Role of parathyroid hormone-related peptide in hypercalcemia of malignancy and osteolytic bone disease

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Tumors cause multiple effects on the skeleton and on calcium homeostasis, but they do so in specific patterns which are becoming better defined as the mediators responsible become more fully characterized. Metastatic bone disease occurs in the majority of patients with advanced cancer, and is particularly frequent in breast, lung and prostate cancers, which are the most common of all human tumors. Approximately 1 000 000 people die each year in Western Europe and the United States from these three malignancies, and the majority of these people have bone metastases. Bone is the third most common site of metastatic disease in tumors of all types and the second most common in breast and prostate cancer. In this review, the role of the tumor peptide parathyroid hormone-related peptide (PTH-rP) in the effects of cancer on the skeleton will be discussed.

Effects of tumors on the skeleton and calcium homeostasis

Osteolytic bone disease

Osteolytic bone disease is the most common effect of malignant disease on the skeleton. Osteolytic bone disease is due to an increase in osteoclastic bone resorption, and is usually not matched by a compensatory increase in new bone formation. It occurs frequently in patients with breast and lung cancer, and is very characteristic of the unique form of bone disease associated with myeloma. The steps involved in the formation of an osteolytic bone metastasis are multiple, but to date most attention has been focused on the final step, namely the increase in osteoclastic bone resorption. Osteolytic bone disease is responsible for catastrophic consequences in the patient with malignant disease - intractable bone pain, susceptibility to fracture following trivial injury or even spontaneously, hypercalcemia and nerve compression syndromes, the most serious of which is spinal cord compression.

Osteoblastic bone disease

Osteoblastic bone disease is much less common than osteolytic bone disease, and occurs mostly frequently in patients with carcinoma of the prostate and urinary tract, but is also seen in patients with carcinoma of the breast and other tumors, and particularly with Hodgkin’s disease and with carcinoid syndromes. The increase in new bone formation is due to the tumor cells stimulating osteoblast activity. In some cases this occurs at sites of previous resorption, and in others it occurs on previous resting trabecular bone surfaces. Less is known of the mechanisms which are involved, in part because of a lack of suitable animal models. However, a number of important growth factors with powerful bone stimulatory activity have been identified and are probably responsible, either alone or in combination.

Mixed osteolytic and osteoblastic lesions

In truth, this is probably the most common effect of solid tumors on the skeleton, although osteolysis is usually much more apparent than the osteoblastic response. In most patients, there is an increase in serum alkaline phosphatase and enhanced uptake of radionuclide scanning agents, which reflects an increase in osteoblastic activity, although morphologically and radiologically osteolysis is by far the most dominant lesion.

Hypercalcemia

Hypercalcemia occurs under several different circumstances in patients with malignant disease. It is almost always associated with a marked increase in osteoclastic bone resorption, which is the primary abnormality. However, there is also often an increase in renal tubular calcium reabsorption, and in some patients an impairment in glomerular filtration is also an important pathogenic factor in the pathophysiology of hypercalcemia. There are
two distinct clinical syndromes, the humoral hypercalcemia of malignancy (HHM), and hypercalcemia based on widespread osteolysis. In fact, these syndromes overlap and in many patients the mediators responsible are the same.

Hypocalcemia
Hypocalcemia occurs under two circumstances, namely in patients with multiple osteoblastic lesions in which calcium from the extracellular fluid is presumably being utilized for the mineralization of new bone which is being rapidly formed, and in tumor lysis syndromes, where tumors release intracellular ions such as phosphate, potassium and uric acid (Mundy 1990). Where intracellular phosphate release is high, this may lead to a rapid fall in serum calcium. Hypocalcemia of severe degree is not common in patients with malignant disease involving the skeleton.

Oncogenic osteomalacia
Oncogenic osteomalacia occurs in association with mesenchymal tumors such as osteomas and hemangio-pericytomas, which presumably produce an as yet unidentified phosphaturic substance which causes a syndrome reminiscent in many ways of sex-linked hypophosphatemic rickets (Drezner 1996). These patients usually have benign tumors and so the syndrome may be present for many years before the tumor is discovered. Occasionally, however, it occurs in patients with solid tumors such as carcinoma of the prostate, and the course may be more rapid and the osteomalacia superimposed on metastatic bone disease. There are about 100 reported cases in the medical literature.

