INTRODUCTION

In a 1941 case report in the New England Journal of Medicine, Fuller Albright first postulated that tumors associated with hypercalcemia might elaborate a PTH-like humor. Work in the 1980s and 1990s subsequently led to the biochemical characterization of a specific syndrome of humoral hypercalcemia of malignancy (HHM) and the fulfillment of Albright's predictions by the isolation of PTH-related protein (PTHrP) and the characterization of its gene. We now understand that calcitriol and calcitriol analogs act by binding to the vitamin D receptor (VDR) and stimulating the expression of PTHrP. PTHrP and PTH are related molecules that can both stimulate the same type I PTH/PTHrP receptor (PTH1R). PTHrP usually serves a local autocrine, paracrine, or intracrine role and normally does not circulate. However, in patients with HMM, PTHrP does reach the circulation and mimics the systemic actions of PTH. Another chapter will discuss malignancy-associated hypercalcemia in detail. This chapter will outline the normal physiology of PTHrP.

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Chapter 26. Parathyroid Hormone–Related Protein

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PTHrP GENE AND THE PTH/PTHrP GENE FAMILY

PTHrP is encoded by a single-copy gene located on the short arm of chromosome 12. The human gene consists of eight exons and at least three promoters. Alternative splicing at the 3' end of the gene gives rise to three different classes of mRNA coding for specific translation products of 139, 141, or 173 amino acids. The physiological significance of these different PTHrP transcripts remains unclear, and in rodents and lower vertebrates such as birds and fish, the gene has a much simpler structure. PTHrP mRNA has been found in almost every organ at some time during its development or functioning. Many different hormones and growth factors regulate the transcription and/or stability of PTHrP mRNA. As with PTH, the calcium-sensing receptor (CaR) has been found to regulate PTHrP gene expression in many cells. Another common theme is the observation that mRNA levels are induced by mechanical deformation. The reader is referred to other reviews for a comprehensive discussion of the sites and regulation of PTHrP expression.

The PTHrP and PTH genes share structural elements and sequence homology, suggesting that they are related genes. The exon/intron organization of that portion of both genes encoding the prepro sequences and the initial portion of the mature peptides is identical. Furthermore, there is high sequence homology at the amino-terminal portion of both genes such that the peptides share 8 of the first 13 amino acids and a high degree of predicted secondary structure over the next 21 amino acids. These common sequences allow both peptides to bind and activate the same PTH1R, which ultimately explains the ability of PTHrP to cause hypercalcemia in HhH. The above-mentioned structural similarities together with the location of the two genes on related chromosomes in the human genome (short arm of chromosome 11 for PTH; short arm of chromosome 12 for PTHrP) indicate that the two genes arose from a common ancestor through a process of gene duplication. The recent demonstration of two PTH genes and two PTHrP genes in several species of fish suggests that these genes split from their common origin before the radiation of the fishes during evolution. Furthermore, fish contain a separate gene, PTH-L, that is intermediate between the PTHrP and PTH genes in its characteristics and may represent their original ancestor. Thus, PTHrP is a member of an ancient family of PTH peptides that seems to be larger and more diverse in lower vertebrates than in mammals.

NUCLEAR PTHrP

Similar to the proopiomelanocortin (POMC) gene, the primary translation product of PTHrP can undergo a variety of post-translational processing events to give rise to an overlapping series of biological peptides. The prepro sequences from amino acids 1-36 to 1 direct the nascent protein into the endoplasmic reticulum so that it can enter the secretory pathway, after which they are removed. The primary sequence of PTHrP contains clusters of basic amino acids that act as recognition sequences for processing enzymes responsible for generating different PTHrP peptides in a cell type–specific manner. The details of PTHrP processing and the biological significance of the different PTHrP peptides are not entirely clear, but several specific secreted forms of PTHrP have been defined. First, PTHrP(1-36) is secreted from several cell types. In addition, longer forms of amino-terminal containing PTHrP are secreted from keratinocytes and mammary epithelial cells and circulate in patients with cancer and during lactation. The amino terminus is necessary for interaction with the PTHrR. The secretion of midregion peptides including amino acids 38-94, 95-95, and 38-95 has also been described. The biology of these specific secretory forms is unclear, but the midregion of PTHrP stimulates placental calcium transport and modulates renal bicarbonate handling, and this portion of the molecule contains nuclear localization signals (see below). Finally, C-terminal fragments consisting of amino acids 107-128 and 109-138 have been described. These peptides have been suggested to inhibit osteoclast function and stimulate osteoblast proliferation.

