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Parathyroid Hormone-Related Peptide and Malignancy

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1. INTRODUCTION

Parathyroid hormone-related peptide (PTHrP) was originally isolated as a causal factor for hypercalcemia of malignancy (HM), one of the most frequent paraneoplastic syndromes. The association of hypercalcemia with malignancy was originally assumed to be the result of tumor invasion of bone with resultant osteolysis (1,2), but subsequent studies demonstrated an association of hypercalcemia with cancer, even when the tumor had not metastasized to bone. In a careful clinical analysis of a case of renal cell carcinoma with metastases, it was noted that hypercalcemia was associated with hypophosphatemia (3). It was therefore postulated, because lysis of bone should liberate both calcium and phosphate, that the tumor was producing a factor that was both hypercalcemic and phosphaturic, analogous to parathyroid hormone (PTH) (3). The concept arose that tumors might “ectopically” produce PTH, which is normally expressed only in the parathyroid gland. The term “pseudohypoparathyroidism” was therefore employed to describe a syndrome in which cancers had not metastasized to bone, but were associated with hypercalcemia and other PTH-like biochemical abnormalities (4). Certain biochemical alterations were, however, found to differ in primary hyperparathyroidism and “pseudohyperparathyroidism,” including a higher level of serum calcium in the latter and a tendency in the latter toward an alkalosis rather than an acidosis. The development of sensitive bioassays for PTH-like bioactivity confirmed the presence of PTH-like material

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in the tumors and serum of patients with pseudohyperparathyroidism (5). Although analyses of tumors for PTH-protein (6) and mRNA-encoding PTH (7) failed to detect PTH in this syndrome, a PTH-like substance was subsequently isolated and cloned from several tumors (8–10). This material was referred to initially as both PTH-like peptide and PTH-related peptide and is now known by the term “PTH-related peptide” (PTHrP).

2. MOLECULAR CHARACTERISTICS OF PTHrP AND ITS GENE EXPRESSION AND REGULATION

Parathyroid hormone-related peptide is a member of a gene family, which encompasses PTH, PTHrP, and a hypothalamic peptide, tuberoinfundibular peptide 39 or TIP39 (11) (Fig. 1). The human gene encoding PTHrP is assigned to the short arm of chromosome 12, whereas that for PTH is located on the short arm of chromosome 11. Chromosomes 11 and 12 carry other functionally related genes and are thought to have arisen from a common ancestral gene. Similarities in the structural organization of the PTH and PTHrP genes exist in that corresponding exons encode similar functional domains. Furthermore, PTH and PTHrP share limited but biologically important amino-acid-sequence homology in their NH₂-terminal domains, where most of the best documented bioactivity is believed to reside. Both PTH-like and PTHrP-like peptides have been found in many species as far back as teleosts (13).

The human PTHrP gene spans more than 14 kb of DNA and contains a minimum of 7 exons and 3 promoters. Alternative promoter usage and/or different splicing patterns account for heterogeneous PTHrP mRNA species. These species encode secretory proteins with mature isoforms up to 139, 141, and 173 amino acids. Consequently, amino acid identity exists in all three forms of position 139 (13). The significance of the carboxyl heterogeneity remains uncertain because there is no consistent evidence that tissue-specific or developmental splicing patterns occur. Tumor-specific promoter utilization has been suggested as a possible explanation of why many malignancies express PTHrP mRNA and protein but only a subset of cancer patients in fact secrete PTHrP in sufficient quantity to develop hypercalcemia (14). In some studies, a general increase in transcription has been suggested rather than enhanced single-promoter usage with alternative splicing to account for PTHrP overproduction in cancer. Region-specific promoter demethylation (15) and gene amplification (16) have also been noted to enhance PTHrP expression in certain malignancies. A number of studies have examined the molecular regulation of PTHrP gene expression. A variety of growth factors, including epidermal growth factor (EGF) (17), insulin-like growth factor (IGF)-1 (18), and transforming growth factor (TGF)- β (19) have been shown to stimulate PTHrP expression, whereas 1,25-dihydroxyvitamin D [1,25(OH)₂D] and androgens have been shown to inhibit its expression (20,21) (Fig. 2). Growth factors produced in a paracrine/autocrine mode by a PTHrP-producing neoplasm or released from surrounding host cells when tumors invade the skeleton or soft tissues might play an important role in enhancing PTHrP production by the tumor cells (19) (Fig. 3).

