American Journal of Pathology, Vol. 150, No. 3, March 1997

Review

Parathyroid-Hormone-Related Peptides

A New Class of Multifunctional Proteins

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Parathyroid-hormone-related peptides (PTHrPs) are a novel class of peptide hormones, isolated and cloned in 1987.[1–3] These peptides share marked amino-terminal homology with PTH (8 of the first 13 amino acids are identical) and bind to a common PTH-PTHrP receptor.[4–8] PTHrPs were first isolated from tumors associated with the syndrome of humoral hypercalcemia of malignancy, and compelling evidence suggests that, in the vast majority of the patients, PTHrPs are responsible for the hypercalcemia that is one of the hallmarks of the syndrome.[9] Synthetic and recombinant PTHrPs have been documented in a number of laboratories to mimic the effects of PTH on the classical PTH target tissues, bone and kidney.[10] Specifically, PTHrPs bind to PTH receptors and stimulate adenylate cyclase in these tissues and also stimulate osteoclastic bone resorption. The findings that PTHrPs stimulate renal (nephrogenous) AMP production *in vitro* and stimulate bone resorption *in vitro* provide evidence that PTHrPs are capable of reproducing the two other hallmarks of the clinical humoral hypercalcemia of malignancy syndrome, namely, increases in nephrogenous AMP excretion and in osteoclastic bone resorption.[9]

Using molecular hybridization and imunohistochemical techniques, PTHrPs have also been localized in tumors not associated with the development of hypercalcemia.[11–19] The detection of PTHrP mRNA transcripts and peptide in a wide range of normal tissues[14,20–25] raised the question of whether it plays a physiological role in the biology of nonneoplastic tissues, different from its role in the regulation of calcium balance. Current evidence suggests an important role in growth and differentiation of neoplastic as well as nonneoplastic cells.[14,26–39] Although the physiological actions of PTHrP are thought to be mainly autocrine or paracrine, lactation is a normal situation in which plasma levels are increased and in which PTHrP can function as a real hormone.[40]

The PTH and PTHrP genes are thought to have arisen from a common ancestral gene, through an ancient chromosomal duplication event. The human gene for PTHrP has been mapped to the short arm of chromosome 12 and spans more than 15 kb of genomic DNA.[41,42] By alternate splicing, it can serve as template for three isoforms of pre-pro-PTHrP, giving rise to three initial translation products composed of 139, 141, or 173 amino acids.[43] In rodents, only one major form of PTHrP is produced; it is composed of 141 amino acids in the rat[23,44] and 139 amino acids in the mouse.[41] PTHrP undergoes extensive endoproteolytic post-translational processing to yield a family of secretory forms of the peptide.[45–52] Post-translational processing of PTHrP is tissue specific; different PTHrP-producing cell types appear to secrete different forms of the peptide.[45,50] At present, at least three major secretory forms of the peptide have been shown to exist: an amino-terminal species,[45,48–50] a mid-region species,[49,50] and a carboxyl-terminal species.[50,53–55] These different secretory forms appear to exert different biological actions; eg, PTHrP 67–86 amide stimulates calcium transport across the placenta,[56] and PTHrP 107–111 (osteostatin) inhibits osteoclastic resorption in the rat.[57,58] In addition, there is recent evidence for the existence of different subtypes of the

Accepted for publication January 3, 1997.

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PTH/PTHrP receptor on different cell types.[59] These subtypes of the receptor have different signaling mechanisms.[59,60]

Several studies have shown that the gene encoding PTHrP shares some features with members of the immediate-early gene family.[30,33–35,42,61] One of these features is rapid induction by serum, growth factors and cycloheximide.[30,33–35,42,61] Previously performed induction studies yielded different results according to the cell cultures used, providing evidence that there is species and cellular specificity in the regulation of PTHrP gene expression.[30,34] The promotor region of the human PTHrP gene is GC-rich and contains many CCGCCC and GGGCGG motifs.[42] The promotors of genes involved in cellular growth, such as those encoding H-Ras I, epidermal growth factor receptor, insulin, and insulin-like growth factor II, contain many of these sequence motifs. Moreover, the 3′-untranslated region of the PTHrP gene contains multiple AUUUA motifs.[42] Such motifs contribute to mRNA instability and are common in mRNA involved in cellular growth and/or differentiation, such as proto-oncogenes, lymphokines, and cytokines.[23,42] There is a marked interspecies conservation of these AUUUA motifs in the PTHrP gene, implying their functional importance.[42] Consequently, both promotor and 3′-untranslated regions of the human PTHrP gene have structural features that may indicate a possible functional role for human PTHrP in cellular proliferation and differentiation.