PTHrP RECEPTORS

The amino terminus of PTHrP binds to and activates the PTH1R, a prototypical, seven transmembrane-containing, G protein–coupled receptor (GPCR), which is a member of class B of the large family of GPCRs. As with the PTH/P gene, PTH1R is one of several related PTH receptor genes that most likely arose through gene duplication events. Although PTH can bind to other receptors in this family, PTHrP can only interact with the PTH1R. This receptor has been described to couple to both Gs and Goq and signal through the cAMP and protein kinase A pathway and through the generation of inositol phosphates, diacylglycerol, and intracellular calcium transients.

The vast majority of studies in vitro suggest that this receptor binds PTHrP and PTH with equal affinity and that both peptides generate identical biological effects. This is also true when amino-terminal fragments of PTH and PTHrP are infused into animals. However, the human PTH1R may respond differently to PTH and PTHrP. Human subjects subjected to continuous infusion of the two peptides for 72 h were found to become hypercalcemic, with lower doses of PTH1(1-34) than PTHrP1(1-36). In these same studies, PTHrP was also much weaker than PTH at stimulating the renal 1-α-hydroxylase enzyme producing 1,25-dihydroxyvitamin D. This may be explained by physical differences in the binding of the two peptides to different conformational states of the receptor, so that the duration of cAMP production is shorter for PTHrP(1-36) than for PTH(1-34).

The existence of biological actions for midregion and C-terminal peptides of PTHrP implies the possibility of additional receptors for these forms of PTHrP. However, no such receptors have been identified to date.

Nuclear PTHrP

Immunohistochemical studies have localized PTHrP to the nucleus of many different cell types. There are several potential mechanisms by which PTHrP can avoid secretion and remain in the cell. Once in the cytoplasm, PTHrP seems to shuttle into and out of the nucleus in a regulated fashion. This is dependent on a specific nuclear localization sequence (NLS) located between amino acids 84 and 93 and requires binding to microtubules and a specific shuttle protein known as importin β1, which allows PTHrP to translocate the nuclear pore. Nuclear export is facilitated by a related shuttle protein known as CRM1 and likely requires a different recognition sequence in the C-terminal region of the peptide.

The regulation of nuclear trafficking of PTHrP is not fully understood, but phosphorylation at Thr465 by the cell cycle–regulated, cyclin-dependent kinase, p34cdc2, seems to regulate nuclear import in a cell cycle–dependent fashion. The function of nuclear PTHrP remains obscure, but it has been described to bind RNA and in some cells PTHrP localizes to the nucleolus. This has led to the suggestion that it may be involved in regulating RNA trafficking, ribosomal dynamics, and/or protein translation. In cell lines, nuclear PTHrP has been implicated in the regulation of proliferation and/or
apoptosis. Whatever the exact function(s) of nuclear PTHrP, it is likely to be important, because replacement of the endogenous mouse PTHrP gene with a mutant version encoding a protein that cannot enter the nucleus is lethal.55

**PHYSIOLOGICAL FUNCTIONS OF PTHrP**

PTHrP has been found in at least some cells of almost all organs. Like many growth factors or cytokines, a variety of effects have been ascribed to PTHrP. The reader is referred to more comprehensive reviews for a complete discussion of these findings.68,9 What follows is a brief outline of areas where PTHrP has been rigorously documented to have physiological effects in intact organisms.

**Skeletone**

Biological functions of PTHrP have been discovered through the study of genetically altered mice, the first of which involved disruption of the PTHrP gene by homologous recombination.250 Lack of PTHrP results in alterations in chondrocyte differentiation in the growth plates of long bones and in costal cartilage that lead to short-limbed dwarfism and a shield chest that interferes with breathing and causes perinatal death. Disrupting the PTHIR gene generates a similar phenotype, and overexpressing PTHrP or a constitutively active PTHIR within growth plate chondrocytes in transgenic mice produces the opposite effect.27-29 These animal models have documented that amino-terminal PTHrP acts through the PTHIR to coordinate the rate of chondrocyte differentiation to maintain the orderly growth of long bones during development.280 As shown in Fig. 1, the growth plate consists of columns of proliferating and differentiating chondrocytes that progressively enlarge to prehypertrophic and hypertrophic chondrocytes, which secrete matrix and undergo apoptosis to form a calcified scaffold that is remodeled into bone in the primary spongiosum. 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, acts on its receptor located on proliferating and prehypertrophic cells 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 (Fig. 1).80