3. PTHrP ACTIONS

3.1. PTHrP as a Polyhormone

Parathyroid hormone-related peptide has been postulated to be a polyhormone and diverse biological actions have been ascribed to its amino (NH₂)-terminal, midregion,

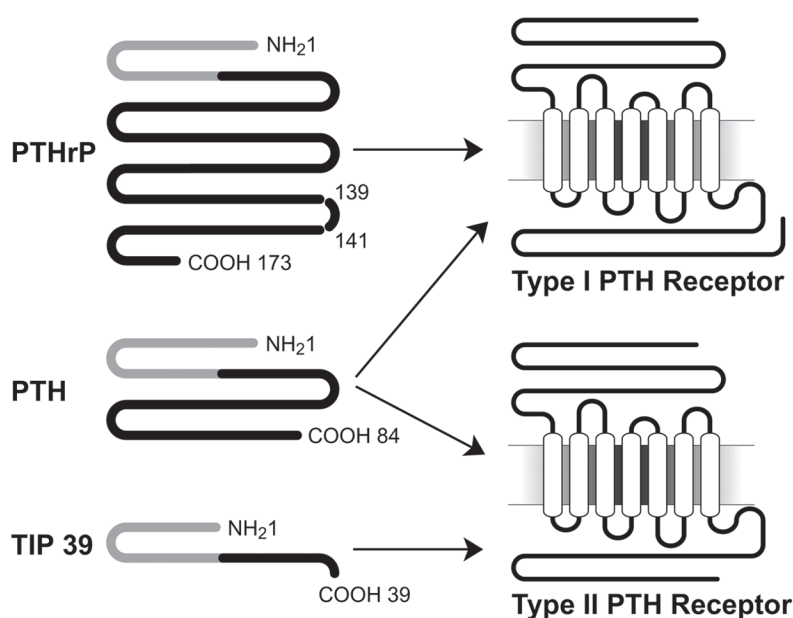


Fig. 1. PTH ligand and PTH receptor families. PTHrP is a member of a gene family that includes parathyroid hormone (PTH) and tuberoinfundibular peptide (TIP39). Amino acid sequence homology is restricted to the amino-terminal domains (shaded regions) of these hormones. Human PTHrP can occur as isoforms of 139, 141, or 173 amino acids, whereas PTH is an 84-amino-acid peptide. The plasma membrane target tissue receptors for these peptides are two G-protein-coupled receptors that are also members of a single gene family. PTHrP and PTH interact with the Type I PTH receptor, and PTH and TIP39 interact with the Type II receptor.

and carboxyl regions. The carboxyl region has been shown, in some studies, to exert an osteoclast inhibitory role (22). A mid-region domain has been demonstrated to contain a nuclear localization sequence that might direct the molecule (23), via the use of the importin B system (24), into the nucleus and then to the nucleolus, where it might alter cell growth, differentiation, and/or apoptosis. Indeed, several studies have demonstrated the presence of intranuclear PTHrP both in tissues *in vitro* and *in vivo*. Since nascent PTHrP contains a leader sequence (25) that ordinarily would direct the molecule into the secretory pathway, several cellular routes have been reported that could lead to the presence of PTHrP in the cytoplasm and enable its nuclear import. These pathways include the use of an alternate translational start site that would exclude expression of the leader sequence (25), internalization of secreted PTHrP (26), and back-transport of PTHrP from the secretory system to the cytoplasm, where it could be available for nuclear import or degraded by the ubiquitin–proteasome pathway (27). Future studies employing “knock-in” technology might be useful for understanding the role of the midregion and carboxyl regions of PTHrP to its biological functions *in vivo*. The majority of the well-documented bioactivity of PTHrP is present within its NH₂-terminal domain so that, in analogy with synthetic PTH (1–34), synthetic PTHrP (1–34), or synthetic PTHrP (1–36) appear to mimic many of the effects of the full-length PTHrP molecule. Sequence homology between PTH and PTHrP is restricted to 8 of the first 13 amino acids at the NH₂-

