# Parathyroid Hormone-Related Protein: An Update

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PTHrP was identified as a cause of hypercalcemia in cancer patients 25 yr ago. In the intervening years, we have learned that PTHrP and PTH are encoded by related genes that are part of a larger "PTH gene family." This evolutionary relationship permits them to bind to the same type 1 PTH/PTHrP receptor, which explains why humoral hypercalcemia of malignancy resembles hyperparathyroidism. This review will outline basic facts about PTHrP biology and its normal physiological functions, with an emphasis on new findings of the past 5–10 yr. The medical and research communities first became aware of PTHrP because of its involvement in a common paraneoplastic syndrome. Now, research into the basic biology of PTHrP has suggested previously unrecognized connections to a variety of disease states such as osteoporosis, osteoarthritis, and breast cancer and has highlighted how PTHrP itself might be used in therapy for osteoporosis and diabetes. Therefore, the story of this remarkable protein is a paradigm for translational research, having gone from bedside to bench and now back to bedside. (***J Clin Endocrinol Metab*** **97: 2947–2956, 2012)**



Twenty-five years have passed since the isolation of PTHrP and the cloning of its gene (*PTHLH*) (1–4). The discovery of PTHrP was the culmination of many years of work directed at understanding the pathophysiology of hypercalcemia in cancer patients and was anticipated as early as 1941, when Fuller Albright postulated that tumors might produce a PTH-like humor (5). Intensive work in the 1980s and 1990s led to the biochemical identification of the syndrome of humoral hypercalcemia of malignancy (HHM), which in turn led to the characterization of PTHrP and the understanding that PTHrP and PTH are related molecules that can both stimulate the same type I PTH/PTHrP receptor (PTHR1) (5–7). As a result, when PTHrP is secreted by tumors, it can mimic its cousin, PTH, and lead to excessive bone resorption and hypercalcemia. After its isolation, it became obvious that PTHrP was widely expressed, especially during development, which prompted efforts to understand its normal functions. Although the discovery of PTHrP 25 yr ago "solved" the clinical riddle of HHM, defining its normal function(s) remains an ongoing puzzle. We now know that PTHrP is critically important to the physiology of some specific tissues, but it is remarkable how little we really understand of its function(s) in all the various tissues that express the *PTHLH* gene. The goal of this review is to outline and update basic facts of PTHrP biology and to highlight more recent knowledge about contributions of PTHrP to physiology and pathophysiology. Space limitations require selectivity in the areas that will be discussed, and readers are referred to longer reviews for a more complete discussion (5–7).

## The *PTHLH* Gene and the *PTH* Gene Family

Human PTHrP is encoded by a single gene on the short arm of chromosome 12 (2–4, 6). Alternative splicing generates many different mRNA species, which encode three separate isoforms of 139, 141, or 173 amino acids. From the outset, it was recognized that the *PTHLH* and *PTH* genes were related. The exon/intron organization of that portion of both genes encoding the pre-pro sequences and the initial portion of the mature peptides are identical. Furthermore, the portions of both genes encoding the amino-termini of secreted PTH and PTHrP are highly homol-

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Abbreviations: ECD, Extracellular domain; HHM, humoral hypercalcemia of malignancy; IHH, Indian hedgehog; PTHR1, type I PTH/PTHrP receptor.