In the remainder of this discussion, we will discuss what is known currently of the physiological role of PTH-rP, and then its role specifically in osteolytic bone disease, the various forms of hypercalcemia associated with malignancy, and osteoblastic bone disease.

PTH-rP - discovery and physiological role
PTH-rP was discovered in a search for the systemic factors involved in HHM. This search was based on the original observations of Albright which were reported in the case records of the Massachusetts General Hospital. Albright noted that a patient with a renal carcinoma and skeletal involvement had a hypercalcemic syndrome which resembled that of primary hyperparathyroidism, and suggested that the tumor produced a parathyroid hormone (PTH)-like factor. The primitive assays of PTH-like factors in the 1970s, a number of studies reported increases in PTH radioimmunoassayable material (Benson et al. 1974). This was clearly not identical chemically to native PTH produced by the parathyroid glands, and at that time it was thought that an altered form of PTH was responsible.

Stewart et al. (1980) showed with careful measurements of nephrogenous cAMP that in patients with the HHM, there was an increase in this parameter suggesting a PTH-like syndrome. The PTH radioimmunoassays had improved by this time, but circulating immunoreactive PTH was not increased in these patients. The authors concluded that a PTH-like peptide rather than PTH itself was responsible for hypercalcemia.

PTH was finally excluded as a common cause for HHM by Simpson et al. (1983), who showed in a series of hypercalcemic patients that there was no detectable PTH messenger RNA in the tumor extracts. Subsequently, a number of groups embarked on approaches using adenylate cyclase assays in renal tubular cells or slices in an effort to identify the mediator with PTH-like biological activity. Attempts at purification of the PTH-like activity led to the discovery of a peptide with similarities to PTH in the N-terminal sequence. Although first reported by Moseley et al. (1987), there were also two other almost simultaneous reports of identical findings by Stewart et al. (1987) and by Strewler et al. (1987). These three groups identified this activity in lung cancer, a renal cancer and interestingly in a breast cancer extract. The peptide was soon cloned and expressed and its biological effects were studied in detail. It was found to have very similar properties to those of PTH.

PTH-rP has 70% homology to the first 13 amino acids of the N-terminal portion of PTH (Suva et al. 1987), binds to PTH receptors (Abou-Samra et al. 1992) and mimics the biological effects of PTH (Horiuchi et al. 1987, Yates et al. 1988). Specifically, it stimulates adenylate cyclase in renal and bone systems (Burtis et al. 1987, Horiuchi et al. 1987, Kemp et al. 1987, Strewler et al. 1987, Yates et al. 1988), increases renal tubular reabsorption of calcium and osteoclastic bone resorption (Strewler et al. 1987, Yates et al. 1988), enhances renal phosphate excretion by inhibiting tubular reabsorption (Horiuchi et al. 1987, Kemp et al. 1987, Sartori et al. 1987, Yates et al. 1988) and stimulates the 1α-hydroxylase enzyme in renal tubular cells responsible for production of 1,25-dihydroxyvitamin D (1,25-(OH)2D3) from 25-hydroxyvitamin D (Horiuchi et al. 1987). PTH-rP has been found in a variety of tumor types associated with hypercalcemia including squamous, renal islet cell, and even breast carcinoma (Danks et al. 1989, Asa et al. 1990). Although the majority of squamous cell carcinomas produce PTH-rP (Dunne et al. 1993), the capacity to cause hypercalcemia may depend on the level of PTH-rP gene expression, which in turn may be determined by differential transcription of the PTH-rP gene promoter (Wysolmerski et al. 1996). The regulation of PTH-rP is complex and factors such as prolactin
(Thiede 1989), epidermal growth factor (EGF) (Komera et al. 1991, Ferrari et al. 1994, Sebag et al. 1994, Southby et al. 1996), insulin (Sebag et al. 1994), insulin-like growth factor-I (Rizzoli et al. 1994, Sebag et al. 1994) and -II (Sebag et al. 1994), transforming growth factor-α (TGF-α) (Burton & Knight 1992), TGF-β (Kiriyma et al. 1992, Zakalik et al. 1992, Merryman et al. 1994, Southby et al. 1996), angiotensin II (Pirola et al. 1993) and stretch (Daifotis et al. 1992) have been shown to increase expression while glucocorticoids (Glatz 1996), angiotensin II (Pirola et al. 1994, Merryman et al. 1994, Southby et al. 1996), angiotensin II (Pirola et al. 1993) and stretch (Daifotis et al. 1992) have been shown to increase expression while glucocorticoids (Glatz et al. 1994, Rizzoli et al. 1994, Sebag et al. 1994) and 1,25-(OH)₂D₃ (Kremer et al. 1991, Sebag et al. 1994) decrease it. Estrogen has been shown to increase PTH-rP expression in uterine tissue and in vitro studies suggest that an estrogen response element is present in the PTH-rP gene (Lu et al. 1989, Thiede et al. 1991).