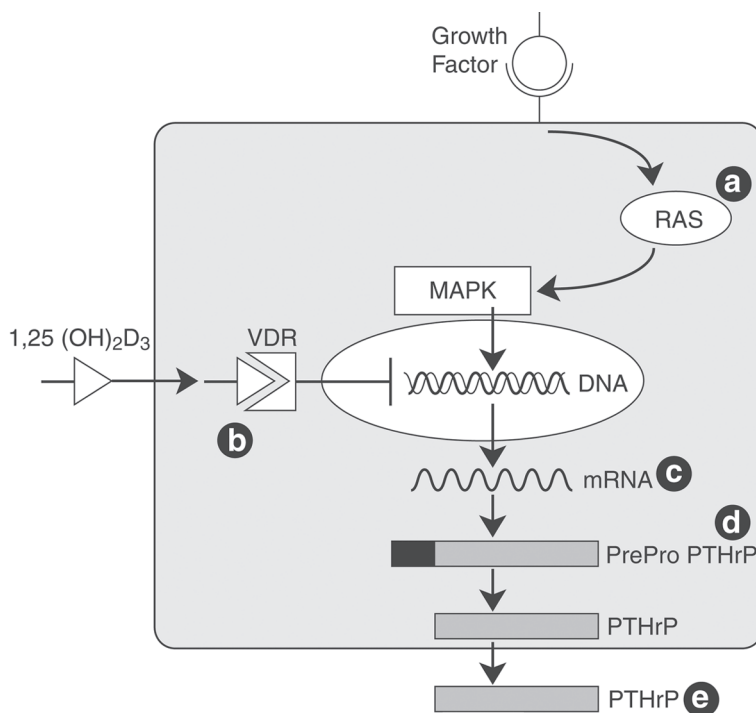


Fig. 2. Regulation of PTHrP production. Growth factors might stimulate PTHrP production by increasing gene transcription via the RAS–mitogen-activated protein kinase (MAPK) pathway whereas 1,25-dihydroxyvitamin D₃ [1,25 (OH)₂D₃] might act via the vitamin D receptor (VDR) to inhibit PTHrP gene transcription. The mRNA translation product, Pre Pro PTHrP, must first be processed by a furin-like enzyme to remove a “leader” or “pre pro” amino acid sequence, and the mature PTHrP molecule can then be secreted. Sites of potential inhibition of PTHrP include (a) RAS inactivation (via farnesyl transferase inhibitors), (b) use of low calcemic vitamin D analogs to inhibit PTHrP gene transcription, (c) use of antisense RNA to reduce PTHrP translation, (d) use of furin antagonists to inhibit PTHrP processing, and (e) use of inhibitors or antibodies to interfere with PTHrP action.

terminus including those at positions 1 and 2, which are critical for the activation of adenylate cyclase. This limited homology, as well as conformational similarities in the nonhomologous 14–34 sequence permits the 1–34 domain of PTHrP and of PTH to bind to a common receptor with equal affinity (Fig. 1). To date, no receptor for domains other than the NH₂-terminal domain have been identified for PTHrP.

3.2. PTHrP Receptor and Postreceptor Signaling

The NH₂-terminal domains of PTHrP and of PTH bind to a common seven-transmembrane-spanning receptor that is linked by G proteins to both the adenylate cyclase and phospholipase C signaling pathways (28,29). With the discovery of a second receptor which binds PTH, the receptor common to both PTH and PTHrP has been termed the type I PTH/PTHrP receptor (PTR). The second (type II) receptor has weak affinity for PTHrP but binds PTH and TIP39 (30) (Fig. 1). In view of its primary expression in the brain and