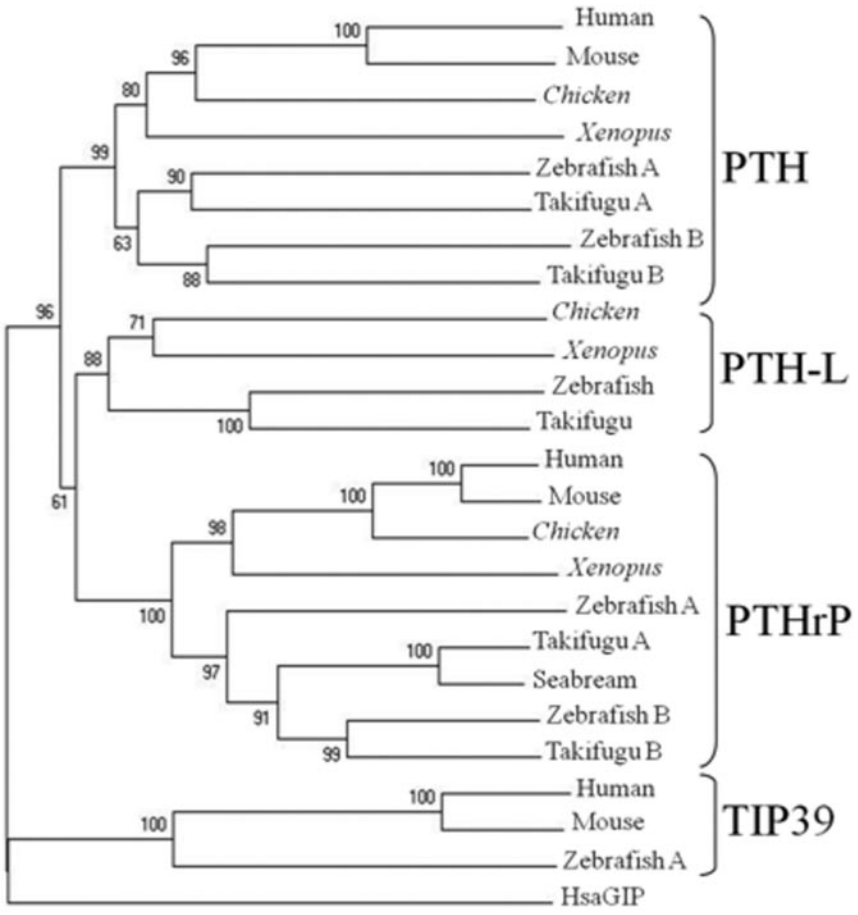


**FIG. 1.** Dendrogram of the consensus phylogenetic hierarchy of the *PTH* gene family among the species depicted. Note that the *TIP39*, *PTHLH*, *PTH-L*, and *PTH* genes cluster as separate clades together across species and appear to arise as separate members of the family early in vertebrate evolution. [Reproduced from P. L. Pinheiro *et al.*: Gene structure, transcripts and calciotropic effects of the PTH family of peptides in *Xenopus* and chicken. *BMC Evol Biol* 10: 373, 2010 (9) with permission.]

ogous, such that the peptides share eight of the first 13 amino acids and a similar secondary structure over the next 21 amino acids. These homologous sequences allow both peptides to bind and activate the same receptor, which ultimately explains why PTHrP causes hypercalcemia in HHM. In the past 10 yr, it has become evident that the "*PTH* gene family" also includes the *PTH-L* and tuberoinfundibular peptide 39 (*TIP 39*) genes (8, 9) (Fig. 1). All members of the *PTH* gene family emerged concurrent with the evolution of vertebrates, probably due to duplications of a single ancestral gene. In fish, there are two separate *PTHLH* and *PTH* genes as well as a single *PTH-L* gene (8–11). However, during evolution, this gene family became smaller in size; amphibians and birds retain only one *PTH* and one *PTHLH* gene along with the *PLH-L* gene, whereas mammals have one *PTH* and one *PTHLH* gene and have lost the *PTH-L* gene (9). All species appear to have only one *TIP-39* gene (8, 9, 12). Sequence comparisons of the different genes reveals areas of highly conserved amino acids in the amino-terminal, receptor-binding portions of each family member, as well as conservation of the structural organization of the genes. Of all the family members, the *PTHLH* gene demonstrates the most complex genomic organization and the most interspecies variability, perhaps reflecting the diverse functions of PTHrP in different tissues (6, 9).

## PTHrP Peptides and Receptors

The primary translation product of PTHrP is processed into an overlapping series of biological peptides (6). The basics of PTHrP processing were elucidated many years ago, but the details of cell-specific PTHrP processing and the biological significance of the different PTHrP peptides are still poorly understood. PTHrP 1–36 is secreted from several cell types (6, 13), but longer forms of amino-terminal-containing PTHrP are also secreted from keratinocytes and mammary epithelial cells and circulate in patients with cancer and during lactation (14–16). The secretion of midregion peptides including amino acids 38–94, 38–95, and 38–101 has also been described (6, 17). The biology of these specific secretory forms is unclear, but midregion PTHrP stimulates placental calcium transport and modulates renal bicarbonate handling, and this portion of the molecule contains nuclear localization signals (see Nuclear PTHrP) (18–20). Finally, C-terminal fragments consisting of amino acids 107–138 and 109–138 have been described. These peptides have been suggested to inhibit osteoclast function and stimulate osteoblast proliferation (6, 19).