The human PTH-rP 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. The combination of three promoters, one 5’ alternative splicing event and alternative 3’ splicing produces PTH-rP isoforms of 139, 141 or 173 amino acids as well as multiple distinct PTH-rP messenger RNA species (Southby et al. 1996). Although there is significant sequence homology across species up to amino acid 111, there is no interspecies sequence homology beyond that point (Martin et al. 1991). Differential utilization of the promoters and of the 3’ alternative splicing pathways among bone, breast, kidney and lung cell lines has been demonstrated (Southby et al. 1996). Dexamethasone decreases while EGF and TGF-β increase 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 (Southby et al. 1996).

Similar to PTH, PTH-rP undergoes endoproteolytic posttranslational processing that results in several secretory forms: (1) an amino-terminal PTH-rP (1-36); (2) a mid-region species that begins at amino acid 38 that has an undefined carboxyl terminus (Burtis et al. 1992, Soifer et al. 1992), and (3) a carboxyl-terminal species that is recognized by an antibody directed against the 109-138 region (Burtis et al. 1990, 1992, Orloff et al. 1993). The arrangement of the abundant basic residues in the protein sequence suggest that members of the subtilisin family of endoproteases such as furin (Liu et al. 1995), PC 1-3, PC-2, PACE-4 and PACE-8 (Brazzani et al. 1996) are responsible for posttranslational processing (Soifer et al. 1992, Orloff et al. 1994, Plawner et al. 1995). Glycosylation of an amino-terminal PTH-rP species produced by keratinocytes has also been demonstrated (Wu et al. 1991). Posttranslational processing of PTH-rP as well as the receptor and signal transduction pathways employed by the mature secretory forms of PTH-rP have been extensively reviewed by Orloff et al. (1994). Regulation of cell secretion of PTH-rP may be cell-specific, since PTH-rP is expressed in neuroendocrine cells in a regulated fashion whereas it is expressed constitutively in non-neuroendocrine cell types such as squamous cell carcinoma (Plawner et al. 1995).

PTH-rP is widely distributed (Danks et al. 1989, Asa et al. 1990) and there is accumulating evidence that it has a role in normal physiology quite distinct from that of PTH (reviewed in Strewler & Nissenson 1994, Guse & Mundy 1996, Philbrick et al. 1996). It shares identical effects with those of PTH on bone cells and on renal tubular cells. In other words, it seemed to be a perfect mimic of PTH. A number of possibilities were raised for a physiological role including potential effects in calcium translocation between the fetus and placenta, calcium translocation in breast milk, a role in parturition, and a role in the keratinocyte and skin physiology. PTH-rP appears to be important in the development of the normal skeleton (Karaplis et al. 1994, Lanske et al. 1996), regulation of placental calcium transport (Kovacs et al. 1995), establishment of bone metastasis in breast cancer (Guse et al. 1996), and autocrine regulation of the growth of some tumors (Burton et al. 1990). In the skeleton, null mutant PTH-rP mice have provided significant insight into the role of PTH-rP in the developing skeleton (Karaplis et al. 1994). These mice died postnatally from asphyxia and exhibited widespread abnormalities of endochondral bone development, including diminished chondrocyte proliferation, associated with premature maturation and differentiation of chondrocytes and accelerated bone formation from cartilage templates. Similarly, mice homozygous for the PTH/PTH-rP receptor gene null mutation had identical skeletal pathology but many were early embryonic lethals, a finding that suggested the phenotype of receptor loss was more severe than that of only PTH-rP loss (Lanske et al. 1996). Consistent with this, transgenic mice in which PTH-rP was overexpressed in prehypertrophic chondrocytes by the use of a mouse collagen type II promoter which targeted the gene to these cells had impaired chondrocyte differentiation and endochondral ossification (Weir et al. 1996) and had the short-limbed phenotype similarly observed in humans with Jansen’s metaphyseal chondrodysplasia (Schipani et al. 1995), which is caused by activating mutations in the PTH/PTH-rP receptor.

