 carcinoma, line A in young and adult rats: effects of etidronate. Oncology 47:160–165
325. **Hall DG, Stoica G** 1994 Effect of the bisphosphonate risedronate on bone metastases in a rat mammary adenocarcinoma model system. J Bone Miner Res 9:221–230
326. **Centrella M, Horowitz MC, Wozney JM, McCarthy TL** 1994 Transforming growth factor β gene family members and bone. Endocr Rev 15:27–39
327. **Derynck R** 1994 TGF-β-receptor-mediated signaling. Trends Biochem Sci 19:548–553
328. **Massagué J, Hata A, Liu F** 1997 TGF-β signalling through the Smad pathway. Trends Cell Biol 7:187–192
329. **Arteaga CL, Dugger TC, Hurd SD** 1996 The multifunctional role of transforming growth factor (TGF)-β3 on mammary epithelial cell biology. Breast Cancer Res Treat 38:49–56
330. **Nørgaard P, Hougaard S, Poulsen HS, Spang-Thomsen M** 1995 Transforming growth factor-β and cancer. Cancer Treat Rev 21: 367–403
331. **Wieser R, Attisano L, Wrana JL, Massagué J** 1993 Signaling activity of transforming growth factor β type II receptors lacking specific domains in the cytoplasmic region. Mol Cell Biol 13:7239–7247
332. **Wrana JL, Attisano L, Wieser R, Ventura F, Massagué J** 1994 Mechanism of activation of the TGF-β receptor. Nature 370:341–347
333. **Yin JJ, Taylor SD, Dallas M, Massagué J, Mundy GR, Guise TA** 1996 Dominant negative blockade of the transforming growth factor β type II receptor decreases breast cancer-mediated osteolysis. J Bone Miner Res 11[Suppl 1]:S139 (Abstract 180)
334. **Sasaki A, Williams P, Mundy GR, Yoneda T** 1994 Osteolysis and tumor growth are enhanced in sites of increased bone turnover *in vivo*. J Bone Miner Res 9[Suppl 1]:S294 (Abstract B257)
335. **Yoneda T, Williams P, Dunstan C, Chavez J, Niewolna M, Mundy GR** 1995 Growth of metastatic cancer cells in bone is enhanced by bone-derived insulin-like growth factors (IGFs). J Bone Miner Res 10[Suppl 1]:P269 (Abstract)
336. **Orr W, Varani J, Gondex MK, Ward PA, Mundy GR** 1979 Chemotactic responses of tumor cells to products of resorbing bone. Science 203:176–179
337. **Mundy GR, DeMartino S, Rowe DW** 1981 Collagen and collagen fragments are chemotactic for tumor cells. J Clin Invest 68:1102–1105
338. **Doerr ME, Jones JI** 1996 The role of integrins and extracellular matrix proteins in the insulin-like growth factor I-stimulated chemotaxis of human breast cancer cells. J Biol Chem 271:2443–2447
339. **Paling MR, Pope TL** 1988 Computed tomography of isolated osteoblastic colon metastases in the bony pelvis. J Comput Tomogr 12:203–207
340. **Kingston JE, Plowman PN, Smith BF, Garvan NJ** 1986 Differentiated astrocytoma with osteoblastic skeletal metastases in a child. Child Nerv System 2:219–221
341. **Gamis AS, Egelhoff J, Roloson G, Young J, Woods GM, Newman R, Freeman AI** 1990 Diffuse bony metastases at presentation in a child with glioblastoma multiforme. A case report. Cancer 66:180–184
342. **McLennan MK** 1991 Case report 657: malignant epithelial thymoma with osteoblastic metastases. Skeletal Radiol 20:141–144
343. **Giordano N, Nardi P, Vigni P, Palumbo F, Battisti E, Gennari C** 1994 Osteoblastic metastases from carcinoid tumor [letter]. Clin Exp Rheumatol 12:228–229
344. **Liaw CC, Ho YS, Koon-Kwan NG, Chen TL, Tzann WC** 1994 Nasopharyngeal carcinoma with brain metastasis: a case report. J Neurooncol 22:227–230
345. **Pingi A, Trasimeni G, Di Biasi C, Gualdi G, Piazza G, Corsi F, Chiappetta F** 1995 Diffuse leptomeningeal gliomatosis with osteoblastic metastases and no evidence of intraaxial lesions. Am J Neuroradiol 16:1018–1020
346. **Pederson RT, Haidak DJ, Ferris RA, Macdonald JS, Schein PS** 1976 Osteoblastic bone metastasis in Zollinger-Ellison syndrome. Radiology 118:63–64