The amino-terminus of PTHrP binds to and activates the PTHR1, a prototypical member of class B of the large family of seven transmembrane-spanning G protein-coupled receptors (8, 21, 22). The receptor couples to both $G\alpha_s$ and $G\alpha_{q11}$ and signals via the cAMP/protein kinase A pathway as well as through the generation of inositol phosphates, diacylglycerol, and intracellular calcium transients (8, 21). Binding of PTHrP 1–36 or PTH 1–34 to the receptor conforms to a model described for other class B, G protein-coupled receptors, in which portions of the C-terminal half of each peptide bind to the extracellular

domain (ECD) of the receptor, whereas the initial few amino acids of either peptide interact with the receptor's transmembrane domains to initiate signaling (8, 22). It was initially thought that PTH and PTHrP bind and activate the PTHR1 identically. However, studies in humans documented that PTH infusions are more potent at raising circulating calcium and 1,25-$(OH)_2$ vitamin D levels than PTHrP infusions (23). Furthermore, biochemical and biophysical data have recently demonstrated that PTH and PTHrP bind to the PTHR1 differently and generate a different temporal pattern of downstream signals. Binding of PTH to the receptor favors a conformational state known as $G^0$, which allows the receptor to undergo multiple rounds of G protein activation, generating more cAMP over a longer period of time compared with binding of PTHrP, which favors a more labile RG conformational state (22, 24). Consistent with these findings, the three-dimensional crystal structures of PTH 1–34 and PTHrP 1–36 bound to the ECD of the PTHR1 suggest that PTH binds more tightly to the active receptor (25). Portions of both peptides from amino acids 14 through 30 form an amphipathic helix, which binds to a specific cleft in the receptor's ECD (Fig. 2). However, the $\alpha$-helix formed by PTH is slightly longer than the one formed by PTHrP. As a result, PTH fits more tightly into the receptor's binding cleft than PTHrP, perhaps explaining why it can maintain the receptor in a more active conformation for longer periods of time.

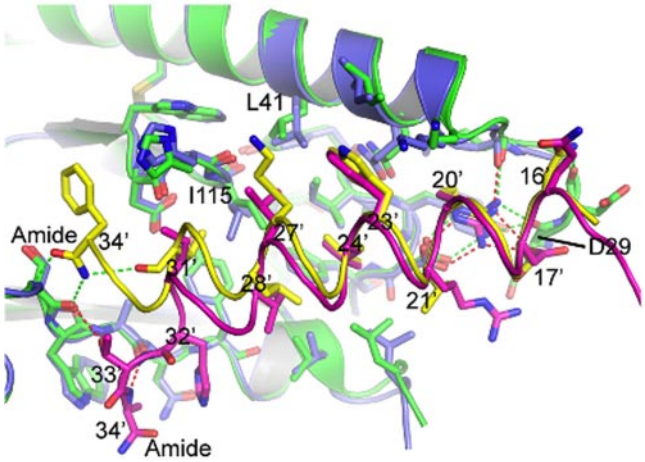


**FIG. 2.** Three-dimensional model of PTHrP (*magenta*) or PTH (*yellow*) binding to the ECD of the PTHR1. *Numbers* refer to the respective amino acids of each peptide. Selected side chains are shown as *sticks*, and the hydrogen bonds between PTHrP and the ECD are shown as *red dashed lines*, whereas the hydrogen bonds between PTH and the ECD are shown as *green dashed lines*. Note that the helical structure of both peptides within the binding pocket is identical from amino acids 16 through 28. However, after that point they diverge, and the longer helix in PTH fits into the binding pocket more tightly. [Originally published in A. A. Pioszak *et al.*: Structural basis for parathyroid hormone-related protein binding to the parathyroid hormone receptor and design of conformation-selective peptides. *J Biol Chem* 284:28382–28391, 2009 (25), with permission. © The American Society for Biochemistry and Molecular Biology.]