PTH-rP appears to be important in the normal physiology of the breast (Thiede & Rodan 1988, Ratcliffe 1992) as it is expressed in lactating mammary tissue (Thiede & Rodan 1988) and secreted into milk at concentrations 10⁴-10⁸ times greater than plasma concentrations of humans with malignancy-associated hypercalcemia (Budayr et al. 1989). Increased plasma PTH-rP concentrations have been documented in several patients with the rare condition of lactational hypercalcemia (Khosla et al.

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normal host cells and to extracellular matrix through substratum communications. Cancer cell adhesion to and metastasis. CAMs mediate cell-to-cell and cell-to-several important events involved in cancer cell invasion and E-cadherin are particularly likely to play a key role in process. Cell adhesion molecules (CAMs) such as laminin extracellular structures is critical for the metastatic development of tumor cells from the primary site to the eventual bone metastasis involves molecular interactions between the tumor cells and host cells, and each represents potential sites for therapeutic intervention (Liotta & Kohn 1990, Zetter 1990). The final step in the process, namely bone destruction, is due to osteoclastic bone resorption. This has been shown using scanning electron microscopy on sections of bone adjacent to tumor cells in the vertebral bodies (Boyd et al. 1986). Histological sections of breast cancer metastatic to bone also show tumor cells adjacent to bone resorbing osteoclasts (Taube et al. 1994). The initial step, namely shedding of tumor cells from the primary site, involves detachment of tumor cells from adjacent cells, followed by invasion of adjacent tissue in the primary organs. The cells then enter tumor capillaries (stimulated by specific angiogenesis factors produced by the tumor) and via these capillaries reach the general circulation (Weiss et al. 1989). The steps involved in entering the tumor blood vessels at the primary site are similar to those which are involved in exit from the vasculature in the bone marrow cavity. These steps include the attachment of the tumor cells to basement membrane, the secretion of proteolytic enzymes which enable tumor cells to disrupt the basement membrane, and then migration of the tumor cells through the basement membrane (Liotta et al. 1980, 1986, Liotta & Steeg 1990).

Attachment of tumor cells to other cells and to extracellular structures is critical for the metastatic process. Cell adhesion molecules (CAMs) such as laminin and E-cadherin are particularly likely to play a key role in several important events involved in cancer cell invasion and metastasis. CAMs mediate cell-to-cell and cell-to-substratum communications. Cancer cell adhesion to normal host cells and to extracellular matrix through CAMs has been shown to regulate tumor cell invasiveness and proliferation (Albelda & Buck 1990).

At the primary site, loss of CAMs causes disruption of the interconnections between cancer cells and promotes the detachment of cancer cells from the primary tumor that results in initiation of local invasion and eventually in the development of metastasis. In contrast, at the metastatic site, elevated expression of CAMs might be a prerequisite for cancer cells to arrest through the attachment to extracellular matrix. Subsequently, expression of CAMs might be diminished, to free cancer cells from direct contact-mediated regulation by host immune cells. More recent studies have demonstrated that metastatic breast and ovarian cancers show heterogeneous expression of E-cadherin (Oka et al. 1993) and E-cadherin expression in these cancer cells may be reversibly modulated according to culture conditions in vitro (Hashimoto et al. 1989) and environmental factors in vivo (Mareel et al. 1991). Therefore, cancer cells may express either decreased or increased levels of CAMs depending on the stage of metastasis development and sites of metastasis. We think it likely that the CAMs E-cadherin and laminin are involved in bone metastasis (see below).

Integrins are the most abundant CAMs and are responsible for a variety of cell-cell and cell-matrix interactions (Hynes 1992), and have been implicated in hematogenous dissemination (Nip et al. 1992). In cancer metastasis, integrins have been shown to mediate cancer cell attachment to vascular endothelial cells and to matrix proteins such as laminin and fibronectin which underlie endothelium, an initial step in tumor colonization (Albelda & Buck 1990). For example, it has been demonstrated that A375 human melanoma cells express high levels of the \( \alpha_v \beta_3 \) integrin (vitronectin receptor) on the cell surface when they bind to and invade the basement membrane matrix matrigel (Selfoot et al. 1992).