347. **George J, Lai FM** 1995 Metastatic cervical carcinoma presenting as psoas abscess and osteoblastic and lytic bony metastases. Singapore Med J 36:224–227
348. **Koutsilieris M** 1995 Skeletal metastases in advanced prostate cancer: cell biology and therapy. Crit Rev Oncol Hematol 18:51–64
349. **Thalmann GN, Anezinis PE, Chang S, Zhau H, Kim EE, Hopwood VL, Pathak S, von Eschenbach AC, Chung LWK** 1994 Androgen-independent cancer progression and bone metastasis in the LNCaP model of human prostate cancer. Cancer Res 54:2577–2581
350. **Greenberg NM, DeMayo F, Finegold MJ, Medina D, Tilley WD, Aspinall JO, Cunha GR, Donjacour AA, Matusik RJ, Rosen JM**

1995 Prostate cancer in a transgenic mouse. Proc Natl Acad Sci USA 92:3439–3443
351. **Gingrich JR, Barrios RJ, Morton RA, Boyce BF, DeMayo FJ, Finegold JJ, Angelopoulou R, Rosen JM, Greenberg NM** 1996 Metastatic prostate cancer in a transgenic mouse. Cancer Res 56:4096–4102
352. **Achbarou A, Kaiser S, Tremblay G, Ste-Marie L-G, Brodt P, Goltzman D, Rabbani SA** 1994 Urokinase overproduction results in increased skeletal metastasis by prostate cancer cells *in vivo*. Cancer Res 54:2372–2377
353. **Charhon SA, Chapuy MC, Delvin EE, Valentin-Opran A, Edouard CM, Meunier PJ** 1983 Histomorphometric analysis of sclerotic bone metastases from prostatic carcinoma special reference to osteomalacia. Cancer 51:918–924
354. **Simpson E, Harrod J, Eilon G, Jacobs JW, Mundy GR** 1985 Identification of a messenger ribonucleic acid fraction in human prostatic cancer cells coding for a novel osteoblast-stimulating factor. Endocrinology 117:1615–1620
355. **Martínez J, Silva S, Sántibañez JF** 1996 Prostate-derived soluble factors block osteoblast differentiation in culture. J Cell Biochem 61:18–25
356. **Koutsilieris M, Rabbani SA, Bennett HP, Goltzman D** 1987 Characteristics of prostate-derived growth factors for cells of the osteoblast phenotype. J Clin Invest 80:941–946
357. **Robey PG, Young MF, Flanders KC, Roche NS, Kondaiah P, Reddi AH, Termine JD, Sporn MB, Roberts AB** 1987 Osteoblasts synthesize and respond to transforming growth factor-type-β *in vitro*. J Cell Biol 105:457–463
358. **Lyons RM, Keski-Oja J, Moses HL** 1988 Proteolytic activation of latent transforming growth factor-β from fibroblast-conditioned medium. J Cell Biol 106:1659–1665
359. **Miyazano K, Hellman U, Wernstedt C** 1988 Latent high molecular weight complex of transforming growth factor β1. Purification from human platelets and structural characterization. J Biol Chem 263:6407–6415
360. **Noda M, Rodan GA** 1987 Type beta transforming growth factor (TGF beta) regulation of alkaline phosphatase expression and other phenotype-related mRNAs in osteoblastic rat osteosarcoma cells. J Cell Physiol 133:426–437
361. **Noda M, Yoon K, Prince CW, Butler WT, Rodan GA** 1988 Transcriptional regulation of osteopontin production in rat osteosarcoma cells by type β transforming growth factor. J Biol Chem 263:13916–13921
362. **Noda M** 1989 Transcriptional regulation of osteocalcin production by transforming growth factor-β in rat osteoblast-like cells. Endocrinology 124:612–617
363. **Ignotz RA, Massague J** 1986 Transforming growth factor-beta stimulates the expression of fibronectin and collagen and their incorporation into the extracellular matrix. J Biol Chem 261:4337–4345
364. **Sato K, Kasono K, Fuji Y, Kawakami M, Tsushima T, Shizume K** 1987 Tumor necrosis factor type α stimulates mouse osteoblast-like cells (MC3T3–E1) to produce macrophage-colony stimulating activity and prostaglandin E2. Biochem Biophys Res Commun 145: 323–329
365. **Ignotz R, Endo T, Massague J** 1987 Regulation of fibronectin and type 1 collagen mRNA levels by transforming growth factor-beta. J Biol Chem 262:6443–6446
366. **Sporn MB, Roberts AB, Wakefield LM, de Crombrugghe B** 1987 Some recent advances in the chemistry and biology of transforming growth factor-β. J Cell Biol 105:1039–1045
