

PTH-Related Peptide (PTHrP) in Hypercalcemia

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ABSTRACT

It is now 20 years since the tumor-associated factor parathyroid hormone-related peptide was identified. Since then, there have been significant changes in the understanding of the hypercalcemic syndromes associated with malignancy and with the role of this peptide in normal physiology as well as this specialized pathologic setting. Parathyroid hormone-related peptide has become a useful diagnostic tool in the differential diagnosis of hypercalcemia, and approaches to inhibit its expression or its effects by malignant cells hold promise for treating the hypercalcemia and osteolysis associated with some cancers.

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Parathyroid hormone (PTH)-like factors responsible for the syndrome of hypercalcemia of malignancy were first proposed by Albright¹ in the 1940s, on the basis of clinical observations in a single patient with hypercalcemia as a result of renal cell carcinoma. Albright suggested that the clinical syndrome in his patient resembled that of primary hyperparathyroidism, and the most likely explanation was that a PTH-like factor produced by the tumor was responsible. This thinking held sway for the next 40 yr, and with the identification of PTH and the development of antisera and immunoassays for its measurement in the 1960s and 1970s, many investigators searched for PTH in association with solid tumors. This search was by and large negative, other than for some confusion caused by the original antisera used for identification of PTH by RIA, and some investigators reported that PTH—or at least PTH-like molecules—were produced by tumors associated with malignancy.^{2–4} In retrospect, these assays may have been weakly recognizing PTH-related peptide

(PTHrP), which is closely related at its N-terminal end to native PTH.

The situation was clarified further in the 1980s, when it was found that patients with hypercalcemia of malignancy frequently had increased nephrogenous cAMP and, moreover, had a circulating factor that increased adenylate cyclase activity in cultured bone cells or renal membranes in much the same manner that PTH did but was clearly not PTH.^{5,6} Thus, it was concluded that a PTH-like factor rather than native PTH was produced by tumors associated with hypercalcemia. In the late 1980s, the active principle responsible for this syndrome was identified as PTHrP, a peptide produced by tumors with close homology in the N-terminal sequence to PTH itself. In fact, PTHrP was found to have arisen after gene duplication of PTH, after which both gene products developed independently as two molecules with different structural complexities and mechanisms of control. Despite these differences, PTHrP exerts cellular effects through the binding and activation of the receptor

that it shares with PTH, the type 1 PTH receptor.^{5,7–10}

In the 1990s, further observations on PTHrP showed that it was produced not only by squamous cell carcinomas and other tumors associated with the syndrome of humoral hypercalcemia of malignancy but also by nonhypercalcemic tumors that caused local osteolysis, such as breast cancer,^{11,12} and in fact was responsible for the osteolysis associated with breast cancer in some models.¹³

After its identification and molecular cloning in the late 1980s, attention then turned to the physiologic role of PTHrP. Genetic mouse studies, by the mid-1990s widely used to characterize the physiologic roles of molecules in bone biology, showed that PTHrP was responsible for normal endochondral bone formation and controlled cartilage proliferation at the growth plate. Perichondral cells and chondrocytes synthesize PTHrP at the ends of the cartilage mold in the growth plate.^{14–16} PTHrP acts to prevent chondrocyte differentiation, thus delaying the appearance of postmitotic hypertrophic chondrocytes. PTHrP expression in the growth plate is controlled by Indian Hedgehog and its downstream mediators in the Gli family, through a negative feedback relationship. The Gs and Gq

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family of heterotrimeric G proteins mediates the effects of PTHrP on chondrocytes to limit their differentiation by the downstream cyclin-cdk inhibitor p57 that is suppressed to maintain chondrocyte proliferation and by the transcription factor Sox9.^{17–19} These effects of PTHrP at the growth plate seem to serve its major physiologic role.

PTHrP has been shown to play a local role in normal osteoblast function.²⁰ Osteoblast-specific ablation of PTHrP in mice results in osteoporosis and an impairment of bone formation, suggesting a paracrine function. Thus, PTHrP may regulate normal osteoblast (and possibly osteoclast) differentiation and activity in the bone microenvironment,²¹ and PTHrP is being developed as a potential anabolic agent for osteoporosis with similar bone stimulatory effects to those of PTH.^{22,23} It also has other local cytokine-type roles of uncertain significance, including in the breast, urinary bladder, uterus, vascular smooth muscle, hair follicles, and skin. It also transfers calcium across the placenta from mother to fetus.^{24–26} Multiple fragments of PTHrP that have biologic activities have been described, but none of these has been confirmed as being important either physiologically or *in vivo*, and they still remain controversial and a matter of investigation.

With the observations that PTHrP plays an important role in both humoral hypercalcemia of malignancy and localized osteolysis associated with metastatic cancer, our concepts of the syndrome of hypercalcemia of malignancy have changed considerably. Up to the mid-1990s, it was thought that humoral hypercalcemia of malignancy was due to a circulating factor (namely PTHrP) and that localized osteolysis was due either to local cytokines or to direct effects of the tumor cells to cause localized bone destruction.²⁷ With the observation that PTHrP may be responsible for both syndromes, at least in many patients, it has become clear that hypercalcemia of malignancy represents a spectrum, with PTHrP acting as the circulating factor when it is produced in excessive amounts by some

tumors (and thereby causing humoral hypercalcemia) but under other circumstances acting as a local factor when produced by metastatic tumor cells in the bone microenvironment and causing osteolytic bone metastases (Table 1).