PTHrP is also produced in other cartilaginous sites such as the perichondrium that surrounds the costal cartilage and the subarticullar chondrocyte population immediately subjacent to the hyaline articular lining the joint space.31,32 In both of these sites, PTHrP seems to prevent hypertrophic differentiation of chondrocytes and the inappropriate encroachment of bone into these structures.31,32 It is the failure of this function that leads to the shield chest noted in the PTHrP knockout mice.

In addition to its functions in cartilage, PTHrP has important anabolic functions in bone. Heterozygous PTHrP-null mice are normal at birth but develop trabecular osteopenia with age.33 In addition, selective deletion of the PTHrP gene from osteoblasts results in a decreased bone mass, reduced bone formation and mineral apposition, and a reduction in the formation and survival of osteoblasts.34 These data suggest that PTHrP acts as an important local anabolic factor in the skeleton. Osteoblast cell lines in culture produce PTHrP and its production in vitro can be stimulated by mechanical deformation, raising the possibility that it may be involved in mediating the anabolic response to skeletal loading. However, despite the clear phenotype in the osteoblast-specific PTHrP-

**Mammary Gland**

Not long after its discovery, PTHrP mRNA was found to be expressed in the lactating breast, and PTHrP protein was measured in high concentrations in milk.35,36 It is now known that PTHrP has important functions during breast development, is involved in regulating systemic calcium metabolism during lactation, and contributes to the pathophysiology of breast cancer.

Like other epidermal appendages, the mammary gland initially forms 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. These processes are regulated by a series of sequential and reciprocal interactions between the epithelial cells in the bud and ducts and adjacent mesenchymal cells in the stroma.37 In mice, as soon as the mammary bud begins to form, epithelial cells produce PTHrP, which interacts with the PTHIR expressed on surrounding mesenchymal cells. This interaction is necessary for proper differentiation of the dense mammary mesenchyme that surrounds the embryonic mammary bud so that these mesenchymal cells differentiate into a mature milkproducing gland. The function of PTHrP in the mammary gland is not yet understood, but its role in regulating osteoblast function suggests that it may have a role in regulating bone remodeling in this organ.
ormal cells can maintain the mammary fate of the epithelial cells, initiate outgrowth of the duct system, and stimulate the formation of the specialized epidermis that comprises the nipple.\(^{(58)}\) PTHrP- or PTH1R-knockout mice lack mammary glands because loss of PTHrP signaling interrupts the vital cross-talk between epithelial and mesenchymal cells (Fig. 2). The formation of the breast in human fetuses is similar to the formation of the fetal mammary gland in mice, and PTHrP is necessary for the formation of breast epithelium in humans as well.\(^{(39)}\)

During puberty, the mammary duct system expands to fill out the surrounding fatty stroma to form the mature virgin breast. This phase of mammary development is governed by systemic hormones, which induce the formation of terminal end buds (TEBs) at the tips of the prepubertal ducts. These bulbus structures serve as the sites of active proliferation, differentiation, and stromal remodeling as the ducts lengthen and branch. During puberty, PTHrP seems to interact with stromal cells surrounding the TEBs to regulate the growth of the mammary ducts in response to estrogen.\(^{(10,41)}\)

PTHrP is also made by breast epithelial cells during lactation, and large quantities are secreted into milk.\(^{(16,38)}\) Although its function in milk is unclear, PTHrP is secreted from the lactating breast into the circulation, where it participates in the regulation of systemic calcium metabolism. Milk production requires a great deal of calcium, an important source of which is the maternal skeleton. Elevated rates of bone resorption and rapid bone loss are well documented in both nursing women and rodents.\(^{(32)}\) During lactation, elevated levels of PTHrP 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.\(^{(15,42)}\) In addition, mammary-specific disruption of the PTHrP gene during lactation reduces circulating PTHrP levels, lowers bone turnover, and preserves bone mass, showing that the lactating breast secretes PTHrP into the circulation to increase bone resorption.\(^{(16)}\) The lactating breast also expresses the CaR, which signals to suppress PTHrP secretion in response to increases in calcium delivery to the breast.\(^{(12)}\) These interactions define a classical endocrine negative feedback loop, whereby mammary cells secrete PTHrP to mobilize calcium from the bone. Calcium, in turn, feeds back to inhibit further PTHrP secretion from the breast. Therefore, during lactation, the breast and bone engage in a conversation, which leads to the mobilization of skeletal calcium stores to ensure a steady supply of calcium for milk production. Interestingly, fish PTHrP fulfills a similar function to mobilize calcium stored in scales to be used during egg production.\(^{(40)}\) Thus, this reproductive function of PTHrP is ancient, and the actions of PTHrP during lactation may represent one of the principal evolutionary pressures that resulted in PTHrP and PTH retaining the use of the same PTH1R.