Diverse lines of research have given additional evidence for a function for PTHrP as a growth and differentiation factor. First is the fact that PTHrP expression in embryonic and fetal tissues is widespread, yet clearly localized, and that the pattern of PTHrP expression changes during gestation.[27–29,37,38] Several recent studies indicate that the PTHrP/PTHrP receptor signaling system serves as a para- or autocrine mechanism for parietal endoderm differentiation in the early preimplantation embryo, thus constituting the earliest hormone receptor system involved in embryogenesis defined to date.[31,62] Moniz et al[29] showed widespread immunostaining of PTHrP in the central and peripheral nervous system and in the epithelia lining the gastrointestinal tract, urogenital tract, and the pulmonary system in 8- to 12-week-old human fetuses. Staining in lung epithelia and kidney progressively diminished with gestational age. The musculoskeletal system revealed a changing pattern especially with respect to the development of cartilage. In both the lung and the developing skeletal system, a changing pattern of expression was seen in immature mesenchyme, which initially stained strongly and subsequently lost immunoreactivity.[27] Campos et al[28] showed a similar widespread though clearly localized immunoreactivity in fetal rat tissues. Lee et al[37] studied the expression of PTHrP and its receptor mRNAs in rat fetuses of 15 to 20 days of gestation. Both PTHrP and its receptor mRNAs were expressed not only in the skeleton but also in many extraskeletal tissues, such as choroid plexus, ear, lungs, tooth buds, heart, and skin. In these extraskeletal tissues, PTHrP mRNA was expressed mainly in surface-lining cells, whereas its receptor mRNA was expressed mainly in adjacent mesenchymal cells. This spatial relationship suggests paracrine roles for PTHrP and its involvement in epithelial-mesenchymal-like interactions. In enchondral bones, the PTHrP/PTHrP receptor mRNA expression changes, but this paracrine localization pattern is maintained throughout development. We studied developing human liver from 16 weeks of gestation until birth and postnatal livers from 1 day of age until 14 years of age.[38] PTHrP immunoreactivity was weakly present in the most immature form of bile ducts, the ductal plate, whereas bile ducts already incorporated in the mesenchyme of portal tracts showed stronger immunoreactivity. PTHrP immunoreactivity became stronger with gestational age, and bile ducts in neonatal livers showed strong immunoreactivity. In children from the age of 2 to 3 years old, PTHrP immunoreactivity progressively diminished and was no longer found after the age of 4 years. It is known that the human liver has an adult-type architecture by the age of 5 years.[63] All adult biopsies were consistently negative for PTHrP. These data suggest that PTHrP plays a physiological role during normal human liver development and that this peptide may function as a growth and differentiation factor for growing and maturing bile ducts, especially at the stage of remodeling of the ductal plate and incorporation of bile ducts in the mesenchyme of portal tracts. This is concordant with a role for PTHrP in epithelial-mesenchymal interactions.

PTHrP appears to function as a regulator of growth and development not only during embryogenesis but also during adult life. PTHrP functions as an autocrine growth factor or growth modulator in many cell lines, including renal carcinoma cells,[26] breast cancer cell lines,[64] lymphocytes,[65] fibroblasts,[32] osteoblasts, chondrocytes, bile duct phenotype-human liver epithelial cells,[39] vascular smooth muscle cells,[66] and keratinocytes.[67] For example, using both human native PTHrP (purified from a breast carcinoma) and synthetic PTHrP 1–36, Insogna et al[32] demonstrated that PTHrP induces epidermal-

growth-factor-dependent transformation of a fibroblast cell line with a concomitant significant increase in the production of fibronectin, a property it shares with transforming growth factor-β. Human liver epithelial cell lines with a bile duct phenotype express PTHrP (whereas human liver epithelial cell lines with a hepatocellular phenotype do not), and this PTHrP expression is rapidly inducible by different inductors and growth factors,[39] supporting the concept that PTHrP is a member of the growth-factor-regulated early response gene family. Moreover, antiserum against PTHrP can reduce the growth rate of the human liver epithelial cell lines with a bile duct phenotype and not of those with a hepatocyte phenotype.[39] Additional data indicate a role of PTHrP as a terminal differentiation factor in human keratinocytes.[68] A polyclonal PTHrP antiserum inhibits cell growth in a human renal carcinoma cell line, known to secrete PTHrP *in vitro*.[26] In contrast, $PTHrP^{1-34}$ appears to function as an autocrine growth inhibitor in lymphocytes,[65] in vascular smooth muscle cells,[66] and in a human keratinocyte cell line.[67] By transfecting this human keratinocyte cell line with a full-length antisense cDNA sequence to PTHrP (and thereby blocking PTHrP synthesis and secretion), a decrease in cell doubling time and an increase in thymidine incorporation was observed.[67] One concludes, therefore, that endogenous PTHrP can act either as an effective inhibitor or as a promotor of cell growth, depending on the portion of the molecule that is secreted and on the target tissue.

