A Novel Parathyroid Hormone-Related Gene Product

T. J. Martin

J. A. Danks

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A parathyroid hormone-related protein (PTHrP) has been invoked as being responsible for the humoral hypercalcemia of malignancy. Eight of the first 13 amino acids of PTHrP are identical with those in PTH, but there is no other significant homology. The PTHrP gene is located on chromosome 12, whereas that for PTH is on chromosome 11, and the two genes are probably related by a duplication process. Antisera against PTHrP(1-34), which cross-react poorly or not at all with PTH, and antisera against other parts of PTHrP not homologous to PTH were used in immunocytochemistry, using a peroxidase-antiperoxidase method, to identify PTHrP in the cytoplasm of cells in a series of unselected parathyroid adenomata. The study was based on our evidence that PTHrP is produced by fetal parathyroid and may be the predominant calcium-regulating hormone in the mammalian fetus. Glands from five patients with parathyroid hyperplasia secondary to chronic renal failure also stained positively for PTHrP. No evidence was obtained for PTHrP in sections from five patients with primary parathyroid chief cell hyperplasia or in a small group of patients with the multiple endocrine neoplasia type 1 or type 2 syndromes. (Henry Ford Hosp Med J 1989;37:187-9)

Humoral hypercalcemia of malignancy, a common syndrome in patients with cancer, is thought to be due to the elaboration by the tumor of factor(s) which act on bone to stimulate resorption and on kidney to decrease calcium and increase phosphorus excretion (1). Many common biochemical characteristics exist between humoral hypercalcemia of malignancy and primary hyperparathyroidism. Since clinical evidence and preliminary radioimmunoassays suggested that the responsible factor was similar to parathyroid hormone (PTH), the search began to identify the PTH-like agent responsible for humoral hypercalcemia of malignancy. The search ended in 1987 with the purification, cloning, sequencing, and expression of a previously unrecognized PTH-related protein (PTHrP) (2-4).

Characterization of PTHrP

Purification of PTHrP was achieved from conditioned medium produced by a lung cancer cell line (BEN) originally derived from a patient with squamous cell carcinoma of the lung associated with hypercalcemia (2). Amino-terminal sequence information led to the cloning of PTHrP cDNA (3) and prediction of a protein of 141 amino acids, including a 36 amino acid prepro-sequence.

PTHrP resembles PTH in many of its biological actions in that it stimulates cAMP production only in PTH target tissues and this action is prevented by antagonists of PTH. PTHrP is immunologically distinct from PTH since antisera against PTH, which completely inhibit the biological effects of PTH, have no effect on the activity of PTHrP (5,6).

The limited homology at the amino-terminal region of the mature protein (eight of the first 13 identical) between PTHrP and PTH is sufficient to account for the interaction of PTHrP with the PTH receptor since the amino-terminal region of PTH interacts with the PTH receptor. Studies with recombinant PTHrP(1-141), PTHrP(1-108), and PTHrP(1-84) have shown that each of these proteins has PTH-like actions similar to those of the synthetic PTHrP peptides first studied (5). Furthermore, on a molar basis, each of the longer preparations of PTHrP is equipotent with PTHrP(1-34) in activating adenylate cyclase in PTH responsive cells (5). Therefore, as is the case with PTH, the major actions of PTHrP on kidney and bone are carried out by the amino-terminal portion of the molecule within the first 34 amino acids.

Human PTHrP Gene

PTHrP cDNA clones have also been isolated from libraries derived from two human renal cell carcinoma cell lines and have been shown to contain essentially the same PTHrP coding sequence (4,5). However, the cDNAs differed in that they contained divergent carboxyterminal coding regions and 5'- and 3'-untranslated regions. This suggested that alternatively spliced mRNAs produce multiple cDNA species encoding PTHrP. This was confirmed when the PTHrP gene was cloned (8,9), revealing a gene structure more complex than that of PTH. The PTHrP gene is located on chromosome 12 (4,8) whereas that for PTH is on chromosome 11. With the PTHrP gene, alternate promoters produce multiple mRNA species with different 5'-untranslated
Physiological Functions of PTHrP

It is clear that PTHrP is a previously unrecognized hormone. It has been localized by immunohistology in the spinous keratinocyte layer of normal skin and in 100% of a variety of unselected squamous cell cancers (6). PTHrP appears to be a marker of epithelial cells but is not exclusively confined to them, since it is produced also by the cells of adult T-cell leukemia associated with the retrovirus human T-lymphotropic virus type 1 (10). In the skin PTHrP may have a local function in differentiation of keratinocytes, but this remains to be determined.

Before the structure of PTHrP was known, we considered that it might be a fetal form of PTH and obtained evidence for the production of PTHrP by ovine placenta and fetal parathyroid (11,12). Extracellular fluid calcium levels in the mammalian fetus are maintained by an active pump of calcium from mother to fetus, making calcium available for the growing fetal skeleton (11). Experiments were carried out in which placenta were perfused at the end of pregnancy in ewes whose fetuses had been parathyroidectomized several weeks earlier (11). In these circumstances the active calcium pump in the placenta is greatly reduced. We showed that it could be restored by partially or highly purified PTHrP (11) and by recombinant PTHrP but not by PTH or by PTHrP(1-34). Evidence clearly showed that the fetal parathyroid gland is a source of PTHrP (12).