**Placenta**

During pregnancy, calcium must be actively transported across the placenta from mother to fetus. Furthermore, the responsible placental pump maintains a higher calcium concentration in the fetus compared with the mother, so that calcium must be transported against a gradient.\(^{(42)}\) In PTHrP-/- mice, this gradient is lost, and PTHrP-deficient fetuses are relatively hypocalcemic, suggesting that fetal PTHrP is important in mediating placental calcium transport from the mother.\(^{(20)}\) The source of the PTHrP is likely the placenta itself and placental production of PTHrP has been shown to be regulated by the CaR.\(^{(44,45)}\) Interestingly, experiments in sheep and mice have shown that it is midregion PTHrP, not the amino-terminal portion, that is responsible for placental calcium transport.\(^{(18,20)}\)

**Smooth Muscle and the Cardiovascular System**

PTHrP is expressed in many different smooth muscle cell beds.\(^{(59)}\) In these sites, mechanical deformation seems to increase the expression of PTHrP, which, in turn, acts in an autocrine or paracrine fashion through the PTH1R to relax the muscle cell or structure that has been stretched.\(^{(49,96,48)}\) In accommodative structures such as the stomach, bladder, or uterus, this feedback loop may be important in allowing for gradual filling. In the vasculature, PTHrP is induced by vasoconstrictive agents and stretch itself and acts as a vasodilator to

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**FIG. 2.** PTHrP regulates mesenchymal cell fate during embryonic mammary development. (A) During normal mammary development, PTHrP is secreted by epithelial cells within the forming mammary bud (dark circles) and interacts with the immature dermal mesenchyme (gray ovals) to induce formation of the dense mammary mesenchyme (light squares). These cells, in response to PTHrP, maintain the fate of the mammary epithelial cells, initiate branching morphogenesis, and induce the formation of the specialized nipple skin (dark squares). (B) In PTHrP- or PTH1R-knockout embryos, the mammary bud forms, but the mammary mesenchyme does not. As a result, the mammary epithelial cells revert to an epidermal fate (dark ovals), morphogenesis fails, and the nipple never forms. (Adapted with permission from Company of Biologists: Foley J, Dunn P, Hong J, Cosgrove J, Dreyer BE, Rimm D, Dunbar, ME, Philbrick WM, Wysolmerski JJ 2001 Parathyroid hormone-related protein maintains mammary epithelial fate and triggers nipple skin differentiation during embryonic breast development. Development 128:513-525.)

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resistance vessels. Given these actions, PTHrP may act as a local modulator of blood flow.

PTHrP regulates the proliferation of vascular smooth muscle cells. Secreted amino-terminal PTHrP can inhibit the proliferation of vascular smooth muscle cells by acting through the PTH1R on the cell surface. However, the midregion and C-terminal portions of PTHrP, acting in the nucleus, stimulate the proliferation of these cells. This latter effect results from destabilization of a cell cycle regulatory protein known as p27kip1, which leads to progression through the G1/S checkpoint. This pathway seems to be active during development, because the rate of proliferation of smooth muscle cells in the aorta of PTHrP−/− embryos is reduced. Furthermore, PTHrP expression is upregulated by vascular damage after balloon angioplasty and in atherosclerotic lesions in rodents and in humans. Several studies have suggested that PTHrP plays an important role in the response of these cells to injury and may contribute to the pathophysiologic development of a neointima after angioplasty.