## Nuclear PTHrP

Transcription of the *PTHLH* gene can be initiated downstream from the originally described start site to bypass the signal peptide, allowing PTHrP to avoid secretion and remain in the cell (19, 26). Once in the cytoplasm, PTHrP shuttles into and out of the nucleus in a regulated fashion. This depends on a classic nuclear localization sequence located between amino acids 84 and 93 and specific proteins that mediate entry into and export from the nucleus (19, 26, 27). Regulation of its nuclear trafficking is not fully understood, but phosphorylation of PTHrP at $Thr^{85}$ by the cell cycle-regulated, cyclin-dependent kinase $p34^{cdc2}$ appears to mediate nuclear import in a cell cycle-dependent fashion (19). It has also been shown that PTHrP can bind to its receptor at the cell surface and be transported into the nucleus after internalization (26). Therefore, nuclear PTHrP signaling might be able to operate in a paracrine fashion as well as through a cell autonomous pathway.

The function(s) of nuclear PTHrP are a focus of active investigation, and we understand very little about its actions in the nuclear microenvironment. PTHrP has been described to bind to RNA, and in some cells, it localizes to the nucleolus, suggesting that it may be involved in regulating RNA trafficking, ribosomal dynamics, and/or protein translation (19, 26). In cultured cells, nuclear PTHrP influences cell proliferation and/or apoptosis and often appears to oppose the effects of secreted PTHrP. In breast, colon, and prostate cancer cells the nuclear pathway appears to stimulate cell proliferation, protect cells from apoptosis or anoikis, and stimulate cell migration, whereas secreted PTHrP inhibits cell proliferation and promotes cell death (28–30). Nuclear PTHrP has been shown to stimulate the proliferation of vascular smooth muscle cells *in vitro* and *in vivo* by inducing the expression of c-myc and skp2, which in turn reduce levels of the cell-cycle inhibitor, p27 (31, 32). In contrast, secreted PTHrP has the opposite effects.

Recent studies *in vivo* suggest that nuclear PTHrP has important functions. Two groups independently replaced the endogenous mouse PTHrP gene with truncated versions of PTHrP that exclude its nuclear localization signals and C terminus (33, 34). The deletion of the entire C-terminal portions of the gene in these experiments complicates the interpretation of the results somewhat because it is difficult to know whether the resulting phenotypes are due to loss of nuclear signaling, or to loss of the C-terminal

peptides, or both. Nevertheless, in both cases, loss of nuclear and C-terminal PTHrP did not cause many of the typical developmental defects noted in the original PTHrP or PTHR1 knockout mice but did cause growth failure, premature osteoporosis, reduced hematopoiesis, altered energy metabolism, and ultimately, premature death at about 2 wk of age. There was a decrease in the proliferation of chondrocytes, osteoblasts, neurons, and bone marrow cells, and an increase in apoptosis or senescence at these sites as well as in the thymus and spleen. Loss of midregion and C-terminal PTHrP was associated with increased expression of senescence markers such as p21 and $p16^{INK4a}$ and decreased expression of Bmi-1, which is involved in stem/progenitor cell maintenance (33, 34). This phenotype is consistent with the previous observations in cell culture and suggests that nuclear PTHrP may participate broadly in the regulation of cell proliferation and survival, as well as stem/progenitor cell maintenance or self-renewal.

## PTHrP in Physiology and Disease

PTHrP is found in many cell types and organs and has been suggested to have many functions at those sites. Space does not permit a full discussion of these data. What follows is a selective description of the sites in which we have the most detailed understanding of the effects of PTHrP on specific organs *in vivo*.

### The skeleton

Animal models of PTHrP overexpression and underexpression have documented that amino-terminal PTHrP acts through the PTHR1 to coordinate the rate of chondrocyte differentiation to maintain the orderly growth of long bones during development (35). As illustrated in Fig. 3, the growth plate consists of columns of proliferating and differentiating chondrocytes that progressively enlarge to prehypertrophic and then hypertrophic chondrocytes. PTHrP is secreted primarily by immature chondrocytes at the top of the columns in response to another molecule known as Indian hedgehog (IHH) produced by differentiating hypertrophic chondrocytes. PTHrP, in turn, activates the PTHR1 located on proliferating and prehypertrophic cells to maintain their proliferation and to slow their rate of differentiation into hypertrophic cells. In this manner, IHH and PTHrP act in a local negative feedback loop to regulate the rate of chondrocyte differentiation (35).