Finally, gene-targeting studies provided unequivocal evidence for the important roles of PTHrP in growth and differentiation. Targeted overexpression of PTHrP in the keratinocytes of transgenic mice affects hair follicle development, resulting in the disappearance of hair follicles in abdominal skin.[69] Mice homozygous for PTHrP gene ablation die shortly after birth and display a multitude of skeletal defects, characterized by abnormalities in enchondral bone formation, due to diminished proliferation and inappropriate differentiation of chondrocytes to the hypertrophic phenotype.[70,71] An intriguing recent study presents evidence that PTHrP promotes some of its cellular effects by translocating to the nucleolus.[72] Localization of transiently expressed PTHrP to the nucleolus is dependent on the presence of a highly basic region at the carboxy terminus of the molecule that bears homology to nuclear targeting sequences identified within human retroviral regulatory proteins. Endogenous PTHrP also localizes to the nucleolus in osseous cells *in vitro* and *in vivo*.[72] Moreover, forced expression of PTHrP in chondrocytic cells increases their proliferative capacity[73] and delays apoptosis induced by serum deprivation[72]; the latter effect depends on the presence of an intact nuclear targeting signal. These findings demonstrate a unique intracellular mode of PTHrP action and a novel mechanism by which this peptide growth factor may modulate cell proliferation and programmed cell death. This mechanism could explain the diminished proliferative capacity and altered terminal differentiation observed in growth plate chondrocytes from fetal mice homozygous for PTHrP gene ablation.

PTHrP appears to be tightly linked to the reproductive process. PTHrP is produced by lactating mammary tissue,[23] uterus,[74] placenta, amnion, and chorion-decidua[56,75]; in addition, abundant quantities are found in both milk[40,76] and amniotic fluid.[77] PTHrP appears to stimulate a placental calcium pump that is responsible for maintaining a relative fetal hypercalcemia during pregnancy.[56] The precise role of PTHrP during lactation remains to be determined. PTHrP has been shown to relax uterine smooth muscle and its uterine expression is induced by stretch and depends on uterine occupancy.[74] PTHrP expression and action in the rat uterus is regulated by steroids, and this regulatory mechanism may promote myometrial contraction before parturition[78]

In fact, PTHrP has been detected in a wide variety of types of smooth muscle of both vascular and nonvascular origin and functions as a smooth muscle relaxant.[14,23,79–84] In hollow organs such as the bladder, uterus, gut, and gastric fundus, PTHrP probably helps in adapting to luminal distention.[79,80,85]

Kelly[86] has described the existence of two secretory pathways in eukaryotic cells: the regulated and the constitutive secretory pathways. In the regulated pathway, the proteins are packaged into secretory granules, whereas this is not the case in the constitutive pathway, where the secretory peptides are found in the endoplasmic reticulum. Recent experimental work showed that all three secretory forms of PTHrP are secreted *via* the regulated pathway in *bona fide* neuroendocrine cell types. In contrast, all three secretory peptides are secreted in a constitutive fashion by nonendocrine cells.[87] This work indicates that a single neuroendocrine peptide may be secreted *via* either the regulated or the constitutive pathway in a cell-specific fashion. However, in this issue of the American Journal of Pathology, Matsushita et al[88] show that in parathyroid adenoma cells, which are classical endocrine cells, PTHrP is secreted through both the regulated and the constitutive pathway. In contrast, PTH, which is present in

the same secretory granules as PTHrP, is not present in the rough endoplasmic reticulum, indicating that PTH is secreted only *via* the regulated pathway. Previous work of our group on human liver showed that bile duct epithelial cells, which one would consider as constitutively secreting cells, can obtain a neuroendocrine phenotype in certain pathological conditions. In conditions of chronic cholestasis or in regenerating liver, small bile ductules proliferate at the border of the portal tracts. These reactive bile ductules, which are immunoreactive for PTHrP, express chromogranin-A immunoreactivity and contain dense-cored secretory granules.[89–91] The dense-cored secretory vesicles in reactive bile ductules are much scarcer than in classical endocrine cells. Taking these findings together, it appears that the distinction between endocrine-type cells and nonendocrine-type cells is not as sharp as we used to think.

In conclusion, the PTHrP molecule can have several bioactivities involved in calcium regulation, of which the PTH-like action, confined to its amino-terminal region, is the most characterized. In addition to the endocrine effects of PTHrP, a growing body of evidence suggests a local role for PTHrP in epithelial growth and differentiation. Although the placental transport (confined to the central sequences of the molecule) and osteoclast inhibitory actions (confined to the carboxyl-terminal domain) can be clearly demonstrated with synthetic and recombinant PTHrP preparations, there is still much to learn about the significance of the non-PTH-like actions of PTHrP in normal physiology. The molecular synthetic and processing events that generate the different secretory forms of PTHrP and the cellular mechanisms that target these secretory forms to either the regulated or the constitutive secretory pathway require additional study.

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