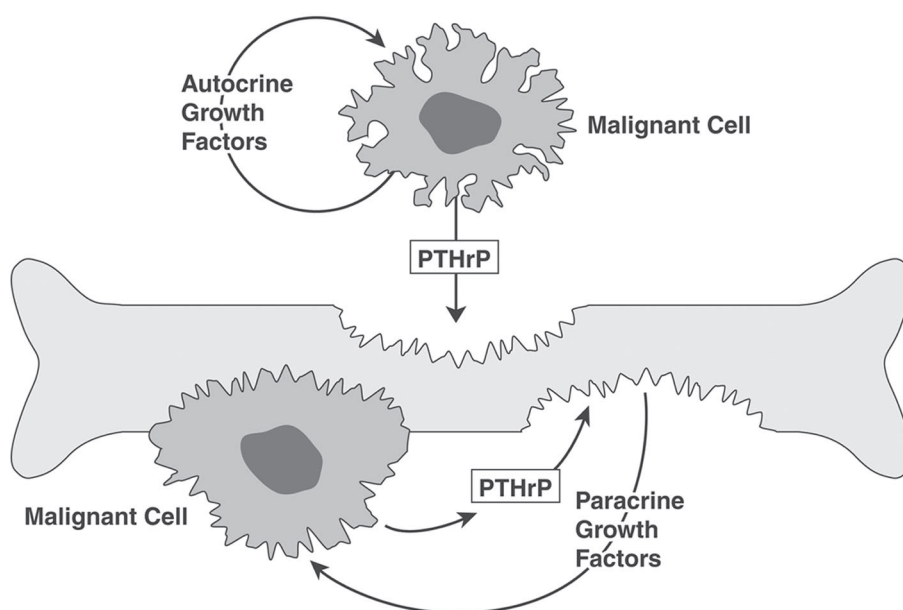


Fig. 3. Endocrine and paracrine effects of PTHrP on tumor-induced bone resorption. Growth factors released by malignant cells that have not metastasized to bone might stimulate PTHrP production and secretion in an autocrine mode; PTHrP might then function in an endocrine manner to resorb bone. Alternatively, PTHrP that is released by malignant cells that have colonized bone can locally resorb bone and release growth factors that can act in a paracrine mode to enhance PTHrP production.

outside of calcium-regulating tissues, it appears to be the primary receptor for TIP39 and its role, if any, in calcium and skeletal homeostasis remains to be determined.

In addition to the traditional signaling molecules cAMP/protein kinase A and calcium/diacylglycerol/protein kinase C, increasing work in recent years has identified other signaling molecules, presumably linked directly or via crosstalk to the PTR, including phospholipase D (31), MAP kinase (32), and, possibly, nitric oxide (33).

3.3. Physiologic Roles of PTHrP

Parathyroid hormone-related peptide effects on cell growth and differentiation and the Type I PTR are expressed in a variety of cells and tissues beginning in early embryogenesis. In vitro and in vivo studies in animals have shown that PTHrP can alter the growth, differentiation, and differentiated functions of a variety of different normal cells and tissues, including, for example, keratinocytes (34), mammary cells (35), brain cells (36), smooth muscle cells (37), respiratory epithelial cells (38), renal cells (39), and pancreatic β cells (40). In some tumor situations, PTHrP has also been shown to exert growth-promoting effects. However, a profound physiologic effect of PTHrP has been demonstrated, via studies of targeted gene ablation, on endochondral bone formation. Normal growth and differentiation of the cartilaginous growth plate appears critically dependent on the action of PTHrP (41). In studies of postnatal animals, PTHrP appears important

for normal bone formation (42). The physiologic effects of PTHrP are almost certainly subserved through local paracrine/autocrine effects. When PTHrP is overproduced in neoplasia and enters the circulation, its endocrine role in HM largely mimics the effects of circulating PTH on the kidney and on bone (43).