367. **Noda M, Camilliere JJ** 1989 *In vivo* stimulation of bone formation by transforming growth factor-β. Endocrinology 124:2991–2994
368. **Marcelli C, Yates AJP, Mundy GR** 1990 *In vivo* effects of human recombinant transforming growth factor beta on bone turnover in normal mice. J Bone Miner Res 5:1087–1096
369. **Mackie EJ, Trechsel U** 1990 Stimulation of bone formation *in vivo* by transforming growth factor β: remodeling of woven bone and lack of inhibition by indomethacin. Bone 11:295–300
370. **Erlebacher A, Derynck R** 1996 Increased expression of TGF-β2 in osteoblasts results in an osteoporosis-like phenotype. J Cell Biol 132:195–210
371. **Marquardt H, Lioubin MN, Ikeda T** 1987 Complete amino acid sequence of human transforming growth factor type beta 2. J Biol Chem 262:12127–12130
372. **Muir GH, Butta A, Shearer RJ, Fisher C, Dearnaley DP, Flanders KC, Sporn MB, Colletta AA** 1994 Induction of transforming growth factor beta in hormonally treated human prostate cancer. Br J Cancer 69:130–134
373. **Steiner MS, Zhou Z-Z, Tonb DC, Barrack ER** 1994 Expression of transforming growth factor-β1 in prostate cancer. Endocrinology 135:2240–2247
374. **Eklov S, Funa K, Nodgren H, Olofsson A, Kanzaki T, Miyazono K, Nilsson S** 1993 Lack of the latent transforming growth factor beta binding protein in malignant, but not benign prostatic tissue. Cancer Res 53:3193–3197
375. **Steiner MS, Barrack ER** 1992 Transforming growth factor-beta 1 overproduction in prostate cancer: effects on growth *in vivo* and *in vitro*. Mol Endocrinol 6:15–25
376. **Jones JI, Clemmons DR** 1995 Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 16:3–34
377. **Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A** 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I and type 1 IGF receptor. Cell 75:59–72
378. **Canalis E** 1994 Editorial: skeletal growth factors and aging. J Clin Endocrinol Metab 78:1009–1010
379. **Canalis E, Gabbitas B** 1995 Skeletal growth factors regulate the synthesis of insulin-like growth factor binding protein-5 in bone cell cultures. J Biol Chem 270:10771–10776
380. **Gabbitas B, Canalis E** 1995 Bone morphogenetic protein-2 inhibits the synthesis of insulin-like growth factor-binding protein-5 in bone cell cultures. Endocrinology 136:2397–2403
381. **Dong Y, Canalis E** 1995 Insulin-like growth factor I and retinoic acid induce the synthesis of IGF-binding protein 5 in rat osteoblastic cells. Endocrinology 136:2000–2006
382. **Cohen P, Peehl DM, Lamson G, Rosenfeld RG** 1991 Insulin-like growth factors (IGFs), IGF receptors and IGF binding proteins in primary cultures of prostate epithelial cells. J Clin Endocrinol Metab 73:401–407
383. **Cohen P, Peehl DM, Rosenfeld RG** 1994 The IGF axis in the prostate. Horm Metab Res 26:81–84
384. **Cohen P, Graves HC, Peehl DM, Kamarei M, Giudice LC, Rosenfeld RG** 1992 Prostate specific antigen (PSA) is an insulin-like growth factor binding protein-3 (IGFBP-3) protease found in seminal plasma. J Clin Endocrinol Metab 75:1046–1053
385. **Conover CA, Perry JE, Tindall DJ** 1995 Endogenous cathepsin D-mediated hydrolysis of insulin-like growth factor-binding proteins in cultured human prostatic carcinoma cells. J Clin Endocrinol Metab 80:987–993
386. **Koutsilieris M, Frenette G, Lazure C, Lehoux JG, Govindan MV, Polychronakos C** 1993 Urokinase-type plasminogen activator: a paracrine factor regulating the bioavailability of IGFs in PA-III cell-induced osteoblastic metastases. Anticancer Res 13:481–486
387. **Cohen P, Peehl DM, Stamey TA, Wilson KF, Clemmons DR, Rosenfeld RG** 1993 Elevated levels of insulin-like growth factor-binding protein-2 in the serum of prostate cancer patients. J Clin Endocrinol Metab 76:1031–1035
