Tumor Products and the Hypercalcemia of Malignancy

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Introduction

One of the most interesting aspects of the hypercalcemia of malignancy is that it is unlikely that the hypercalcemia is due to the secretion by the tumor cells of one gene product which causes osteoclastic bone resorption and hypercalcemia, but rather that multiple tumor products work in concert on bone and kidney to overwhelm the normal compensatory mechanisms which guard calcium homeostasis so carefully. Moreover, although tumors secrete multiple factors which affect calcium metabolism, in the great majority of nonparathyroid gland tumors it is clear that parathyroid hormone itself is not one of these factors.

Our understanding of the mechanisms of hypercalcemia of malignancy has advanced steadily over the last five years. This has occurred in part because of the application of the emerging techniques of molecular biology to this field. The use of complementary DNA (cDNA) probes for detecting gene expression and the availability of recombinant tumor products for testing in biological assays have clarified some of the mechanisms by which tumors affect bone cell function. Moreover, investigators have realized that understanding the mechanisms of tumor-induced hypercalcemia may not only lead to increasing our knowledge of this important clinical problem, but since production of these factors by tumors probably represents aberrations of normal physiological mechanisms, clarification of their mode of action may lead to new insights into normal bone remodeling.

Tumors associated with hypercalcemia of malignancy do not represent a homogeneous group, and there is no single unifying mechanism that can explain all cases of hypercalcemia. However, it is likely that similar mechanisms are responsible in similar types of tumors (1). In the hematologic malignancies (~15–20% of the total), local bone-resorbing factors are responsible for extensive osteolytic bone destruction and hypercalcemia usually occurs in association with impaired glomerular filtration. In a second group, the solid tumors associated with advanced osteolytic metastases, hypercalcemia rarely occurs unless the tumor is widespread and there is extensive local bone destruction. The most common example of this group is breast cancer (~25% of the total). A third group is comprised of solid tumors such as squamous cell carcinoma of the lung, head, and neck, carcinoma of the kidney, and carcinoma of the ovaries, where the primary mechanism is increased bone resorption caused by tumor secretion of a circulating stimulator or stimulators of osteoclast activity. This syndrome is called the humoral hypercalcemia of malignancy (HHM)[1] and comprises ~55% of the total. These patients may or may not have metastatic bone disease.

Hematologic malignancies

The most widely recognized example of a hematologic malignancy associated with destructive bone lesions and hypercalcemia is myeloma, but recently a subset of T cell lymphoma has been described which is very frequently associated with hypercalcemia (2). This lymphoma is caused by the exogenous retrovirus human T cell lymphotrophic virus (HTLV)-Type 1, and occurs particularly in the southern islands of Japan, the Caribbean basin, and among blacks in the southeastern United States. The mechanisms by which lymphoproliferative cells affect bone metabolism are complex. Early studies suggested that cultured myeloma cells and lymphoid cell lines derived from patients with myeloma produced an osteoclast-activating factor (OAF), a factor with similar or identical biological and chemical characteristics to a lymphokine secreted by antigen or mitogen-activated normal leukocytes (3–5). However, the entity "OAF" probably comprises a family of bone-resorbing cytokines rather than one discrete factor. Lymphocyte products such as colony-stimulating factor of the granulocyte-macrophage series (CSF-GM), lymphotoxin, and gamma interferon have important effects on bone cell metabolism in vitro (MacDonald, B., D. Bertolini, and M. Gowen, unpublished observations). Both CSF-GM and CSF-1 (a different glycoprotein which acts on cells of the macrophage lineage) cause proliferation of osteoclast progenitors, although they do not seem to have the capacity to activate preexisting osteoclasts (MacDonald, B., and G. D. Roodman, unpublished observations). Lymphotoxin is a potent osteoclast activator and may represent a portion of the bone-resorbing activity seen in crude activated leukocyte culture supernatants or myeloma cell cultures (Bertolini, D., G. Nedwin, and G. Mundy, unpublished observations). Leukocyte interferon has the capacity to inhibit osteoclastic bone resorption (6–7). Thus, bone resorption produced by lymphoproliferative diseases may represent the net difference between bone resorption stimulators and bone resorption inhibitors produced by malignant lymphoid cells. However, there are also other bone-resorbing factors which may be produced by lymphoid cells. Bone-resorbing activity is present in preparations rich in the monocyte product interleukin (IL)-1 (8), and this could account for the hypercalcemia associated with some monocytic malignancies. Moreover, it is possible that 1,25-dihydroxyvitamin D may be produced by some normal or abnormal cells in the bone marrow cell microenvironment.