3.4. Renal Effects of PTHrP

In view of its prominent effect on stimulating adenylate cyclase in the kidney, PTHrP, as with PTH, enhances renal cell intracellular cAMP, a fraction of which enters the renal tubular lumen and is excreted as a nephrogenous portion of urinary cAMP. Consequently, in PTHrP-associated HM, nephrogenous cAMP (NcAMP) in the urine is elevated (44). Cyclic AMP appears to mediate many of the cellular responses to PTHrP, as it does to PTH, including the phosphaturic response. This response appears to occur via enhanced protein kinase A but also protein kinase C-mediated internalization of the (Type II) Na/PO₄ cotransporter leading to diminished phosphate reabsorption (45). PTHrP-induced stimulation of calcium reabsorption, predominantly via active transcellular transport in the ascending limb of the loop of Henle and in the distal tubule, is another critical renal effect that seems important for the development and maintenance of the hypercalcemia in HM (46). Mobilization of calcium from bone resorption might be responsible of the episodes of severe hypercalcemia observed in more advanced stages of the disease.

A third major effect of PTHrP in the kidney is its effect on the renal 1 α hydroxylase enzyme. Intravenous administration of NH₂-terminal fragments of PTH or PTHrP both in animals (47) and humans results in an elevation in serum 1,25(OH)₂D. Additionally, it has been suggested that a positive correlation might also exist between 1,25(OH)₂D and PTHrP in the early stages of HM (48). Nevertheless, serum 1,25(OH)₂D concentrations are often suppressed in the terminal stages of HM, when the patient is severely hypercalcemic (44). It is possible that non-NH₂-terminal domains of PTHrP could be inhibitory on the renal 1 α hydroxylase enzyme, that additional inhibitory materials might be cosecreted with PTHrP by the tumor, or that severe hypercalcemia *per se* might inhibit the enzyme. These possibilities remain to be definitively explored. Finally, whether HCO₃ reabsorption by the kidney can be handled differently by PTHrP and by PTH leading to a mild metabolic alkalosis in HM vs a mild metabolic acidosis in primary hyperparathyroidism also remains to be clarified. To date, few major differences have been observed in PTHrP and PTH effects of the kidney in controlled animal studies or in humans, suggesting that other mechanisms might converge to modulate kidney function in the patient with HM and advanced neoplasia.

3.5. Skeletal Actions of PTHrP

Both PTHrP and PTH bind *in vivo* to cells of the osteoblastic phenotype (49), which express the Type I PTR. Each peptide can enhance both osteoblastic bone formation and osteoclastic bone resorption through this interaction. The mechanism of osteoclastic bone resorption involves the enhancement of expression, in osteoblastic stromal cells, of the cytokine, receptor activator of nuclear factor- κ B (RANK) ligand (RANKL), which can then bind to its cognate receptor RANK on cells of the hematopoietic lineage (Fig. 4) (50). RANKL is a member of the tumor necrosis factor (TNF) family of cytokines and RANK transduces the RANKL signal via second messengers such as TRAF6. This interaction then promotes differentiation and fusion of mononuclear