388. **Kanety H, Madjar Y, Dagan Y, Levi J, Papa MZ, Pariente C, Goldwasser B, Karasik A** 1993 Serum insulin-like growth factor-binding protein-2 (IGFBP-2) is increased and IGFBP-3 is decreased in patients with prostate cancer: correlation with serum prostate-specific antigen. J Clin Endocrinol Metab 77:229–233
389. **Tennant MK, Thrasher JB, Twomey PA, Birnbaum RS, Plymate SR** 1996 Insulin-like growth factor-binding protein-2 and -3 expression in benign human prostate epithelium, prostate intraepithelial neoplasia, and adenocarcinoma of the prostate. J Clin Endocrinol Metab 81:411–420
390. **Koutsilieris M, Polychronakos C** 1992 Proteinolytic activity against IGF-binding proteins involved in the paracrine interactions between prostate adenocarcinoma cells and osteoblasts. Anticancer Res 12:905–910
391. **Watt KW, Lee P-J, M'Timkulu T, Chan WP, Loor R** 1986 Human prostate-specific antigen: structural and functional similarity with serine proteases. Proc Natl Acad Sci USA 83:3166–3170
392. **Stamey TA, Yang N, Hay AR, McNeal JE, Frieha FS, Redwine E**

1987 Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N Engl J Med 317:909–916
393. **Murphy GP** 1995 The second Stanford conference on international standardization of prostate specific antigen assays. Cancer 75:122–128
394. **Zagars GK, von Eschenbach AC** 1993 Prostate-specific antigen. An important marker for prostate cancer treated by external beam radiation therapy. Cancer 72:538–548
395. **Henttu P, Liao S, Vihko P** 1992 Androgens up-regulate the human prostate-specific antigen messenger ribonucleic acid (mRNA), but down-regulate the prostatic acid phosphatase mRNA in the LNCaP cell line. Endocrinology 130:766–772
396. **Oesterling JE** 1991 Prostate specific antigen: a critical assessment of the most useful tumor marker for adenocarcinoma of the prostate. J Urol 145:907–923
397. **Chybowski FM, Keller L, Bergstralh EJ, Oesterling JE** 1991 Predicting radionuclide bone scan findings inpatients with newly diagnosed, untreated prostate cancer: prostate specific antigen is superior to all other clinical parameters. J Urol 145:313–318
398. **Oesterling JE** 1993 Using PSA to eliminate the staging radionuclide bone scan. Significant economic implications. Urol Clin North Am 20:705–711
399. **Oesterling JE** 1995 Using prostate-specific antigen to eliminate unnecessary diagnostic tests: significant worldwide economic implications. Urology 46[3 Suppl A]:26–33
400. **Lorente JA, Morote J, Raventos C, Encabo G, Valenzuela H** 1996 Clinical efficacy of bone alkaline phosphatase and prostate specific antigen in the diagnosis of bone metastasis in prostate cancer. J Urol 155:1348–1351
401. **Yu H, Giai M, Diamandis EP, Katsaros D, Sutherland DJA, Levesque MA, Roagna R, Ponzone R, Sismondi P** 1995 Prostate-specific antigen is a new favorable prognostic indicator for women with breast cancer. Cancer Res 55:2104–2110
402. **Monne M, Croce CM, Yu H, Diamandis EP** 1994 Molecular characterization of prostate-specific antigen messenger RNA expressed in breast tumors. Cancer Res 54:6344–6347
403. **Yu H, Diamandis EP, Levesque M, Sismondi P, Zola P, Katsaros D** 1994 Ectopic production of prostate specific antigen by a breast tumor metastatic to the ovary. J Clin Lab Anal 8:251–253
404. **Yu H, Diamandis EP, Sutherland DJ** 1994 Immunoreactive prostate-specific antigen levels in female and male breast tumors and its association with steroid hormone receptors and patient age. Clin Biochem 27:75–79
405. **Fielder PJ, Rosenfeld RG, Graves HC, Grandbois K, Maack CA, Sawamura S, Ogawa Y, Sommer A, Cohen P** 1994 Biochemical analysis of prostate specific antigen-proteolyzed insulin-like growth factor binding protein-3. Growth Regul 4:164–172