In recent years, many details about the network of signals though which PTHrP exerts it effects on chondrocyte differentiation have emerged. The mechanisms by which

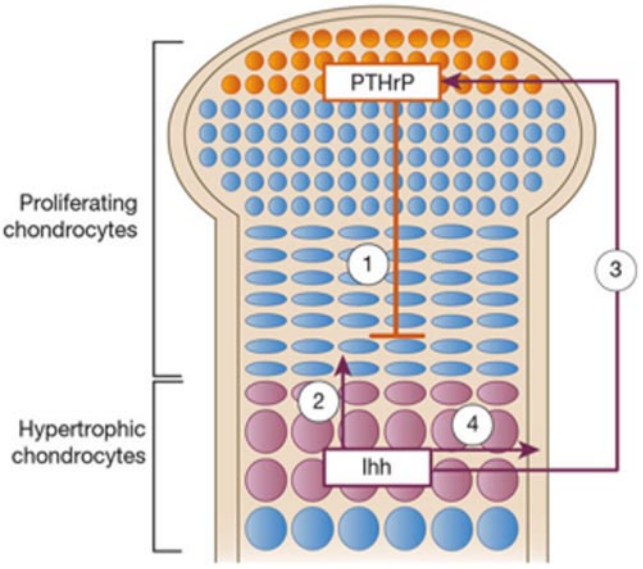


**FIG. 3.** PTHrP and IHH act as part of a negative feedback loop regulating chondrocyte proliferation and differentiation. The chondrocyte differentiation program proceeds from undifferentiated chondrocytes at the end of the bone to proliferative chondrocytes within the columns and then to prehypertrophic and terminally differentiated hypertrophic chondrocytes nearest the primary spongiosum. PTHrP is made by undifferentiated and proliferating chondrocytes at the ends of long bones. It acts through the PTH1R on proliferating and prehypertrophic chondrocytes to delay their differentiation, maintain their proliferation, and delay the production of IHH, which is made by hypertrophic cells (see 1 in figure). IHH, in turn, increases the rate of chondrocyte proliferation (2) and stimulates the production of PTHrP at the ends of the bone (3). IHH also acts on perichondrial cells to generate osteoblasts in the bone collar (4). [Reproduced from H. M. Kronenberg: Developmental regulation of the growth plate. *Nature* 423:332–336, 2003 (94), with permission. © Nature Publishing Group.]

IHH increase PTHrP expression at the ends of the growth plate are not entirely clear, but this process is likely mediated in part through direct effects of IHH and in part indirectly through TGF-$\beta$ signaling (35–37). Two groups have shown that IHH increases PTHrP expression in the growth plate by antagonizing the activity of the transcription factor, Gli3 (38, 39). PTHrP acts on chondrocytes through the PTH1R, primarily by stimulating $G\alpha_s$, cAMP production, and protein kinase A activity, which in turn, mediate a series of downstream events including the phosphorylation of SOX9, the inhibition of p57 expression, the induction of Gli3, Bcl-2, and cyclin D1 expression, and eventually, the phosphorylation and degradation of Runx2 and Runx3, two transcription factors necessary for chondrocyte differentiation (35, 40). Recently, PTHrP has also been shown to modulate chondrocyte differentiation by regulating the movement of histone deacetylase 4 (HDAC4) into the nucleus, which in turn regulates the activity of a network of transcription factors such as Zfp521, MEF2, and Runx2 (41–43). This pathway is required for actions of PTHrP because deletion of Zfp521

rescues the abnormal growth plate phenotype in mice that overexpress a constitutively active PTHR1 (43). Furthermore, it has recently been appreciated that mutations in the *PTHrP* and *HDAC4* genes cause a form of chondrodysplasia known as brachydactyly type E, which mimics bony abnormalities found in patients with mutations in the *GNAS* gene, suggesting that all three genes are in the same genetic pathway (44–46). These studies demonstrate that PTHrP signaling regulates a web of downstream events that affect chondrocyte proliferation and differentiation during development. A recent study employing genetic strategies to disrupt the PTHrP gene in chondrocytes during postnatal life documented that PTHrP also regulates chondrocyte differentiation and maintenance in the mature growth plate (47). This raises the intriguing possibility that loss of PTHrP signaling might contribute to growth plate closure during the adolescent growth spurt, especially because it has been shown that IGF-I also regulates PTHrP production by chondrocytes in the growth plate (48).