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1. *Abbreviations used in this paper:* EGF, epidermal growth factor; HHM, humoral hypercalcemia of malignancy; IL, interleukin; PDGF, platelet-derived growth factor; PTH, parathyroid hormone; TGF, transforming growth factor.

Macrophages have the capacity to convert 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D (9–10). A similar phenomenon has been shown in HTLV-Type I infected lymphocytes (Fetchick, D., D. Bertolini, and J. Dunn, unpublished observations) and recently there have been several reports indicating that some patients with adult T cell lymphoma have increased serum 1,25-dihydroxyvitamin D concentrations (11). Since 1,25-dihydroxyvitamin D has striking effects on the immune system, such as inhibition of lymphocyte mitogenesis, inhibition of IL-2 production, and possibly stimulation of IL-1 production by macrophages (12–13), then the number of local stimulators and inhibitors which may influence bone resorption are protean and it will require much more work to determine the relative importance of each.

The role of renal mechanisms in the pathogenesis of hypercalcemia associated with hematologic malignancies is not clear, but may be important. Extensive bone destruction is common in most patients with myeloma, but hypercalcemia is seen only in some cases (about 20%). There is no positive correlation between OAF production by bone marrow myeloma cells and serum calcium concentration (14). However, there is a good correlation between renal failure and hypercalcemia, and hypercalcemia occurs most commonly in those patients with impaired glomerular filtration. It is possible that hypercalcemia occurs in patients with myeloma who have extensive bone destruction and subsequent entry of calcium into the extracellular fluid but who do not have the capacity to excrete this calcium in the urine because of impaired glomerular filtration.

Solid tumors and extensive localized osteolysis

Breast cancer is often associated with hypercalcemia, but the mechanisms may be different from other solid tumors. Hypercalcemia does not occur early in the course of the disease and is always associated with extensive bone destruction (15). Although breast cancer cells can destroy bone directly in vitro (16), careful examination of bone surfaces by scanning electron microscopy suggests that stimulation of osteoclastic resorption is the major mechanism (Boyde, A., and G. Mundy, unpublished observations). The mechanism by which breast cancer cells stimulate osteoclast activity is unknown, although prostaglandin secretion by the tumor cells may be important. It has long been recognized that administration of estrogens or antiestrogens to patients with breast cancer is sometimes associated with acute exacerbations of hypercalcemia. When cultured human breast cancer cells are incubated with these agents in vitro, bone-resorbing activity and prostaglandins of the E series are released (17). Release of bone-resorbing activity (and prostaglandins) is inhibited by indomethacin, suggesting that the bone-resorbing activity is in fact due to the prostaglandins. In vitro studies of tumor cell migration have also clarified the mechanisms involved in the attraction of tumor cells toward bone surfaces. Organ cultures of resorbing bone release factors which are chemotactic for human and animal breast cancer cells (18). These chemotactic factors may represent Type I collagen or its fragments released from bone by the resorption or remodeling process (19).

Solid tumors and HHM

Most of the interest in the hypercalcemia of malignancy over the last few years has been focussed on solid tumors associated with the HHM. Most cases of HHM are not caused by authentic parathyroid hormone (PTH). Most nonparathyroid gland tumors do not have the capacity to secrete PTH, certainly not in amounts sufficient to cause hypercalcemia. We have not been able to detect PTH messenger RNA (mRNA) in most nonparathyroid tumors associated with hypercalcemia (20). Using a sensitive and specific assay which employs base pair hybridization of PTH cDNA with PTH mRNA, it is possible to identify PTH mRNA in as little as 100 μg of parathyroid tissue. We examined 1,000 times that amount of tumor tissue (100 mg) in over 25 tumors associated with hypercalcemia and we have been able to detect PTH mRNA in only one of these. Lack of sensitivity of this technique could not account for not finding PTH mRNA if it is present in sufficient amounts to encode enough PTH to cause hypercalcemia.