406. **Killian CS, Corral DA, Kawinski E, Constantine RI** 1993 Mitogenic response of osteoblast cells to prostate-specific antigen suggests an activation of latent TGF-beta and a proteolytic modulation of cell adhesion receptors. Biochem Biophys Res Commun 192: 940–947
407. **Cramer SD, Chen Z, Peehl DM** 1996 Prostate specific antigen cleaves parathyroid hormone-related protein in the PTH-like domain: inactivation of PTHrP-stimulated cAMP accumulation in mouse osteoblasts. J Urol 156:526–531
408. **Rabbani SA, Desjardins J, Bell AW, Banville D, Mazar A, Henkin J, Goltzman D** 1990 An amino-terminal fragment of urokinase isolated from a prostate cancer cell line (PC-3) is mitogenic for osteoblast-like cells. Biochem Biophys Res Commun 173:1058–1064
409. **Liu DF, Rabbani SA** 1995 Induction of urinary plasminogen activator by retinoic acid results in increased invasiveness of human prostate cancer cells PC-3. Prostate 27:269–276
410. **Dunstan CR, Garrett IR, Adams R, Burgess W, Jaye M, Youngs T, Boyce R, Mundy GR** 1995 Systemic fibroblast growth factor (GFG-1) prevents bone loss, increases new bone formation and restores trabecular microarchitecture in ovariectomized rats. J Bone Miner Res 10[Suppl 1]:S198 (Abstract P279)
411. **Matuo Y, Nishi N, Matsui S, Sandberg AA, Isaacs JT, Wada F** 1987 Heparin binding affinity of rat prostate growth factor in normal and cancerous prostate: partial purification and characterization of rat prostatic growth factor in the Dunning tumor. Cancer Res 47:188–192
412. **Mansson PE, Adams P, Kan M, McKeehan WL** 1989 HBGF-1 gene expression in normal rat prostate and two transplantable rat prostate tumors. Cancer Res 49:2485–2494
413. **Nishikawa K, Yoshitake Y, Minemura M, Yamada K, Matuo Y** 1992 Localization of basic fibroblast growth factor (bFGF) in a metastatic cell line (AT-3) established from the Dunning prostatic carcinoma of rat: application of a specific monoclonal antibody. Adv Exp Med Biol 324:131–139
414. **Nakamoto T, Chang CS, Li AK, Chodak GW** 1992 Basic fibroblast growth factor in human prostate cancer cells. Cancer Res 52:571–577
415. **Shain SA, Ke LD, Wong G, Karaganis AG** 1992 Rat prostate cancer cell line-specific production and apparent secretion of heparin-binding growth factors. Cell Growth Differ 3:249–258
416. **Izbicka E, Dunstan C, Esparza J, Jacobs C, Sabatini M, Mundy GR** 1996 Human amniotic tumor that induces new bone formation *in vivo* produces growth-regulatory activity *in vitro* for osteoblasts identified as an extended form of fibroblast growth factor. Cancer Res 56:633–636
417. **Jimi E, Shuto T, Ikebe T, Jingushi S, Hirata M, Koga T** 1996 Basic fibroblast growth factor inhibits osteoclast-like cell formation. J Cell Physiol 168:395–402
418. **Urist MR** 1965 Bone: formation by autoinduction. Science 151:893–899
419. **Harris SE, Bonewald LF, Harris MA, Sabatini M, Dallas S, Feng JQ, Ghosh-Choudhury N, Wozney J, Mundy GR** 1994 Effects of transforming growth factor beta on bone nodule formation and expression of bone morphogenetic protein 2, osteocalcin, osteopontin, alkaline phosphatase, and type I collagen mRNA in long-term cultures of fetal rat calvarial osteoblasts. J Bone Miner Res 9:855–863
420. **Ghosh-Choudhury N, Harris MA, Feng JQ, Mundy GR, Harris SE** 1994 Expression of the BMP 2 gene during bone cell differentiation. Crit Rev Eukaryot Gene Expr 4:345–355
421. **Harris SE, Feng JQ, Harris MA, Ghosh-Choudhury N, Dallas MR, Wozney J, Mundy GR** 1995 Recombinant bone morphogenetic protein 2 accelerates bone cell differentiation and stimulates BMP-2 mRNA expression and BMP-2 promoter activity in primary fetal rat calvarial osteoblast cultures. Mol Cell Differ 3:137–155