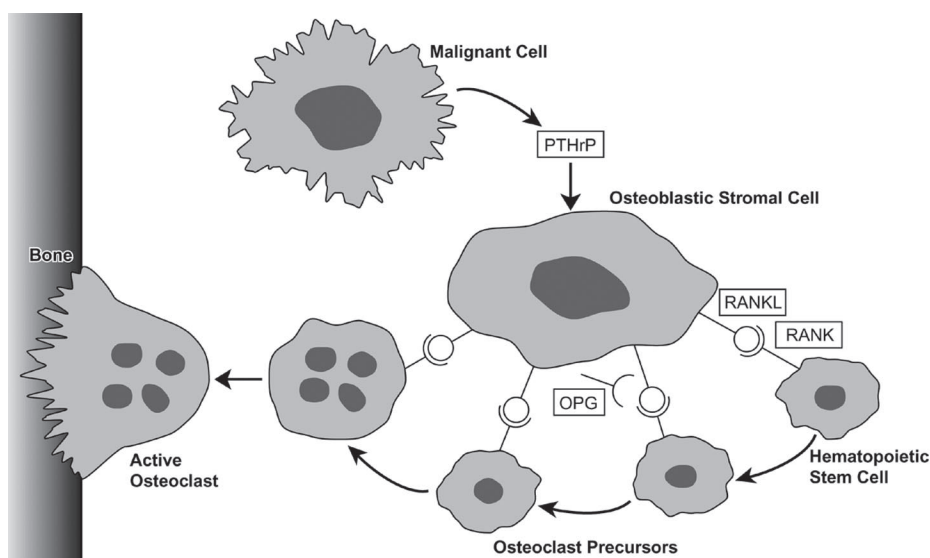


Fig. 4. Role of the RANKL–RANK–OPG system in PTHrP-induced osteoclastogenesis. PTHrP secreted from malignant cells can interact with an osteoblastic stromal cell, causing increased production of RANKL and decreased production of OPG. RANKL binds to its cognate receptor RANK in osteoclast precursor cells, which are of the hematopoietic lineage, causing them to differentiate and fuse to form multinucleated cells that are then activated to form bone-resorbing osteoclasts.

osteoclast precursors to multinucleated cells and then activation of the multinucleated osteoclasts to resorb bone (51,52). Simultaneously, PTHrP (and PTH) can reduce the expression of a soluble decoy receptor for RANKL termed “osteoprotegerin” (OPG) (53) and thereby enhance the capacity of RANKL to interact with RANK (Fig. 4). In HM, PTHrP clearly enhances osteoclastic bone resorption to an extent that exceeds osteoblastic bone formation, thereby causing a net mobilization of calcium from bone and contributing to hypercalcemia.

When PTHrP is released from a tumor that has not yet invaded bone, this might cause diffuse osteopenia, but even when neoplasms such as breast cancer have metastasized to bone, locally released PTHrP might also contribute to local osteolysis in the microenvironment adjacent to the tumor metastasis (Fig. 3). This localized resorption around skeletal metastatic lesions might or might not result in hypercalcemia probably depending on the extent of the metastasis and the capacity of the kidney to clear the increased filtered load of calcium. Just as autocrine growth factors can stimulate PTHrP production in a tumor that has not yet metastasized to bone, growth factors released from bone such as TGF- β can stimulate PTHrP production locally in a paracrine mode (Fig. 3). Although, in animal models of HM, bone formation appears to accompany the accelerated resorption caused by PTHrP, this might not always occur in humans with HM, such that “uncoupled” resorption might occur (54). Whether other tumor products or the extent of hypercalcemia play a role in this discordance remains to be clarified.

Table 1
Causes of Hypercalcemia of Malignancy (HM)

HM with overproduction of PTHrP
Humoral Hypercalcemia of Malignancy (HHM)
Solid tumors with skeletal metastases
Hematopoietic malignancies
HM with overproduction of other factors
Lymphomas with overproduction of 1,25-dihydroxyvitamin D
Malignancies with overproduction of other cytokines
Ectopic hyperparathyroidism

3.6. Spectrum of Tumors Associated With PTHrP Overproduction

In contrast to PTH, whose expression is virtually restricted to the parathyroid gland, PTHrP is widely expressed in a variety of normal fetal and adult tissues. Consequently, it is likely that overproduction of PTHrP by a broad spectrum of tumors likely represents eutopic overexpression, as malignant transformation of these tissues occurs, rather than ectopic expression. However, true ectopic overexpression of PTH as a cause of HM has been documented in a small number of tumors.

The syndrome of HM in the absence of skeletal metastasis (humoral hypercalcemia of malignancy or HHM) has classically been associated with renal cell carcinomas and squamous cell carcinomas derived from a variety of primary sites (Table 1). Once it was demonstrated that PTHrP infusion could induce the biochemical and skeletal abnormalities of HM it was believed that PTHrP overproduction would only be associated with such tumors. With the introduction of molecular biological and immunological techniques to detect PTHrP, it became clear that overexpression of PTHrP and elevated circulating concentrations of this peptide can occur with a much broader histological spectrum of tumors than was originally envisioned. Thus, breast cancers produce PTHrP (55), as do a variety of other tumors, including endometrial (56) and colon cancers and even mesotheliomas (57). A variety of endocrine tumors have also been shown to produce PTHrP (58), including pheochromocytomas (59), insulinomas (60), parathyroid adenomas (61), pituitary tumors (62), and thyroid cancers (63). Furthermore, increased circulating concentrations of PTHrP have been detected in some patients with hematological malignancies, especially those with advanced-stage lymphomas (64). In contrast, PTHrP overproduction in multiple myeloma seems less frequent than in other hematologic malignancies. PTHrP can contribute to HM in patients with lymphomas whose hypercalcemia in the past was attributed solely to excess 1,25(OH)₂D. Although not all tumors that show increased expression of PTHrP secrete sufficient PTHrP so that it is detectable in the serum, even with such tumors (e.g., breast cancer), PTHrP released locally can induce osteolysis around metastases and contribute to the localized bone resorption.