PTHrP is also produced in other cartilaginous sites such as the perichondrium that surrounds the costal cartilage and the subarticular chondrocyte population immediately subjacent to the hyaline cartilage lining the joint space (49, 50). In both sites, PTHrP prevents hypertrophic differentiation of the chondrocytes and the inappropriate encroachment of bone into these structures (49–51). It is also prominently expressed at the insertion sites of ligaments and tendons into bone and contributes to modeling of these sites during growth (49, 50).

PTHrP has important anabolic functions in bone. Heterozygous PTHrP-null mice are normal at birth but develop osteopenia with age (52). In addition, selective deletion of the PTHrP gene from osteoblasts results in decreased bone mass, reduced bone formation and mineral apposition, and a reduction in the formation and survival of osteoblasts (53). However, despite the clear phenotype in the osteoblast-specific PTHrP knockout mice, there is disagreement over the PTHrP-expressing population(s) of osteoblasts in the skeleton and even whether the gene is normally expressed within these cells (49, 50). Although secreted PTHrP can affect osteoblasts through PTHR1 signaling, as noted above, loss of the nuclear PTHrP pathway *in vivo* also causes defects in osteoblast proliferation and function and is associated with low bone mass (33, 34). Thus, some of the effects of PTHrP in bone may be mediated through intracrine signaling.

Given the extensive involvement of PTHrP in normal chondrocyte and bone biology, it is not surprising that alterations in PTHrP function are associated with skeletal diseases. The most obvious case is HHM, where circulating PTHrP interacts with PTHR1 receptors in the skeleton to induce widespread bone resorption and produce hypercalcemia. Loss-of-function mutations in the *PTHR1* gene cause Blomstrand's chondrodysplasia, which is associated with bone abnormalities that mimic the PTHrP knockout mouse and cause fetal demise (54). Gain-of-function mutations in the *PTHR1* gene cause Jansen's metaphyseal chondrodysplasia. This is a form of short-limbed dwarfism that results from inhibition of chondrocyte differentiation due to overactive PTHR1 signaling (54). As described previously, loss-of-function mutations in the *PTHLH* gene have recently been shown to cause brachydactyly type E, a syndrome including short stature, shortened metacarpals and metatarsals, and learning disabilities (44, 45). The functions of PTHrP in the maintenance of articular cartilage and modeling at ligamentous and tendinous insertion sites suggest that it may contribute to the pathophysiology of osteoarthritis or enthesopathies. Finally, the anabolic actions of PTHrP on bone suggest that it may be useful in the treatment of osteoporosis. Preliminary studies of PTHrP injections, in fact, demonstrate its efficacy and potential superiority to PTH in this regard (55).

## Mammary gland

Studies in PTHrP and PTHR1 knockout mice demonstrated that PTHrP signaling is required for the formation of mammary glands (56). Fetuses with Blomstrand's chondrodysplasia also lack breast tissue, confirming that PTHrP signaling is required for breast development in humans as well (57). The mammary gland forms in embryos as a bud-like invagination of epidermal cells that grow down into a developing fatty stroma as a branching tube that becomes the mammary duct system. This process is regulated by a series of interactions between the epithelial cells in the bud and ducts and adjacent mesenchymal cells in the stroma (58). In mice and human fetuses, epithelial cells in the mammary bud produce PTHrP, which interacts with the PTHR1 expressed on the surrounding mesenchymal cells. This interaction is necessary for proper differentiation of the mesenchymal cells, which in turn trigger the outgrowth of the epithelial ducts (56). Loss of PTHrP signaling interrupts the vital cross talk between epithelium and mesenchyme resulting in the arrest of development at the bud stage. PTHrP modulates Wnt and BMP signaling as well as the expression of several transcription factors, including the androgen receptor, Msx2, and Lef1 in the mesenchyme, all of which contribute to the outgrowth of the embryonic ducts (59, 60). After birth, PTHrP continues to be expressed at low levels in myoepithelial cells, whereas the PTHR1 is expressed within the periductal stroma. However, a recent study showed that deleting PTHrP specifically from myoepithelial cells failed to result

in defects in development of the duct system or in the formation of alveolar structures in the adult (61).