Attention has shifted recently from PTH to two other types of protein factors which could account for the hypercalcemia of malignancy, the "PTH-like" factors and the transforming growth factors (TGFs). The PTH-like factors are probably a family of proteins which are usually produced in solid tumors associated with hypercalcemia and which bind to some, but not all, PTH receptors (21–23). In fact, they provide convincing evidence that there is more than one class of PTH receptor. They mimic the effects of PTH on some renal tubular functions, notably cyclic AMP generation and inhibition of renal phosphate reabsorption. However, unlike PTH, they do not cause renal bicarbonate wasting and the subsequent hyperchloremic acidosis seen in primary hyperparathyroidism, nor do they increase gut absorption of calcium, serum 1,25-dihydroxyvitamin D concentrations, or rates of bone formation, in contrast with what is seen in primary hyperparathyroidism (24). There is other information which suggests that these factors should not be considered as simple PTH analogues which bind to the PTH receptor and activate it. Their effects to stimulate adenylate cyclase production in cultured rat osteosarcoma cells differ from authentic PTH under the same conditions; with the PTH-like factors, the time course is usually slower, the effect is usually less, and inhibition by the synthetic PTH antagonists usually less complete than against PTH (22). Thus, the question arises, what do these factors do? We think it unlikely that they can be solely responsible for bone resorption for several reasons. Unlike PTH, the bone resorbing factors produced by tumors associated with HHM are not inhibited by synthetic antagonists to PTH (25). However, it is possible that they could work in concert with TGFs on bone resorption, just as PTH itself has a synergistic effect with epidermal growth factor (EGF) to resorb bone (26). It is possible that PTH-like factors and TGFs produced by tumors have a similar synergistic effect on bone resorption, the TGFs working predominantly on osteoclast precursor cell replication and the PTH-like factors on activation of preexisting osteoclasts.

The PTH-like factors are operationally defined by in vitro assays in which they mimic the actions of PTH by stimulating adenylate cyclase (22) or increasing renal cell glucose-6-phosphate-dehydrogenase content (27). Their effects in vitro are inhibited by the synthetic PTH antagonists. They probably represent more than one factor. Whether these PTH-like factors represent excessive production by tumors of a normal, as yet uncharacterized, factor involved in calcium and/or phosphate homeostasis is unknown, but this seems likely. Members of this family could conceivably be involved in the pathogenesis of oncogenic osteomalacia, the syndrome asso-

ciated with renal phosphate wasting and osteomalacia. This syndrome usually occurs in association with benign mesenchymal tumors, although recently it has also been found in patients with carcinoma of the prostate (28). Some of the features of oncogenic osteomalacia, namely renal phosphate wasting and low serum 1,25-dihydroxyvitamin D concentrations, are reminiscent of those seen in HHM and could be due to interaction of a tumor-derived factor with some PTH receptors.

The other family of factors which have been implicated recently in HHM are the TGFs (1, 29). These factors are polypeptide stimulators of cell replication which are released by many tumors, as well as normal tissues and particularly platelets (30) (for review of effects on bone, see reference 1). The evidence is now very strong that TGF alpha, a class of TGF which binds to the EGF receptor, is responsible for bone resorption in several models of HHM (29, 30). Human recombinant or rat synthetic TGF alpha resorb bone in concentrations one to two orders of magnitude less than concentrations of PTH which produce similar effects. Moreover, in several tumor models, the bone-resorbing factor produced by the tumors is inhibited by specific anti-EGF receptor antisera, which inhibits the binding and expression of biological activity of TGF alpha (31, 32). TGF alpha inhibits bone formation in vitro, a similar effect to that which is seen in vivo in patients with HHM (21). In several of the models of HHM, TGF alpha expression is abnormal. The form of TGF alpha produced by these tumors is of higher molecular weight than synthetic or recombinant TGF alpha and may represent aggregation of secreted TGF alpha or incomplete processing (29). Recent data suggest that TGF alpha is encoded by one gene, and is synthesized and inserted into the cell membrane (33, 34). The biologically active moiety may be released by a novel proteolytic mechanism (34). Whether the larger form found in tumors associated with hypercalcemia represents an abnormality in such a proteolytic mechanism or an abnormality in transcription and translation will require further study.