4. HYPERCALCEMIA OF MALIGNANCY: CLINICAL CONSIDERATIONS

4.1. Clinical Manifestations of HM

Hypercalcemia is usually a manifestation of advanced malignancy, as compared with early stages of malignancy. Gastrointestinal manifestations of anorexia, nausea, and vomiting are common in association with hypercalcemia and could lead to dehydration.

Renal involvement, manifested by polyuria and evidence of azotemia caused by dehydration, can also occur. Finally, central nervous system manifestations of weakness progressing toward psychoses, stupor, and coma can ultimately ensue. The acuteness and severity of the hypercalcemia can, therefore, lead to life-threatening consequences if left untreated.

4.2. Diagnosis of PTHrP-Associated HM

The biochemical abnormalities observed with PTHrP-associated HM are similar, but usually more severe than those seen with primary hyperparathyroidism. In particular, the hypercalcemia is generally more pronounced. Its onset is generally acute and the elevation quite marked, with serum calcium concentrations not infrequently greater than 12 mg/dL or 3 mmol/L. Hypophosphatemia, reduced renal phosphate threshold, and increased renal tubular reabsorption of calcium are all seen, as is increased NcAMP excretion. In view of the high filtered load of calcium resulting from bone resorption, urinary calcium excretion might be increased.

Biochemical markers of bone resorption such as Type I collagen crosslinked N-telopeptides and C-telopeptides or pyridinium crosslinks might also be increased (65). In contrast, indices of bone formation such as bone-specific alkaline phosphatase and osteocalcin might not be elevated in HM because of the suppression of formation, whereas these indices are generally elevated in patients with primary hyperparathyroidism in whom formation and resorption are coupled. The most significant biochemical difference between HM and primary hyperparathyroidism, however, and a highly useful diagnostic tool is the concentration of circulating PTH, which is elevated in hyperparathyroidism but suppressed in HM because of hypercalcemia-induced suppression of the parathyroid gland. This is particularly helpful as a tool for differential diagnosis because of the high specificity and sensitivity of modern two-site immunoradiometric PTH assays.

Although occasional cases of true ectopic hyperparathyroidism have been reported, and some tumors might cause HM via overproduction of 1,25-dihydroxyvitamin D or of bone-resorbing cytokines (Table 1), the majority of cases of HM are associated with increased PTHrP production. The use of PTHrP immunoassays should be the most definitive method of diagnosing HM, because most cases of HM will be associated with excess PTHrP secretion. However, the three isoforms of PTHrP appear to undergo complicated posttranslational processing in the tumor cell of origin (66,67) and secreted metabolites may undergo differential metabolic clearance once secreted. As a result of the complexity of this process, multiple forms of bioactive PTHrP have been identified in the plasma of hypercalcemic cancer patients, and the precise character of circulating forms remains to be determined in order to maximize the sensitivity and specificity of PTHrP immunoassays.

In view of the fact the PTHrP bioactivity resides within the NH₂-terminal domain, initial efforts were developed using antisera directed against epitopes in this region. NH₂-terminal immunoassays measured elevated PTHrP not only in the majority of patients with HM but also in some normocalcemic cancer patients, although mean levels in the normocalcemic subjects were lower than in the hypercalcemic (68,69). This could reflect the capacity of such assays to measure bioinactive as well as bioactive NH₂ terminal fragments or the capacity to measure lower concentrations of PTHrP that are insufficient to cause hypercalcemia.