There is now accumulating evidence that PTH-rP production by breast cancer cells in bone is a major factor in the osteoclastic stimulation and bone destruction in patients with advanced metastatic breast cancer. Clinical studies have shown that breast cancer cells produce increased amounts of PTH-rP when they are present in the bone microenvironment (Powell et al. 1991). There is a marked difference between expression of PTH-rP at the primary breast cancer site or soft tissue organ sites other than bone (Powell et al. 1991). The most likely explanation for this is that bone provides the fertile environment for the growth of the tumor cells and also enhances the production of PTH-rP in this microenvironment. One of the major mechanisms by which this may occur is by tumor cell production of PTH-rP. This possibility occurs as a consequence of the release of TGF-\( \beta \) by resorbing bone, which enhances PTH-rP production by breast cancer cells. We have found that by

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1990, Lepre et al. 1993), in addition to some breast-feeding mothers (Grill et al. 1992). Thus, PTH-rP may be responsible for mobilizing calcium from maternal bone for use in milk production and it may be the implicating factor in lactation-associated bone loss.

**PTH-rP and osteolytic bone disease (or predominantly osteolytic bone disease)**

Osteolytic bone disease is the most common effect of tumor cells on the skeleton. It is found more commonly than osteoblastic disease and is almost universal in patients with advanced carcinoma of the breast and carcinoma of the lung. Breast cancer is usually thought of most frequently in association with osteolytic bone disease, since unlike lung cancer it rarely causes hypercalcemia without widespread bone metastases, and osteolytic metastases are more common in patients with advanced breast cancer than with any other solid tumor.

Each of the multiple steps which are involved in the metastasis of tumor cells from the primary site to the eventual bone metastasis involves molecular interactions between the tumor cells and host cells, and each represents potential sites for therapeutic intervention (Liotta & Kohn 1990, Zetter 1990). The final step in the process, namely bone destruction, is due to osteoclastic bone resorption. This has been shown using scanning electron microscopy on sections of bone adjacent to tumor cells in the vertebral bodies (Boyde et al. 1986). Histological sections of breast cancer metastatic to bone also show tumor cells adjacent to bone resorbing osteoclasts (Taube et al. 1994). The initial step, namely shedding of tumor cells from the primary site, involves detachment of tumor cells from adjacent cells, followed by invasion of adjacent tissue in the primary organs. The cells then enter tumor capillaries (stimulated by specific angiogenesis factors produced by the tumor) and via these capillaries reach the general circulation (Weiss et al. 1989). The steps involved in entering the tumor blood vessels at the primary site are similar to those which are involved in exit from the vasculature in the bone marrow cavity. These steps include the attachment of the tumor cells to basement membrane, the secretion of proteolytic enzymes which enable tumor cells to disrupt the basement membrane, and then migration of the tumor cells through the basement membrane (Liotta et al. 1980, 1986, Liotta & Steeg 1990).

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rendering human breast cancer cells unresponsive to TGF-β by stable transfection with a dominant negative TGF-β receptor, the tumor cells form less bone metastases and secrete reduced amounts of PTH-rP (Yin et al. 1996). Thus, this evidence is consistent with the notion that there is a bidirectional interaction between TGF-β in active form produced in bone as a consequence of bone resorption, and PTH-rP production by breast cancer cells. Increased PTH-rP production leads to increased osteoclast formation and bone resorption, which in turn leads to increased amounts of active TGF-β, which subsequently lead to increased amounts of PTH-rP expression by the breast cancer cells. Breaking this vicious cycle is very important for making the bone marrow microenvironment a less favorable soil for the growth of tumor cells. These observations suggest that PTH-rP may not only be a systemic mediator of the HHM syndrome, but also a local mediator of osteoclastic bone resorption and bone destruction, which may or may not be associated with hypercalcemia. It raises the possibility that neutralization of PTH-rP in the bone microenvironment may be a useful therapy for patients with metastatic breast cancer.

