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Physiological roles of parathyroid hormone-related protein

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Summary. *Background:* Parathyroid hormone related peptide (PTHrP) is widely expressed in a variety of normal fetal and adult tissues. *Aim of work:* Review of these normal physiologic functions of PTHrP in each of these tissues. *Method:* Performed literature search on pubmed on articles related to PTHrP and physiologic roles. *Results:* PTHrP is expressed in wide range of sites in the body with roles including relaxation of vessels and smooth muscle cells, and regulation of development. PTHrP also mediates humoral hypercalcemia of malignancy. PTHrP can be falsely elevated in benign conditions. Lastly, PTHrP has a pharmacological role in osteoporosis treatment. *Conclusions:* PTHrP has many various physiological roles besides mediating humoral hypercalcemia of malignancy. (www.actabiomedica.it)

Key words: parathyroid hormones related protein, hypercalcemia, and osteoporosis

Introduction

Hypercalcemia in patients with malignancy was initially attributed to local bone destruction by malignant cells in the 1920s. Fuller Albright, in 1941, was the first to postulate that a tumor can produce a hormone in excess that was similar to parathyroid hormone (PTH). In 1987, the development of bioassays allowed advances in identification and purification of this protein from a patient with squamous cell carcinoma of the lung who had humoral hypercalcemia of malignancy. This protein, given its structural and functional similarity to PTH, was later called parathyroid hormone-related peptide (PTHrP) (1).

The human *PTHLH* gene was subsequently mapped on the short arm of chromosome 12. The translation product of PTHrP is processed into 3 secretory forms including N-terminal, mid-region, and C-terminal regions (2). PTHrP (1-141) shares homology with PTH (1-34) at the N-terminal sequence in which the first 13 residues of the mature protein are identical. Both PTHrP and PTH bind to a common G-protein-coupled receptor called PTH receptor (PTHR1), but dissociate from the receptor by different mechanisms. The mid-region peptide is involved in placental calcium transport and also fetal skeletal development. In contrast, the C-terminal region of PTHrP, which is also called "osteostatin", activates both protein kinase C and also Src-dependent vascular endothelial growth factor receptor 2 (VEGFR2) to promote osteoblast proliferation (3).

PTHrP is expressed in a wide range of normal tissues and organs including bone, cartilage, teeth, stomach, pancreas, cardiovascular-renal system, lungs, bladder, uterus, placenta, and mammary glands whereas PTH is found exclusively in the parathyroid glands. PTHrP has paracrine and autocrine actions on receptors of many different tissues. We will review the normal physiologic functions of PTHrP in each of these tissues in detail below (summarized under table 1). Lastly, we will also discuss humoral hypercalcemia of malignancy, benign conditions with falsely elevated PTHrP, and the pharmacological role of PTHrP in osteoporosis treatment.

Table 1. Summary of the physiological roles of PTHrP by tissues and organs

Site	Proposed Roles
Bone and cartilage	• Promotes bone formation • Inhibits apoptosis of osteoblasts • Regulates chondrocyte maturation
Teeth	• Regulates dental formation and mandibular development
Stomach	• Relaxes the smooth muscle of gastric fundus
Pancreas	• Stimulates insulin production • Regulates pancreatic cell proliferation, apoptosis, and differentiation
Cardiovascular-renal system	• Vasodilator effect of smooth muscle cells in response to mechanical stretch • Heart: positive chronotropic and inotropic effects • Renal: renal vasodilation to increase renal blood flow and eGFR
Lungs	• Stimulates surfactant production • Reduces lipofibroblast to myofibroblast transdifferentiation
Bladder	• Relaxes the detrusor muscles to increase bladder compliance
Uterus	• Induces vasorelaxation in the uterine arteries
Placenta	• Stimulates placenta calcium transport
Mammary gland	• Mediates mammary developments • Secreted in milk

References: (1-23)

Physiologic roles of PTHrP by tissues and organs

Cartilage and bone

PTHrP plays an important role in endochondral bone formation and osteoblast apoptosis inhibition. PTHrP is produced by chondrocytes at the end of long bones. PTHrP stimulates chondrocyte proliferation, osteoblast formation, and subsequently bone formation (1). Mice with PTHrP deficiency died immediately after birth, in contrast, PTHrP haploinsufficient mice were born with low bone mass due to decreased bone formation and also increased osteoblast apoptosis (4). Furthermore, a proposed mechanism is that oxidative stress impairs osteoblast function by causing cellular damage and apoptosis. N- and C-terminal domains of PTHrP has been demonstrated to protect against reactive oxygen species (ROS) production by oxidative stress related agent H_2O_2 in both murine osteoblastic cells and also human osteoblastic cells (3).