422. **Maliakal JC, Asahina I, Hauschka PV, Sampath TK** 1994 Osteogenic protein-1 (BMP-7) inhibits cell proliferation and stimulates the expression of markers characteristic of osteoblast phenotype in rat osteosarcoma (17/2.8) cells. Growth Factors 11:227–234
423. **Sampath TK, Maliakal JC, Hauschka PV, Jones WK, Sasak H, Tucker RF, White KH, Coughlin JE, Tucker MM, Pang RH, Corbett C, Özkaynak E, Oppermann H, Rueger DC** 1992 Recombinant human osteogenic protein-1 (hOP-1) induces new bone formation *in vivo* with a specific activity comparable with natural bovine osteogenic protein and stimulates osteoblast proliferation and differentiation *in vitro*. J Biol Chem 267:20352–20362
424. **Shafritz AB, Shore EM, Gannon FH, Zasloff MA, Taub R, Muenke M, Kaplan FS** 1996 Overexpression of an osteogenic morphogen in fibrodysplasia ossificans progressiva. N Engl J Med 335:555–561
425. **Harris SE, Harris MA, Mahy P, Wozney J, Feng JQ, Mundy GR** 1994 Expression of bone morphogenetic protein messenger RNAs by normal rat and human prostate and prostate cancer cells. Prostate 24:204–211
426. **Pollard M, Luckert MS, Scheu J** 1988 Effects of diphosphonate and x-rays on bone lesions induced in rats by prostate cancer cells. Cancer 61:2027–2032
427. **Chen D, Feng JQ, Feng M, Harris MA, Mahy P, Mundy GR, Harris SE** 1995 Sequence and expression of bone morphogenetic protein 3 mRNA in prolonged cultures of fetal rat calvarial osteoblasts and in rat prostate adenocarcinoma PA III cells. DNA Cell Biol 14:235–239
428. **Harris SE, Boyce B, Feng JQ, Mahy P, Harris M, Mundy GR** 1992 Antisense bone morphogenetic protein 3 (BMP 3) constructions decrease new bone formation in a prostate cancer model. J Bone Miner Res 7[Suppl 1]:S115 (Abstract 92)
429. **Yanagisawa M, Kurihara H, Kimura S, Tomobe Y, Kobayashi M, Mitsui Y, Yazaki Y, Goto K, Masaki T** 1988 A novel potent va-

soconstrictor peptide produced by vascular endothelial cells. Nature 332:411–415
430. **Langenstroer P, Tang R, Shapiro E, Divish B, Opgenorth T, Lepor H** 1993 Endothelin-1 in the human prostate: tissue levels, source of production and isometric tension studies. J Urol 151:495–499
431. **Takuwa Y, Ohue Y, Takuwa N, Yamashita K** 1989 Endothelin-1 activates phopholipase C and mobilizes Ca2+ from extra- and intracellular pools in osteoblastic cells. Am J Physiol 257:E797–E803
432. **Takuwa Y, Masaki T, Yamashita K** 1990 The effects of the endothelin family peptides on cultured osteoblastic cells from rat calvariae. Biochem Biophys Res Commun 170:998–1005
433. **Alam AS, Gallagher A, Shankar V, Ghatei MA, Datta HK, Huang CL, Moonga BS, Chambers TJ, Bloom SR, Zaidi M** 1992 Endothelin inhibits osteoclastic bone resorption by a direct effect on cell motility: implications for the vascular control of bone resorption. Endocrinology 130:3617–3624
434. **Nelson JB, Hedican SP, George DJ, Reddi AH, Piantadosi S, Eisenberger MA, Simons JW** 1995 Identification of endothelin-1 in the pathophysiology of metastatic adenocarcinoma of the prostate. Nature Med 1:944–949
435. **Yamashita J, Ogawa M, Nomura K, Matsuo S, Inada K, Yamashita S, Nakashima Y, Saishoji T, Takano S, Fujita S** 1993 Interleukin 6 stimulates the production of immunoreactive endothelin 1 in human breast cancer cells. Cancer Res 53:464–467
436. **Chackal-Roy M, Niemeyer C, Moore M, Zetter BR** 1989 Stimulation of human prostatic carcinoma cell growth by factors present in human bone marrow. J Clin Invest 84:43–50
437. **Lang SH, Miller WR, Habib FK** 1995 Stimulation of human prostate cancer cell lines by factors present in human osteoblast-like cells but not in bone marrow. Prostate 27:287–293
438. **Kostenuik PJ, Sanchez-Sweatman O, Orr FW, Singh G** 1996 Bone cell matrix promotes the adhesion of human prostatic carcinoma cells via the alpha 2 beta 1 integrin. Clin Exp Metastasis 14:19–26