After embryogenesis, PTHrP is most highly expressed by breast epithelial cells during lactation, and large quantities are secreted into milk (16, 62). Its function in milk is unclear. However, PTHrP is also released from the breast into the circulation, and it regulates systemic calcium and bone metabolism during lactation. The maternal skeleton serves as a source of calcium for milk production, and rapid bone loss is well documented in both nursing women and rodents (63). Elevated levels of PTHrP in the circulation correlate with bone loss in humans, and circulating levels of PTHrP correlate directly with rates of bone resorption and inversely with bone mass in mice (15, 64). Disruption of the PTHrP gene in mammary epithelial cells reduces circulating PTHrP levels, lowers bone turnover, and preserves bone mass, demonstrating that the lactating breast secretes PTHrP into the circulation to increase bone resorption (16). Furthermore, the CaSR suppresses PTHrP secretion from breast cells (65), defining a classical endocrine negative feedback loop: PTHrP mobilizes skeletal calcium, which in turn feeds back to inhibit further PTHrP secretion from the breast (Fig. 4). In fish, circulating PTHrP may mobilize calcium from scales during egg production (66). Thus, the reproductive functions of PTHrP are ancient, and the systemic actions of PTHrP during lactation likely contribute to the evolutionary pressures that resulted in PTHrP and PTH retaining the use of the same PTHR1.

Given the role of PTHrP in increasing bone resorption during lactation, it is not surprising that it also contributes to osteolysis in the setting of bone metastases. In response to the bone microenvironment, breast cancer cells metastatic to the skeleton produce more PTHrP than cells in the primary tumor (67). TGF-$\beta$, released at sites of bone resorption, has been shown to up-regulate PTHrP production through a signaling pathway involving the transcription factor Gli2 (67, 68). In turn, PTHrP increases the production of receptor activator of nuclear factor-$\kappa$B ligand and decreases the production of osteoprotegerin, increasing osteoclast numbers and activity (67). This sets up a feed-forward loop between PTHrP production and bone resorption, contributing to a vicious cycle of osteolysis. In addition, unlike in normal breast cells, CaSR signaling in breast cancer cells stimulates PTHrP production and can synergize with the effects of TGF-$\beta$ (69, 70). Finally, the mechanical stiffness of bone tissue may also increase PTHrP production (71). Thus, several aspects of the bone microenvironment conspire to increase PTHrP production in skeletal metastases.

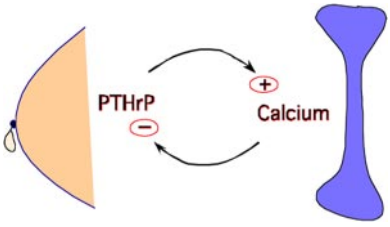


**FIG. 4.** The breast and the skeleton communicate during lactation to provide a steady supply of calcium for milk production. The lactating breast secretes PTHrP into the systemic circulation during lactation. PTHrP interacts with the PTH1R in bone cells to increase the rate of bone resorption and liberate skeletal calcium stores. Mammary epithelial cells in the lactating breast express the CaSR and suppress PTHrP production in response to increased delivery of calcium, defining a classical endocrine negative feedback loop between breast and bone.

The function of PTHrP in primary breast tumors has been less clear than its role in bone metastases. Studies in cultured breast cancer cell lines, animal studies, and clinical studies have shown disparate results (30, 72). Some case series implied that PTHrP expression predicts a more aggressive clinical course (73, 74). However, the largest and best-controlled study suggested just the opposite. Henderson *et al.* (75) examined 526 consecutive cases of breast cancer and found that PTHrP expression was an independent predictor of a more benign clinical course. These results were echoed in a transgenic model of breast cancer reported by Fleming *et al.* (76), who demonstrated that mammary-specific disruption of the *PTHLH* gene increased the incidence of tumors in MMTV-Neu mice. In contrast, Li *et al.* (77) used an identical genetic approach and found that disruption of the *PTHLH* gene dramatically prolonged tumor latency, slowed tumor growth, and reduced metastases caused by transgenic overexpression of the polyoma middle T antigen (MMTV-PyMT mice). The opposing results of these two studies suggest that the molecular context of transformation is critical for determining the actions of PTHrP. Sorting out these mechanistic details will be important, for the *PTHLH* gene was recently identified to be a breast cancer susceptibility locus in a large genome-wide association study (78).