Thus it seemed likely that PTHrP is the hormone of the fetal parathyroid responsible for maintaining extracellular fluid calcium in the fetus by the only means available to it—the placental calcium pump. The realization that PTHrP is indeed likely to be a parathyroid hormone led to two further lines of investigation. First, a clonal rat parathyroid cell line thought to produce PTH was shown to produce predominantly, or even exclusively, PTHrP and to release into medium a form equivalent in size to PTHrP(1-84) (13). Second, immunohistology was used to investigate the possibility that PTHrP is produced by abnormal parathyroid tissue.

PTHRP in Parathyroid Adenoma and Hyperplasia

Primary hyperparathyroidism is usually associated with an adenoma and less commonly with chief cell hyperplasia involving multiple parathyroid glands. Hyperplasia is also a feature of the multiple endocrine neoplasia (MEN) type 1 and type 2 syndromes. In an immunohistochemical study, we used antibodies directed against various sequences of PTHrP to establish the presence of the antigen in adenomatous and in hyperplastic glands from patients with primary hyperparathyroidism and with hyperparathyroidism secondary to chronic renal disease.

Polyclonal antisera against synthetic peptides PTHrP(1-34), PTHrP(50-69), and PTHrP(106-141) were used in a peroxidase-antiperoxidase technique on paraffin sections which has been described in detail (6). The anti-PTHrP(1-34) antisera used in all studies were chosen carefully for lack of any demonstrable cross-reaction against PTH(1-34) even at the very high concentrations used in neutralizing biological activity (6).

Fourteen patients with adenomatous primary hyperparathyroidism had tumors that showed strongly positive staining with anti-PTHrP(1-34) and anti-PTHrP(50-69) but not with anti-PTHrP(106-141). An example is shown in the Figure, which illustrates the lack of staining in the surrounding nonneoplastic parathyroid tissue. Of sections examined from five patients with primary hyperparathyroidism due to hyperplasia, four were negative with all three antisera, and one was faintly positive with anti-PTHrP(1-34). In parathyroid hyperplasia secondary to chronic renal failure, however, the sections of all five patients stained positively with anti-PTHrP(1-34) and anti-PTHrP(50-69).

Sections were also examined from four patients with MEN 1 (provided by Dr. J. Shepherd, Hobart, Tasmania) and from two patients with MEN 2A (provided by Dr. N. Samaan, Houston, TX). All these sections were negative with all antisera.

It is concluded that parathyroid adenoma and secondary hyperplastic glands from chronic renal failure patients are capable of producing PTHrP. To what extent the protein reaches the circulation in these conditions, and how PTHrP might contribute to the disorders of calcium metabolism in these states, if at all, remains unknown. In this study, the PTH levels were elevated in those patients who were assayed (14).

Failure to find evidence of PTHrP in tissue from primary hyperplasia is surprising. The stimulus of hypercalcemia in chronic renal failure seemed sufficient to provoke PTHrP production. Whatever the stimulus to polyclonal expansion in primary hyperplasia, it might be expected that the same cellular
process would occur as in secondary hyperparathyroidism. In MEN 1, a humoral factor is proposed as the stimulus to the parathyroid (15), and it is possible that analogous factors operate in primary hyperplasia and MEN 2. It would certainly be of interest if any stimulus to hyperplasia in these conditions resulted in selective overproduction of PTH but not of PTHrP, whereas in chronic renal failure as a cause of hyperplasia both peptides are produced.

Failure of the carboxyterminal antiserum to detect PTHrP in adenomatous or secondary hyperplastic parathyroid glands is also of interest. The same anti-PTHrP(106-141) antiserum readily detects PTHrP in the spinous keratinocyte layer of normal skin (14). The finding might indicate that a processing mechanism operates in the parathyroid cell, resulting in cleavage of a carboxyterminal fragment, and that this either does not take place in skin or does so to a much lesser extent. It may be relevant that a clonal nontransformed rat parathyroid cell line secretes PTHrP equivalent in size to PTHrP(1-84), with no larger material detectable (13).

The findings with immunohistology complement the observations of Ikeda et al (16) who demonstrated expression of mRNA for PTHrP in seven of eight parathyroid adenomata and in the only patient they examined who had chronic renal failure with secondary hyperparathyroidism. They did not examine tissue from primary hyperplastic glands. The genes for MEN 1 and MEN 2 are linked to chromosome 11 and to chromosome 10, respectively. The growth factor thought to be important in MEN 1 is probably not so in MEN 2 or idiopathic primary hyperplasia. Although the hyperplastic stimulus of chronic renal failure results in identifiable production of PTHrP by the parathyroid, this does not occur in any of the primary hyperplastic syndromes.

Further studies of PTHrP production and secretion by abnormal parathyroid tissue will be of interest, particularly for understanding the molecular mechanisms of the MEN syndromes.

Although early data from analysis of glucose-6-phosphate dehydrogenase isoenzyme in parathyroid tumors indicated that adenomata were multicellular in origin (17), a recent molecular approach suggests that most parathyroid adenomata have a monoclonal origin (18). In the MEN syndromes, hyperplasia precedes tumor formation. Our identification of PTHrP in adenomatous and in renal failure glands but not in those from primary hyperplasia or MEN patients might be due to differing amounts of hormone present in those circumstances or could imply fundamental differences in pathogenesis. Plasma assays of sufficient sensitivity and specificity will be necessary to assess the role of PTHrP in hyperparathyroid syndromes.

References