439. **Morton DM, Barrack ER** 1995 Modulation of transforming growth factor beta 1 effects on prostate cancer cell proliferation by growth factors and extracellular matrix. Cancer Res 55:2596–2602
440. **Rajan R, Vanderslice R, Kapur S, Lynch J, Thompson R, Djakiew D** 1996 Epidermal growth factor (EGF) promotes chemomigration of a human prostate tumor cell line, and EGF immunoreactive proteins are present at sites of metastasis in the stroma of lymph nodes and medullary bone. Prostate 28:1–9
441. **Mundy GR, Bertolini DR** 1986 Bone destruction and hypercalcemia in plasma cell myeloma. Semin Oncol 13:291–299 [Published erratum appears in Semin Oncol 1986; 13:Ixiii]
442. **Mundy GR, Raisz LG, Cooper RA, Schechter GP, Salmon SE** 1974 Evidence for the secretion of an osteoclast stimulating factor in myeloma. N Engl J Med 291:1041–1046
443. **Bardwick PA, Zvaifler NJ, Gill GN, Newman D, Greenway GD, Resnick DL** 1980 Plasma cell dyscrasia with polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes: the POEMS syndrome. Report on two cases and a review of the literature. Medicine 59:311–322
444. **Farhangi M, Merlini G** 1986 The clinical implications of monoclonal immunoglobulins. Semin Oncol 13:366–379
445. **Cozzolino F, Torcia M, Aldinucci D, Rubartelli A, Miliani A, Shaw AR, Lansdorp PM, DiGuglielmo R** 1989 Production of interleukin-1 by bone marrow myeloma cells. Blood 74:380–387
446. **Kawano M, Tanaka H, Ishikawa H, Nobuyoshi M, Iwato K, Asaoku H, Tanabe O, Kuramoto A** 1989 Interleukin-1 accelerates autocrine growth of myeloma cells through interleukin-6 in human myeloma. Blood 73:2145–2148
447. **Garrett IR, Durie BG, Nedwin GE, Gillespie A, Bringman T, Sabatini M, Bertolini DR, Mundy GR** 1987 Production of lymphotoxin, a bone-resorbing cytokine, by cultured human myeloma cells. N Engl J Med 317:526–532
448. **Bataille R, Chappard D, Klein B** 1992 The critical role of interleukin-6, interleukin-1β and macrophage colony-stimulating factor in the pathogenesis of bone lesions in multiple myeloma. Int J Clin Lab Res 21:283–287
449. **Bataille R, Jourdan M, Zhang XG** 1989 Serum levels of interleukin-6, a potent myeloma cell growth factor, as a reflect of dyscrasias. J Clin Invest 84:2008–2011
450. **van Zaanen HCT, Koopmans RP, Aarden LA, Rensink HJAM, Stouthard JML, Warnaar SO, Lokhorst HM, van Oers MHJ** 1996 Endogenous interleukin 6 production in multiple myeloma patients treated with chimeric monoclonal anti-IL6 antibodies indicates the existence of a positive feed-back loop. J Clin Invest 98: 1441–1448
451. **Lokhorst HM, Lamme Tanja de Smet M, Klein S, de Weger R, van Oers R, Bloem AC** 1994 Primary tumor cells of myeloma patients induce interleukin-6 secretion in long-term bone marrow cultures. Blood 84:2269–2277
452. **Feyen JH, Elford P, Di Padova FE, Trechsel U** 1989 Interleukin-6 is produced by bone and modulated by parathyroid hormone. J Bone Miner Res 4:633–638
453. **Harada H, Kawano MM, Huang N, Harada Y, Iwato K, Tanabe O, Tanaka H, Sakai A, Asaoku H, Kuramoto A** 1993 Phenotypic difference of normal plasma cells from mature myeloma cells. Blood 81:2658–2663
454. **Uchiyama H, Barut BA, Chuhan D, Cannistra SA, Anderson KC** 1992 Characterization of adhesion molecules on human myeloma cell lines. Blood 80:2306–2314
455. **Hamilton MS, Ball J, Bromidge E, Franklin IM** 1991 Surface antigen expression of human neoplastic plasma cells includes molecules associated with lymphocyte recirculation and adhesion. Br J Haematol 78:60–65
456. **Barker HF, Hamilton MS, Ball J, Drew M, Franklin IM** 1992 Expression of adhesion molecules LFA-3 and N-CAM on normal and malignant human plasma cells. Br J Haematol 81:331–335
457. **Van Reit I, De Waele M, Remels L, Lacor P, Schots R, Van Camp B** 1991 Expression of cytoadhesion molecules (CD56, CD54, CD18 and CD29) by myeloma plasma cells. Br J Haematol 79: 421–427