PTHrP has been found in cardiomyocytes and co-localizes with atrial natriuretic peptide in granules within atrial cells in the rat heart. In certain genetic backgrounds, the absence of the PTH1R causes widespread cardiomyocyte death during midgestation in developing mice. However, this phenotype is not seen in PTHrP−/− mice. Thus, it is not clear if this lesion reflects the actions of a different ligand for the receptor or alternatively an imbalance between the actions of secreted and intracellular forms of PTHrP as a result of loss of the receptor. In isolated hearts, PTHrP has both positive inotropic and chronotropic effects and may affect coronary blood flow.

Teeth

Developing teeth become surrounded by alveolar bone but must maintain a cavity or crypt that is free from bone to allow for proper morphogenesis. After teeth are formed, they must erupt through the roof of the dental crypt to emerge into the oral cavity. The process of tooth eruption relies on geographically uncoupled bone turnover in which osteoclasts form over the crown of the tooth to resorb the overlying bone and osteoblasts at the base of the tooth propel it upward out of the crypt. In the absence of PTHrP, teeth develop but do not erupt. Normally, just before the onset of eruption, PTHrP is produced by epithelial, stellate reticulum cells and signals to stromal, dental follicle cells to drive the formation of osteoclasts above the crypt. In the absence of PTHrP, these osteoclasts do not appear, eruption fails to occur, and the surrounding bone encroaches leading to impaction of the tooth.

Pancreatic Islets

PTHrP is expressed by all four neuroendocrine cell types within the pancreatic islets. In β cells, it is stored within secretory granules and is co-released with insulin. Pancreatic islets express the PTH1R and β cells respond to PTHrP by activating phospholipase C and increasing intracellular calcium. Overexpression of PTHrP in β cells leads to an increased β-cell mass, hyperinsulinemia, and hypoglycemia, because of the combination of increased β-cell proliferation, increased insulin production, and inhibition of β-cell apoptosis. It is not clear how these actions of PTHrP relate to normal islet physiology, especially because there are no obvious defects in islet development in the PTHrP−/− mice. Nonetheless, the death of these mice at birth has precluded any examination of potential roles of PTHrP in the physiology of islets.

Central and Peripheral Nervous Systems

PTHrP and the PTH1R are both widely expressed within the brain, including regions of the cortex, the cerebellum, and the hippocampus, hypothalamus, and pituitary. In cultured hippocampal neurons, PTHrP has been shown to be secreted in response to calcium influx through L-type calcium channels on depolarization. In turn, PTHrP can act on the PTH1R on these same neurons to dampen L-type channel activity, giving rise to the idea that PTHrP acts in an autocrine/paracrine short feedback loop to protect neurons from damage caused by prolonged or repeated depolarization, so-called "excitotoxicity." The PTHrP−/− mice have been rescued from neonatal death by the reintroduction of transgenic PTHrP into chondrocytes. Consistent with the role of PTHrP in protecting from excitotoxicity, these "PTHrP-rescue" mice were shown to be much more sensitive to kainate-induced seizures.

In addition to neurons, PTHrP has also been shown to be expressed in glia and astrocytes. Interestingly, the PTHrP gene is expressed in fetal and malignant glial cells but not in mature glia in the adult brain. However, its expression can be induced in reactive glia in an injury model in rats. Consistent with the enhanced expression of PTHrP in less differentiated glial cells, in humans, the level of PTHrP expression in glial tumors was shown to correlate with more aggressive behavior of the tumor and was predictive of a poor outcome for the patient. In a similar fashion, a recent report showed upregulation of PTHrP in dedifferentiated Schwann cells after crush injury in the peripheral nervous system (sciatic nerve). PTHrP was shown to inhibit the differentiation of these cells, suggesting that it contributed to maintaining the dedifferentiated state necessary for nerve regeneration.

CONCLUSIONS

PTHrP was discovered as the cause of the clinical syndrome of HHM. It is evolutionarily and functionally related to PTH and shares the same PTH1R. The common use of this receptor, in turn, allows PTHrP to act as a hormone mimicking the actions of PTH during reproduction, a function preserved from fish to mammals. Although the conservation of these relationships through evolution allows PTHrP to cause hypercalcemia when it is secreted into the circulation by tumors, we have come to understand that PTHrP is generally a locally produced and locally acting growth factor that participates in normal development and physiology at many diverse sites. The power of mouse genetics has provided the tools to begin to catalogue the biological functions of PTHrP. However, much remains to be accomplished to understand its functions fully.

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