It should be noted that many patients with breast cancer and hypercalcemia do not have increased plasma PTH-rP or increased nephroenogenous cAMP. However, this does not mean that the absence of these parameters indicates that PTH-rP is unimportant in the bone destruction or hypercalcemia. Rather, it is acting as a powerful local mediator in this situation and may not reach the systemic circulation in sufficient amounts to cause an increase in plasma PTH-rP or nephroenogenous cAMP. This is supported by the fact that neutralizing antibodies to PTH-rP inhibited the development and progression of bone metastasis due to the human breast cancer cell line MDA-MB-231 (Guise et al. 1996).

Although these data suggest that PTH-rP is very important in the pathophysiology of bone destruction associated with metastatic cancer, it is probably not acting alone. Other mediators produced by tumor cells in the bone cell microenvironment such as TGF-α, interleukin (IL)-1α, IL-6 and tumor necrosis factor may also be important. We have found that the effects of PTH-rP on bone are markedly enhanced by the presence of small amounts of IL-6 (De La Mata et al. 1995). Presumably, these factors act at different sites in the osteoclast lineage resulting in potentiation of effects.

Since PTH-rP expression is enhanced in the bone microenvironment in patients with metastatic breast cancer, factors which could be responsible for regulation of PTH-rP expression are obviously of interest. These include EGF, TGF-α, TGF-β and the src and ras proto-oncogenes. EGF has been shown to increase PTH-rP expression in a keratinocyte cell line (Allinson & Drucker 1992, Southby et al. 1995) while TGF-α, a breast cancer tumor product (Nagata et al. 1989), enhanced PTH-rP expression in a human squamous cell carcinoma of the lung (Burton & Knight 1992). Moreover, other tumor-associated factors may modulate the end organ effects of PTH-rP. We have found that TGF-α can enhance the hypercalcemic effects of PTH-rP in an animal model of malignancy-associated hypercalcemia (Guise et al. 1993) and others have demonstrated that TGF-α can modulate the renal and bone effects of PTH-rP (Pizurki et al. 1990, 1991). IL-6 has recently been shown to enhance hypercalcemia and bone resorption mediated by PTH-rP in vivo (De La Mata et al. 1995). Additionally, TGF-β, which is expressed by many breast cancers and presents in high concentrations in the bone microenvironment, has been shown to enhance secretion of and stabilize PTH-rP mRNA in a renal cell carcinoma (Zakalik et al. 1992) as well as in a squamous cell carcinoma (Kiriyama et al. 1992, Merryman et al. 1994). Not only has TGF-β been shown to enhance PTH-rP expression in renal and squamous cell carcinoma, but our studies (Guise et al. 1994) and those of others (Firek et al. 1994, Southby et al. 1996) demonstrate that this relationship also exists in a human breast adenocarcinoma, MDA-MB-231.

The fact that TGF-β is abundant in bone (Hauschka et al. 1986) and can enhance PTH-rP expression by cancer cells makes it an important candidate factor in the establishment and progression of breast cancer metastases to bone. TGF-β is a member of a large superfamilly of proteins that are important regulators of bone cell activity (Czembella et al. 1994). Five isoforms of TGF-β exist and appear to control cell proliferation and differentiation in many human cell types (Derynck 1994). The prototype of these isoforms, TGF-β1, is highly expressed by differentiated osteoblasts and osteoclasts, is stored in bone matrix and released during osteoclastic bone resorption (Pfeilschifer & Mundy 1987). The effects of TGF-β include stimulation of cell proliferation of mesenchymal cells, growth inhibition of epithelial cells, synthesis of extracellular matrix proteins and enhancing cell adhesion. These effects of TGF-β are mediated through complex receptor interactions that have been recently elucidated (Derynck 1994, Wrana et al. 1994). TGF-β binds to a component of the receptor (type II receptor) and this ligand-receptor component complex then recruits and phosphorylates the type I receptor, which in turn initiates signal transduction to downstream substrates recently identified as Smad proteins (Massague et al. 1997). A mutant TGF-β receptor II has been constructed which binds TGF-β1 and presents the complex to receptor II but fails to mediate the biological response (Wieser et al. 1993). Thus, it acts in a dominant negative fashion to block the effects of TGF-β. Use of this mutant receptor is providing significant insights into the pathophysiological