TGF alpha appears at present to be a tumor product in the adult animal or human. It may have important physiological actions in the developing fetus as a growth factor since it has been found in increased amounts in developing rodent embryos (35, 36). However, it has not been convincingly demonstrated yet in normal adult tissues.

Two other members of the growth factor family could potentially be involved in the hypercalcemia of malignancy. These are TGF beta and platelet-derived growth factor (PDGF). TGF beta is a distinct molecule from TGF alpha. It is a homodimer; each subunit of 12.5 kD bound by disulfide bonds. It has the same effects in vitro of maintaining the transformed phenotype in indicator cells and in promoting soft tissue colony formation, but it has no amino acid sequence homology with TGF alpha and it has its own receptor distinct from the EGF receptor (37, 38). Moreover, TGF beta is frequently produced by replicating normal cells as well as tumor cells. The richest source of TGF beta is the alpha granule of the platelet (39). Since TGF beta mRNA is expressed in most tumors associated with hypercalcemia, it is a potential mediator of HHM. In one animal model, we have found partial purification of TGF beta biological activity with bone-resorbing activity (40).

PDGF is also a potential mediator of HHM. One chain of PDGF is closely homologous to the protein encoded by the v-sis oncogene (41, 42), and we have found that the cellular homolog of v-sis is expressed in all of the hypercalcemic tumors which we have studied. This association could be fortuitous, but this strong positive correlation between hypercalcemia and sis expression is tantalizing. Although early reports suggested that preparations of PDGF resorbed bone (43), neither we nor others have been able to confirm this in the rat long bone culture system. However, PDGF may also play a role in bone resorption by working in concert with the other growth factors. For example, it has been shown by Assoian et al. (44) that PDGF, TGF beta, and an EGF-like factor, all derived from platelets, work in concert to promote synergistic anchorage-independent growth of indicator cells in soft agar.

HHM is not the only ectopic hormone syndrome to be associated with growth factor production by a tumor. In some patients with tumor hypoglycemia, hypoglycemia has been ascribed to production of insulin-like growth factor II by the tumor cells, which mimics the action of insulin (45). There are other similarities between tumor hypoglycemia and HHM. In both, the hormone first suspected for being responsible for the syndrome (insulin and PTH, respectively) is rarely if ever produced by nonendocrine tumors. Secondly, it is now clear that nonendocrine tumors do produce growth factors and other products which can interact with the receptors of the "classic" hormones. Whether other ectopic hormone syndromes currently ascribed to "classic" hormones will turn out to be due to tumor products mimicking the biological effects of the hormone by binding to the hormone's receptor remains to be determined.

How then could these two seemingly distinct families of factors, the PTH-like factors and the growth factors, account for the hypercalcemia associated with solid tumors. Both are frequently produced by hypercalcemic tumors, and increasing evidence suggests that both may be involved. Clearly, these two factors could work in concert. Possibly they are encoded by related genes or by a single gene with variable splicing events generating two mRNAs with different functions. Possibly both types of factors are needed for hypercalcemia to occur. It is conceivable that PTH-like factors could work on bone to enhance the TGF bone-resorbing effect, although they do not have a major effect on bone per se. Our current working hypothesis for this syndrome is that the growth factors work primarily to increase bone resorption and inhibit bone formation, and that the PTH-like factors act predominantly on the kidney to inhibit renal phosphate reabsorption and generate cyclic AMP. The most important PTH-like effect on the kidney may be to promote renal calcium reabsorption. There is a growing body of evidence that suggests that impaired renal calcium excretion is important in HHM (46, 47). This has always been apparent in myeloma, where hypercalcemia rarely occurs in the absence of renal failure. Possibly, in HHM, the normal homeostatic mechanism for control of serum calcium can handle an increase in bone resorption caused by a transforming growth factor without a fluctuation in the serum calcium, unless the tumor also produces a factor which increases renal tubular calcium reabsorption and interferes with that compensatory mechanism. Within the framework of this model, we would suggest that cellular oncogene expression (possibly c-sis), leads to activation of several genes, including one encoding the TGFs and one encoding the PTH-like factors. This, in turn, leads to secretion of the corresponding proteins

by the tumor cells, which together produce the hypercalcemic syndrome.

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