## Placenta

Calcium is transported across the placenta from mother to fetus during pregnancy (63). The placenta produces PTHrP, and its secretion is regulated by the CaSR (79, 80). Furthermore, studies in PTHrP$^{-/-}$ mice have demonstrated that PTHrP promotes calcium transport from mother to fetus and is required to maintain normal fetal calcium concentration (20). Of particular interest is that midregion PTHrP, not amino-terminal PTHrP, is responsible for placental calcium transport (18, 20), implying the existence of specific receptors for midregion PTHrP in the placenta. It also appears that PTH1R signaling may mediate placental calcium transfer, but in response to fetal PTH (81). PTHrP and PTH have overlapping functions in

the regulation of fetal mineral homeostasis, and both are necessary to support the normal fetal calcium concentration (82).

### Smooth muscle and the cardiovascular system

PTHrP is produced by smooth muscle cells in response to mechanical deformation and functions in a short feedback manner to relax the stretched muscle (5, 6, 83–85). In the vasculature, PTHrP is induced by vasoconstrictive agents as well as stretch itself, and it acts as a vasodilator in resistance vessels (86). As discussed earlier, nuclear PTHrP appears to be particularly important in regulating vascular smooth muscle proliferation. Nuclear PTHrP signaling may also contribute to vascular pathology. PTHrP expression is up-regulated after balloon angioplasty, which may stimulate smooth muscle proliferation and contribute to neointima formation (31, 32).

### Teeth

Developing teeth are surrounded by bone and must erupt through the roof of the dental crypt to emerge into the oral cavity. Tooth eruption requires the spatial coordination of bone cell activity. Osteoclasts must resorb the bone overlying the crown of the tooth to allow it to emerge, and osteoblasts must form bone at the base of the tooth to propel it upward out of the crypt. PTHrP is normally produced by stellate reticulum cells, and it signals to dental follicle cells to promote the formation of osteoclasts above the crypt. In the absence of PTHrP, these osteoclasts do not appear, eruption fails to occur, and the teeth become impacted (87–89).

### Pancreatic islets

All cells in the pancreatic islets produce PTHrP, and $\beta$-cells respond to PTHrP by activating phospholipase C and intracellular calcium transients (7, 90). Overexpression of PTHrP in $\beta$-cells leads to an increase in islet cell mass, hyperinsulinemia, and hypoglycemia due to increased proliferation, increased insulin production, and inhibition of apoptosis (7, 90, 91). PTHrP also induces proliferation and improves glucose-stimulated insulin secretion in cultured human $\beta$-cells by stimulating a pathway downstream of the PTHR1 that involves PKC-$\zeta$, cyclin E, and cdk2 (92). In recent experiments, daily sc injections of PTHrP 1–36 increased the proliferation of $\beta$-cells in mouse islets *in vivo* and improved glucose tolerance, suggesting that PTHrP administration might prove useful in the maintenance of islet cell mass and the treatment of diabetes (93).

## Conclusions

Elucidation of the pathophysiology of HHM and the identification of PTHrP serve as prime examples of outstanding clinical research. In the past 25 yr, PTHrP has become firmly established in the clinical lexicon, and its measurement has become incorporated into standard clinical practice. However, we still have limited insight into the reasons why some tumors cause this syndrome and others do not. Furthermore, in addition to causing this paraneoplastic syndrome, emerging evidence in breast and other cancers suggests that PTHrP affects the development and/or behavior of tumors directly. We have also learned much about the normal functions of PTHrP in well-studied sites such as bone, cartilage, pancreas, and breast. However, questions regarding the mechanisms by which PTHrP acts at these sites persist, and we have very little knowledge of its role in multiple other tissues in which it is expressed. Clearly, much remains to be done. The recent demonstration that nuclear PTHrP signaling is required for life and the identification of *PTHLH* as a breast cancer susceptibility gene underscore its fundamental roles in biology and disease. Furthermore, truly exciting recent studies in preclinical models and human subjects have demonstrated that administering PTHrP or targeting PTHrP can offer benefit for the treatment of specific human diseases such as osteoporosis, diabetes, and breast cancer. So, in the true spirit of translational research, the investigation of PTHrP continues its journey from bedside to bench and back again.

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