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There is accumulating information which suggests that increased expression of the non-receptor tyrosine kinase src renders breast cancer cells more aggressive. Human breast cancer tissues contain relatively increased src tyrosine kinase activity compared with benign breast tumors or normal breast tissue (Ottenhoff-Kalf et al. 1992). It has also been shown that the tyrosine kinase activity of breast cancer tissue is related to prognosis and metastasis-free survival in patients with breast cancer (Hennipman 1989). More recently, it has been shown that c-src tyrosine kinase under transcriptional control of the murine mammary tumor virus (MMTV) long terminal repeat induces mammary tumors in transgenic mice (Webster et al. 1995). The importance of src in breast cancer is further supported by the finding that mice expressing MMTV/polyoma virus middle T-transgene in a c-src-deficient background rarely show mammary tumor formation, whereas tumor formation is not altered in a c-yes-deficient background (Guy et al. 1994). Although these effects are confined not just to breast cancer (Fanning 1992, Talamonti 1993), current evidence suggests that enhanced src activity in breast cancer cells renders them particularly more aggressive.

The trivial explanation for the relationship between src and aggressive behavior of breast cancer cells is that proliferation of tumor cells is enhanced, and this may in fact be part of the explanation. However, it is also likely that src has other specific deleterious effects in breast cancer cells which may be related to bone metastasis. For example, src tyrosine kinase activity in breast cancer may enhance the expression of PTH-rP, which in turn causes osteolytic bone destruction. A direct relationship between src expression and PTH-rP production has recently been established in cell culture studies with other types of cells. Li & Drucker (1994) have shown that cellular transformation by the v-src oncogene is associated with a marked increase in PTH-rP gene expression in NRK 49F renal cells. Following transfection, the cells secreted increased amounts of immunoreactive PTH-rP, and an increase in PTH-rP mRNA transcripts was confirmed by Northern analysis. There was no significant change in the half-life of PTH-rP mRNA transcripts and nuclear run-on assays showed an increased rate of PTH-rP gene transcription. Thus, the gene encoding PTH-rP is a downstream target for src in these cells. This suggests a potential mechanism for the increased expression of PTH-rP gene expression in breast cancers associated with increased src expression.

PTH-rP has also been implicated recently in the hypercalcemia associated with hematological malignancies. For some years it has appeared that hypercalcemia associated with some T-cell lymphomas is caused by PTH-rP (see below). This syndrome is similar to HHM associated with solid tumors. However, recently it has also been linked to hypercalcemia in some cases of myeloma (Firkin et al. 1996). It has been found in increased circulating concentrations in the blood in three of nine patients, and myeloma cells have been found to express PTH-rP by immunohistochemistry and in situ hybridization.

Osteolytic bone destruction due to metastatic solid tumors or hematological malignancies has received much more attention with the now widespread use of bisphosphonates for this indication. Clinical trials have revealed an approximately 50% reduction in skeletal-related events in patients treated with pamidronate (Berenson et al. 1996, Hortobagyi et al. 1996). Similar effects have been found with other potent bisphosphonates in Europe. These drugs have now become important adjuvant therapy for patients with osteolytic bone disease due to malignancy.

**PTH-rP and HHM**

Hypercalcemia of malignancy occurs in several different settings. These include HHM where a circulating mediator produced by the tumor cells stimulates osteoclastic bone resorption and enhances renal tubular calcium re-absorption by a systemic effect. The second situation is where hypercalcemia occurs in association with extensive localized osteolysis (Table 1). In HHM, PTH-rP is produced by most tumors, and the patients have an increase in nephrogenous cAMP. These patients usually have squamous cell carcinomas of the lung, or head and neck, or adenocarcinomas of the kidney, lung, pancreas or ovary. It also includes some patients with hypercalcemia associated with lymphoma (Motokura et al. 1989). There is an approximate correlation between plasma PTH-rP and hypercalcemia in these patients, and the majority of patients have an increase in osteoclastic bone resorption but without an associated increase in new bone formation. In most patients, the serum 1,25-(OH)₂D₃ concentration is not increased, and the patients have a metabolic alkalosis. Thus, there are some differences between the clinical features of the HHM syndrome and primary hyperparathyroidism, where the majority of patients have a hyperchloremic acidosis, and there is an increase in bone

**Table 1** Tumor syndromes associated with PTHrP production.

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<th>Syndrome</th>
<th>PTHrP Production</th>
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<tr>
<td>Humoral hypercalcemia of malignancy</td>
<td>↑Plasma [PTHrP], [ncAMP]</td>
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<tr>
<td>Localized osteolysis</td>
<td>↑Hypercalcemia</td>
</tr>
<tr>
<td>No plasma [PTHrP] or [ncAMP]</td>
<td></td>
</tr>
</tbody>
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turnover characterized by increased bone formation to go along with increased bone resorption. In patients with primary hyperparathyroidism, the serum 1,25-(OH)₂D₃ concentration is usually increased.