Although PTHrP and PTH share the same receptor, there are differences between the syndromes of humoral hypercalcemia of malignancy and primary hyperparathyroidism. In humoral hypercalcemia of malignancy, bone formation is suppressed, and patients have a metabolic alkalosis rather than hyperchloremic acidosis. Moreover, serum 1,25 dihydroxyvitamin D₃ concentrations are increased in primary hyperparathyroidism and suppressed in cancer. The reasons for these differences are unknown but may be due to other factors produced in conjunction with PTHrP in humoral hypercalcemia of malignancy.^{27,28}

Other cancer syndromes are associated with PTHrP excess. Probably the most important of these is cachexia, which has been emphasized by Ogata and colleagues.²⁹ Humoral hypercalcemia of malignancy induces accumulation of orexigenic peptides, such as neuropeptide Y in the arcuate nucleus of the hypothalamus, and both the cachexia and the mRNA for these peptides are reduced by anti-PTHrP therapies. It is still unclear whether cachexia is in fact due to PTHrP directly or is a consequence of increased tumor burden in the bone marrow.

Since PTHrP is such an important factor in causing these common syndromes associated with malignancy, different attempts have been made to block its biologic activity. One approach was

the development of neutralizing antibodies to PTHrP, based on preclinical studies showing that neutralizing antibodies reduced serum calcium and decreased bone metastases in preclinical models.^{13,30} A second was to use small molecules that inhibit PTHrP transcription. We have identified specific small molecules that inhibit PTHrP transcription by tumor cells.³¹ These molecules, which are antimetabolites and include 6-thioguanine, were identified in a cell-based screening assay and found to reduce osteolysis and lower serum calcium in preclinical models of bone metastasis and hypercalcemia. A third was to develop antagonists of PTHrP binding to the PTH receptor, but this has not led to successful therapeutics so far. Other possibilities include small molecule approaches to inhibit PTHrP signal transduction within tumor cells.³²

The regulation of PTHrP production by malignant cells is an issue of extreme interest. Why do certain cancer cells express a peptide that is physiologically important to cartilage cells of the growth plate? In the growth plate, PTHrP is regulated by the Hedgehog pathway and the Gli family of transcriptional mediators. We believe that similar mechanisms are also involved in cancer, albeit a little more complex. In solid tumors associated with PTHrP expression, it was found that PTHrP transcription is driven by Gli family members similar to what happens in the developing growth plate.^{33,34} The cancer cell uses this usually dormant developmental Hedgehog pathway that is important in embryonic life to enhance its expression of PTHrP, initiate bone resorption, and form a nidus for a bone metastasis. The process is driven by TGF- β , which is released into the bone microenvironment when bone is resorbed, because it is the most abundant growth factor in the bone matrix.^{35,36} This adds another level of understanding to the vicious cycle between tumor cells and osteoclasts in the bone microenvironment in metastatic cancer (Figure 1).^{27,37} Thus, TGF- β released as a consequence of bone resorption in the bone microenvironment stimulates expression of Gli family members in the

Table 1. Tumor syndromes associated with PTHrP production

Humoral hypercalcemia of malignancy hypercalcemia
increased plasma PTHrP and nephrogenous cAMP
Localized osteolysis
\pm hypercalcemia
no increase in plasma PTHrP and nephrogenous cAMP

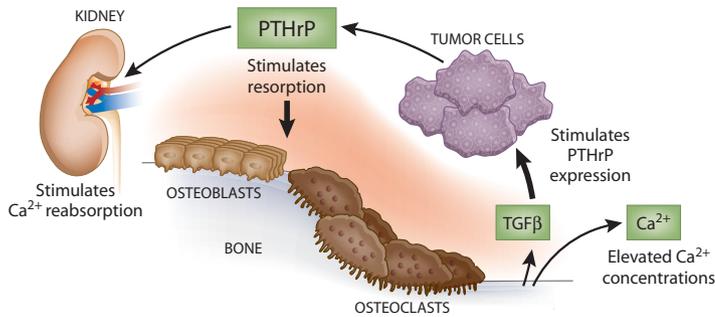


Figure 1. Pathologic roles of PTHrP. PTHrP is the principal factor in cancer-induced bone disease responsible for humoral hypercalcemia of malignancy and localized osteolysis. In the latter case, severe bone loss is initiated and fueled by the “vicious cycle,” whereby tumor-derived PTHrP stimulates osteoclastic resorption. The subsequent release of bone-derived growth factors stimulates tumor growth and PTHrP expression by tumor cells.

Hedgehog pathway, in turn leading to increased PTHrP expression and bone resorption. The resorption of bone favors tumor cell growth and further release of active TGF- β from the bone matrix.

PTHrP is thus an extremely interesting molecule. Derived from the same ancestral gene as PTH, it has a limited physiologic role in embryonic and early postnatal life to regulate cartilage growth in developing long bones. It has no major physiologic role to our knowledge in adult life; however, it does have a major pathologic role, mediating bone destruction and hypercalcemia by certain tumors that have learned to activate the developmental Hedgehog pathway and express PTHrP, which allows the tumor cells to create a “safe harbor” in the form of a bone metastasis. As our knowledge of this interesting process by which cancer destroys bone grows, it seems certain that successful pharmacologic attempts to limit the expression of PTHrP or its effects hold potential benefits for the multitude of patients with advanced cancer.

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DISCLOSURES

None.

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