Teeth

PTHrP is expressed only in the odontoblastic cells of normal dental pulp. During maturation of teeth, the activation of PTH/PTHrP receptor regulates the odontoblastic cells. In an inflamed pulp, PTHrP production is stimulated by pro-inflammatory TNF-α and IL-1β, and found in the vascular zone, pulp stroma, and odontoblastic zones. PTHrP plays an important role in angiogenesis but its function remains controversial (5)

The action of PTHrP is mediated by the p27 pathway which has a negative regulatory effect on dental formation and mandibular development. Sun et al showed that deletion of p27 enhances the dental alveolar bone formation in mice by modulating cyclin E, CDK2, Bmi-1, and antioxidant enzymes (6).

Stomach

The gastric fundus relaxes in response to the deglutition process in order to accommodate the food ingested. PTHrP is found in the gastric fundus and functions to relax the smooth muscle of the gastric fundus. PTHrP inhibits the phasic contraction by release of nitric oxide-cyclic guanosine monophosphate (NO-

cGMP) and also has a direct action on the smooth muscle by increasing cyclic adenosine monophosphate (cAMP) pathways. Both of these mechanisms promote PTHrP-induced relaxation of the gastric fundus (7).

Pancreas

PTHrP is found in the pancreatic islet and ductal cells. PTHrP acts on PTH/PTHrP receptor (PTH1P) to regulate cell proliferation, apoptosis, and differentiation. It has been demonstrated that the overexpression of PTHrP stimulates insulin production, inhibits apoptosis, and also increases pancreatic islet mass (8, 9).

Normally, PTHrP has a very low expression in the exocrine pancreas. However, PTHrP level is elevated in a mouse model of cerulein-induced acute pancreatitis that mimics human pancreatitis as characterized by acinar cell death, edema formation, and inflammatory cell infiltration.

The deletion of the PTHrP gene in acinar cells protects against pancreatitis by decreasing the release of pro-inflammatory cytokines and chemokines. These findings show PTHrP is an important mediator of inflammation and fibrosis associated with acute and chronic pancreatitis (10).

Cardiovascular-renal system

In the cardiovascular system, PTHrP is found in the smooth muscle layer of the vessel, intrarenal arterial tree, and endothelial cells. The expression of PTHrP is induced in the smooth muscle by hypertension, atherosclerosis, and after balloon angioplasty (11).

In response to mechanical stretch, smooth muscle cells produce PTHrP that functions to relax the stretched muscle by its potent vasodilator effect. Serum vasoconstrictors such as angiotensin II can also induce PTHrP mRNA and its protein. If PTHrP is overexpressed in transgenic mice, it can reduce systemic blood pressure as well as decrease vascular responsiveness (12).

PTHrP has both positive chronotropic and indirect inotropic effects when released by atrial and ventricular myocytes (13). PTHrP is also expressed in the renal arterial tree and can induce vasodilation on both afferent and efferent arterioles. Raison et al used smooth muscle specific-PTHrP knockdown to understand the physiologic effect of PTHrP in the cardiovascular-renal system. They found no effect on systemic blood pressure but decreased renal plasma flow and glomerular filtration rate (GFR). Hence, PTHrP plays a role in renal vasodilation by increasing renal blood flow and GFR (14).

Lungs

Stretch sensitive type II epithelial cells in the lungs enhance PTHrP production. PTHrP binds to PTH 1 receptors (PTHR1) to increase the expression of proteins responsible for surfactant phospholipid synthesis including PPAR-γ, leptin, and adipose differentiation related protein (ADRP). PTHrP stimulates surfactant production via a paracrine feedback loop mediated by leptin and also reduces lipofibroblast to myofibroblast transdifferentiation (15).