Thus, from what is currently known, the production of PTH-rP alone cannot explain all aspects of the clinical syndrome of HHM. The reasons for these discrepancies between HHM and primary hyperparathyroidism may be due to pulsatile secretion of PTH and the presumed continuous secretion of PTH-rP, suppression of bone formation and 1α-hydroxylase activity by biologically active PTH-rP fragments or hypercalcemia per se, or the production of other cytokines in patients with malignancies such as IL-6, tumor necrosis factor, IL-1 or TGF-α. IL-6 certainly enhances the effects of PTH-rP on bone resorption (De La Mata et al. 1995). Yoneda et al. (1991) have confirmed that there is production of these mediators by both host immune cells as well as by tumor cells in patients with HHM, and have actually reduced the immunosuppressors by both host immune cells as well as by tumor cells in patients with HHM, and have actually reduced the hypercalcemia in nude mice carrying human tumors with therapy with antibodies to cytokines such as IL-6 and tumor necrosis factor. Patients with the HHM often have a paraneoplastic leukocytosis and cachexia due to the hypercalcemia in nude mice carrying human tumors with therapy with antibodies to cytokines such as IL-6 and tumor necrosis factor. Patients with the HHM often have a paraneoplastic leukocytosis and cachexia due to the production of cytokines either by the tumor cells or by host immune cells (Yoneda et al. 1991).

**PTH-rP and osteoblastic metastases**

Osteoblastic bone lesions caused by cancer occur most frequently in patients with prostate cancer, although they can occur in other solid tumors as well such as breast cancer and Hodgkin’s lymphoma. They even occur occasionally in patients with myeloma and the polyneuropathy, organomegaly, endocrinopathy, M-protein, and skin changes syndrome.

In patients with prostate cancer, it is still not clear whether osteoblastic metastases lead to increase in bone formation on resting bone surfaces or whether they occur in association with an increase in bone destruction. It is probable that both occur in the same patient. The majority of patients with prostate cancer and osteoblastic metastases have an increase in resorption markers which is even more striking than in patients with osteolytic disease (Urwin et al. 1985, Percival et al. 1987, Coleman et al. 1992, Lipton et al. 1993, Demers et al. 1995, Ikeda et al. 1996, Takeuchi et al. 1996). However, histomorphometric studies also show that at least some osteoblastic metastases occur without previous bone resorption.

It is clear in patients with osteoblastic metastases that there are growth factors produced by the metastatic cancer cells which stimulate bone formation, either on resting bone surfaces or at sites of previous resorption. A number of growth factors have been implicated in this bone formation including TGF-β, the bone morphogenetic proteins, fibroblast growth factors (Izbicka et al. 1996), proteases such as plasminogen activator or urokinase type plasminogen activator (Rabbani et al. 1990, Achbarou et al. 1994), and more recently, endothelin-I (Nelson et al. 1995) and prostate specific antigen (PSA) (Chybowski et al. 1991). Although there is evidence for each of these mechanisms, and none has been proven definitively, there are some recent data which have implicated PTH-rP and suggested a potential role for PTH-rP in bone formation. It has long been known that PTH and PTH-rP have an anabolic effect on bone when delivered to bone surfaces intermittently and in low dose (Stewart 1996). It is also known that many prostate cancer cells, at least in culture, produce PTH-rP. More recently, it has been shown that PSA can cleave PTH-rP and it has been postulated that this cleaved PTH-rP may have a role in the osteoblastic response to prostate cancer (L. McCauley, personal communication). PSA has been shown to cleave PTH-rP (~1 to ~141) at the carboxy-terminal phenylalanine 23 and thereby prevents the biological effects of PTH-rP to stimulate cAMP production in an osteoblastic cell line (Cramer et al. 1996). Some breast cancers also express PSA, suggesting that there may be a relationship between expression of PSA and capacity to stimulate osteoblast formation which is in some way related to its proteolytic activity on PTH-rP. These associations will clearly require further study.

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