458. **Kawano MM, Huang N, Tanaka H, Ishikawa H, Sakai A, Tanabe O, Nobuyoshi M, Kuramoto A** 1991 Homotypic cell aggregations of human myeloma cells with ICAM-1 and LFA-1 molecules. Br J Haematol 79:583–588
459. **Huang N, Kawano MM, Harada H, Harada Y, Sakai A, Kuramoto A, Niwa O** 1993 Heterogeneous expression of a novel MPC-1 antigen on myeloma cells: possible involvement of the MPC-1 antigen in the adhesion of mature myeloma cells to bone marrow stromal cells. Blood 82:3721–3729
460. **Huang N, Kawano MM, Mahmoud MS, Mihara K, Tsujimoto T, Niwa O, Kuramoto A** 1995 Expression of CD21 antigen on myeloma cells and its involvement in their adhesion to bone marrow stromal cells. Blood 85:3704–3712
461. **Vacca A, Di Loreto M, Ribatti D, Di Stefano R, Gadaleta-Caldarola G, Iodice G, Caloro D, Dammacco F** 1995 Bone marrow of patients with active multiple myeloma: angiogenesis and plasma cell adhesion molecules LFA-1, VLA-4, LAM-1 and CD44. Am J Hematol 50:9–14
462. **Alsina M, Boyce B, Devlin RD, Anderson JL, Craig F, Mundy GR, Roodman GD** 1995 Development of an *in vivo* model of human multiple myeloma bone disease. Blood 87:1495–1501
463. **Garrett IR, Dallas S, Radl J, Mundy GR** 1997 A murine model of myeloma bone disease. Bone 20:515–520
464. **Berenson JR, Lichtenstein A, Porter L, Dimopoulos MA, Bordoni R, George S, Lipton A, Keller A, Ballester O, Kovacs MJ, Blacklock HA, Bell R, Simeone J, Reitsma DJ, Heffernan M, Seaman J, Knight RD** 1996 Efficacy of pamidronate in reducing skeletal events in patients with advanced multiple myeloma. N Engl J Med 334:488–493
465. **Garrett IR, Dallas SL, Bonewald LF, Boyce BF, Radl J, Mundy GR** 1995 Evidence for bidirectional interactions between osteoclasts and myeloma cells. J Bone Miner Res 10[Suppl 1]:S195 (Abstract P267)
466. **Belch AR, Bergsagel DE, Wilson K, O'Reilly S, Wilson J, Sutton D, Pater J, Johnston D, Zee B** 1991 Effect of daily etidronate on the osteolysis of multiple myeloma. J Clin Oncol 9:1397–1402
467. **Lahtinen R, Laakso M, Palva I, Virkkuned P, Elomaa I** 1992 Randomised, placebo-controlled multicentre trial of clodronate in multiple myeloma. Finnish Leukaemia Group. Lancet 340:1049–1052 [Published erratum appears in Lancet 1992; 340:1420]

468. **Warrell Jr RP, Lovett D, Dilmanian FA, Schneider R, Heelan RT** 1993 Low-dose gallium nitrate for prevention of osteolysis in myeloma: results of a pilot randomized study. J Clin Oncol 11:2443–2451
469. **Mundy GR, Luben RA, Raisz LG, Oppenheim JJ, Buell DN** 1974 Bone-resorbing activity in supernatants from lymphoid cell lines. N Engl J Med 290:867–871
470. **Valentin-Opran A, Charhon SA, Meunier PJ, Edouard CM, Arlot ME** 1982 Quantitative histology of myeloma-induced bone changes. Br J Haematol 52:601–610
471. **Ackery D, Yardley J** 1993 Radionuclide-targeted therapy for the management of metastatic bone pain. Semin Oncol 20:2:27–31 [Published erratum appears in Semin Oncol 1993; 20:551]
472. **Robinson RG, Preston DF, Schiefelbein M, Baxter KG** 1995 Strontium 89 therapy for the palliation of pain due to osseous metastases. J Am Med Assoc 274:420–424
473. **van der Pluijm G, Vloedgraven H, van Beek E, van der Wee-Pals L, Löwik C, Papapoulos S** 1996 Bisphosphonates inhibit the adhesion of breast cancer cells to bone matrices *in vitro*. J Clin Invest 98:698–705
474. **Taube T, Kylmala T, Lamberg-Allardt C, Tammela TL, Elomaa I** 1994 The effect of clodronate on bone in metastatic prostate cancer. Histomorphometric report of a double-blind randomised placebo-controlled study. Eur J Cancer 30A:751–758
475. **Vinholes J, Guo C-Y, Purohit OP, Eastell R, Coleman RE** 1996 Metabolic effects of pamidronate in patients with metastatic bone disease. Br J Cancer 73:1089–1095