In particular, nitric oxide deficiency is commonly seen in many forms of pulmonary diseases. A proposed mechanism is that nitric oxide deficiency directly increases the pulmonary PTHrP expression. Subsequent activation of the pulmonary renin-angiotensin system (RAS) may also increase PTHrP expression in the lung. However, RAS may also modify pulmonary fibrosis independent of PTHrP (16).

Bladder

Relaxation of the detrusor muscle allows normal bladder storage function. PTHrP functions via the PTH/PTHrP receptor 1 (PTH1R) expressed in the detrusor muscle, renal tubules, and blood vessels in normal bladders. In response to mechanical stretch upon bladder retention, PTHrP is released from the detrusor muscle and acts as an inhibitory autocrine factor to suppress spontaneous contractions of mucosa. Thus, PTHrP may increase bladder compliance function to allow more urine storage (17). In contrast, downregulation of PTH1R may be involved in detrusor overactivity seen in end stage bladder disease (18).

Uterus

PTHrP can induce vasorelaxation in the uterine arteries which helps maximize the delivery of blood to the uterus during pregnancy. The proposed mechanism involves upregulation of PTHrP mRNA but downregulation of PTH1R mRNA expression in uterine arteries. Interestingly, the expression of PTHrP and PTH1P is shifted from the medial layer to the intimal layer of the uterine arteries during pregnancy (19). Decreased intrauterine PTHrP level is associated with growth restriction in spontaneously hypertensive rats, which suggests a role for PTHrP in maintaining placental function and fetal growth (20).

Placenta

Fetal plasma calcium levels are significantly higher than maternal calcium levels. PTH level is suppressed but PTHrP level is elevated in the fetal circulation. The mid-region of PTHrP stimulates placental calcium transport to allow calcium to be made available for mineralization of fetal skeleton. In mice with PTHrP gene deletion, fetal serum calcium was significantly reduced compared to mice with wild type PTHrP, which shows that PTHrP regulates fetal calcium homeostasis by disrupting the fetomaternal calcium gradient (21).

Mammary gland

PTHrP is expressed in epithelial cells in the embryonic mammary buds of human fetuses while PTHR1 is expressed in the mesenchymal cells. PTHrP induces the proliferation and differentiation of the mesenchymal cells. Loss of PTHR1 on mesenchymal cells causes mammary development defects. Boras-Granic et al examined mammary development in PTHrP knock-in mice that express a mutant form of PTHrP with a deletion of the C-terminus and nuclear localization signals (NLS). This mutant form of PTHrP impairs mammary mesenchyme differentiation and mammary duct outgrowth but also caused insufficient PTHrP production (22).

During lactation, PTHrP is secreted by mammary glands into the systemic circulation, which activates bone resorption and liberates skeletal calcium stores. Activation of calcium sensing receptor (CaSR) in the mammary epithelial cells during lactation downregulates PTHrP levels in the systemic circulation in response to the increased delivery of calcium. Hence, PTHrP is important in modulating the calcium concentration of breast milk (23).

Clinical Implications of PTHrP

Elevated PTHrP levels associated with malignancy

Elevated PTHrP accounts for 80% of the cases in patients with hypercalcemia of malignancy. PTHrP promotes the formation of osteoclasts to cause bone resorption but does not affect the $1,25(OH)_2D$ levels which is responsible for increased intestinal calcium absorption. Laboratory findings for PTHrP mediated hypercalcemia associated with malignancy include low PTH, high PTHrP, low phosphorus, low or normal $1,25(OH)_2D$, and variable 25(OH)D levels (24). In a large case series of PTHrP mediated hypercalcemia, solid organ malignancies made up 82.6% of the overall cases (25). Similarly, another study also found 85% of the cases are solid organ malignancies (43.4% squamous cell carcinoma, 23.4% adenocarcinoma, 15.7% hepatocellular carcinoma, and 9.6% cholangiocarcinoma) and 14.8% of the cases are hematologic malignancies (26). The results from these two case series are summarized in table 2. Lastly, PTHrP mediated hypercalcemia is associated with a poor prognosis with a median survival of less than 2 months (25).