Immunoassays detecting primarily midregion and carboxyl-terminal epitopes of PTHrP have proven to be of less value clinically in the differential diagnosis of HM (70). Assays that recognize carboxyl-terminal fragments might show elevated levels in patients with renal insufficiency probably reflecting the renal clearance of such fragments rather than their hypersecretion by tumors.

The most prevalent and useful PTHrP assays appear to be two-site immunoradiometric assays that employ one antibody recognizing an NH₂-terminal epitope and a second antibody recognizing a more carboxyl epitope (although generally within the PTHrP [1–86] sequence) (71,72). These tend to be the most sensitive and specific assays for diagnosis and for monitoring therapy. The presence of an elevated concentration of PTHrP with malignancy has however been reported to portend a poor prognosis (73).

4.3. Treatment of HM

The most urgent treatment of HM generally involves treatment of severe, acute hypercalcemia. Because dehydration is an inevitable consequence of the hypercalcemia, treatment should initially begin with rehydration via the use of intravenous saline. Saline infusion will expand the intravascular volume, improve the glomerular filtration rate, and reduce proximal tubular sodium-linked calcium reabsorption. Once the patient is adequately hydrated, therapy can be directed to inhibit bone resorption. Intravenous bisphosphonates (zoledronic acid or pamidronate), which inhibit osteoclastic activity, are currently the most potent antiresorptive agents and the resultant reduction in serum calcium can last for several days to weeks (74). Nevertheless, because the onset of calcium lowering might be delayed for 1 or 2 d, parenteral calcitonin can be concomitantly administered. This peptide hormone will also directly inhibit osteoclastic action but has a peak response at 2–4 h after administration (75).

However, tachyphylaxis could occur after repeated doses of calcitonin. An additional approach, to rapidly reduce the serum calcium once the patient is adequately hydrated, is to administer in moderation, a loop diuretic such as furosemide to inhibit renal calcium reabsorption and promote calciuresis. Consequently, a treatment regimen involving initial rehydration followed by administration of calcitonin and/or furosemide (for rapidity) and intravenous bisphosphonate (for potency) would be most efficacious in correcting hypercalcemia.

Once the hypercalcemia has been corrected, efforts should be directed at reducing tumor burden or at least at inhibiting PTHrP production and action. A variety of approaches have been used with reasonable success to inhibit PTHrP production in animal models, including farnesyl transferase inhibitors to diminish growth factor mediated production (76), furin inhibitors to diminish PTHrP processing from its inert prohormone form to the mature bioactive form (77), and low calcemic vitamin D analogs to suppress PTHrP gene expression (78) (Fig. 2). None of these approaches have yet reached the clinic for application in humans. Antibodies to PTHrP have also been used in animal models with success (46,79) and have undergone early clinical trials in humans (80). Finally, because the RANKL–RANK pathway represents a final common pathway for bone resorption induced by PTHrP as well as by other stimulators of osteoclastogenesis that might be released by tumors (including a variety of cytokines), considerable attention is being paid to the development of inhibitors of this system (81), including OPG analogs,

RANKL production inhibitors, RANK antagonists, and inhibitors of the RANK signaling pathway.

Even when hypercalcemia has not occurred, in view of the apparent role of PTHrP in stimulating osteoclastic bone resorption adjacent to some skeletal metastases, considerable attention is being paid to inhibiting osteoclast production and activity (80), both by antagonizing PTHrP (81) and by employing bisphosphonates (82) or components of the RANKL–RANK–OPG pathway. This approach to altering the bone microenvironment appears to reduce the number of metastases and the untoward events related to metastases and is being assessed in virtually all skeletal metastatic disease. Indeed, it has become the standard of care in metastatic breast cancer.

5. CONCLUSION

The discovery of PTHrP has led to improved understanding of the molecular basis of HM—particularly HM occurring in the absence of significant skeletal metastasis but also HM induced by some tumors metastasizing to bone. This has led to improved ability to diagnose this condition and could ultimately lead to effective therapies to reduce PTHrP production and action both to prevent hypercalcemia and to control malignancies where PTHrP might play a growth-promoting role.

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