Falsely elevated PTHrP in benign conditions

Healthy subjects have undetectable or low N-terminal and C-terminal PTHrP levels. Most clinical laboratories use an assay directed toward C-terminal PTHrP since levels of circulating C-terminal PTHrP are approximately 14 times higher than N-terminal PTHrP, making it easier to detect. Both C-terminal and N-terminal PTHrP levels are elevated in patients with hypercalcemia of malignancy.

However, the renal clearance of C-terminal PTHrP level is dependent on glomerular filtration rate. Serum C-terminal PTHrP can accumulate in pa-

Table 2. PTHrP mediated hypercalcemia associated with malignancy

Solid organ malignancy	Hematologic malignancy
• Squamous cell carcinomas (lung, esophagus, head & neck, manubrium, skin, parotid, vulva, cervix, scrotum, penis and anus) • Adenocarcinoma (cholangiocarcinoma, breast, renal cell, stomach, lung, ovary, duodenum, endometrium, and pancreas) • Hepatocellular carcinoma • Small cell lung carcinoma • Non-small cell lung carcinoma • Urothelial and bladder carcinoma • Medullary thyroid carcinoma • Neuroendocrine tumors • Myxoid sarcoma • Merkel cell carcinoma • Epithelioid hemangioendothelioma	• Multiple myeloma • Non-Hodgkin's lymphoma • Diffuse large B cell lymphoma • Acute leukemia • Chronic myeloid leukemia/small cell lymphoma • Plasma cell leukemia

Adapted from Donovan et al, Jin et al (25,26)

tients with renal failure leading to a falsely elevated PTHrP levels especially if eGFR is less than 20 ml/min. In contrast, N-terminal PTHrP level is low or undetectable in patients with renal disease. Hence, the preferred test to screen for malignancy associated hypercalcemia in patients with kidney disease is N-terminal PTHrP (27). Compared to the healthy adults, PTHrP concentration is also elevated in breastfeeding women and also in postmenopausal women with low body mass index (28).

Pharmacological role of PTHrP

Abaloparatide is a synthetic PTHrP analog with potent anabolic activity that increases bone formation while also reducing bone resorption due to the parathyroid type 1 receptor conformation binding selectivity. The phase 3 Abaloparatide Comparator Trial in Vertebral Endpoints (ACTIVE) trial found the use of subcutaneous abaloparatide over 18 months compared to placebo increased bone mineral density (BMD), and reduced the risk for new vertebral and non-vertebral fractures for postmenopausal women (>65 year old) (29). Furthermore, a post hoc analysis of a subgroup of ACTIVE trial showed abaloparatide therapy was associated with increased BMD and reduced risk for vertebral and non-vertebral fractures in post-menopausal women aged 80 years or older (30). Another double blind placebo controlled trial showed 24 weeks of abaloparatide increases the BMD of the lumbar spine, femoral, and total hip compared to the placebo group. Total hip BMD increase by abaloparatide (2.6% at 80 μg) is greater compared to the teriparatide group (0.5%) and placebo group (0.4%). There was a low incidence of abaloparatide induced hypercalcemia due to lower rates of bone resorption observed in these patients. Other adverse events were similar between all treatment groups and included dizziness, injection site reactions, headache, dyspepsia, syncope, diarrhea, arthralgia, and upper abdominal pain (31). The FDA approved abaloparatide on April 2017 for the treatment of postmenopausal women with osteoporosis. This new osteoporosis therapy is promising given its ability to increase BMD in the lumbar spine, femoral and total hip, safety data, and low incidence of hypercalcemia.

Conclusions

PTHrP is widely expressed and plays a physiological role in a variety of normal fetal and adult tissues. The insights into these roles and the proposed mechanisms come from animal studies through overexpression, mutation, or deletion of PTHrP. Further studies will be needed to elucidate the mechanisms by which PTHrP acts on these tissues. PTHrP is also an important mediator of malignancy-related hypercalcemia in a wide range of solid and hematologic malignancy which is associated a poor prognosis. In an exciting di-

rection, the FDA approved a synthetic PTHrP analog that can increase BMD and reduce the risk for vertebral and non-vertebral fractures for postmenopausal women. Understanding the physiological role and malignancy associated hypercalcemia will help us further develop other therapeutic options in the future.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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