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Role of Parathyroid Hormone/Parathyroid Hormone-Related Peptide on Cell Proliferation in the Gastric Mucosa

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Parathyroid hormone-related peptide (PTHrP) is widely expressed in normal tissues and elicits various functions through the PTH/PTHrP receptor. Relaxation effects of PTHrP on gastrointestinal smooth muscle cells were well documented, but the physiological role on mucosal growth and differentiation is little known. The purpose of this study was to evaluate the expression of PTHrP and PTH/PTHrP receptor in the rat gastric mucosa, and the role of PTHrP on mucosal cell proliferation. Male Wistar rats were used in this study. Localization of PTHrP and PTH/PTHrP receptor were observed by immunohistochemistry and in situ hybridization. Expression of PTH/PTHrP receptor mRNA were examined by RNase protection assay in control and stress condition. Double staining with BrDU incorporation was performed to differentiate cell cycle states. Cell proliferative effect by external PTHrP-(1-34) was evaluated by BrDU incorporation. PTHrP immunopositive cells were encountered in and around the mucosal neck area. PTH/PTHrP receptor immunoreactivity was observed in the gastric mucosa broadly. Cells with stronger expression for PTHrP and its cognate receptor were located in the vicinity of generative zone. But BrDU incorporating cells were negative for both PTHrP and PTH/PTHrP receptor. By RNase protection assay, PTH/PTHrP receptor mRNA expression was weak in a steady state, and the receptor expression increased at stress. External PTHrP-(1-34) did not show cell proliferative effect in a steady state. At stress BrDU incorporation was suppressed significantly, and PTHrP-(1-34) increased BrDU incorporation.

These observations suggest that PTHrP and PTH/PTHrP receptor involve maintenance of mucosal growth and differentiation in the stomach.

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Introduction

Parathyroid hormone-related peptide (PTHrP) was first isolated from human tumor cells and was shown to be responsible for producing hypercalcemia in patients with humoral hypercalcemia of malignancy[1]. PTHrP is the product of a gene that is also expressed in many normal tissues[2,3], and the localization of PTHrP in the stomach has been demonstrated in fetal and adult animals[4,5]. Gene expression of PTH/PTHrP receptor was also demonstrated in many tissues[6] and PTHrP elicits physiological effects through PTH/PTHrP receptor in an autocrine/paracrine fashion. Among the various physiological effects of PTHrP, smooth muscle relaxation in visceral and vascular tissues is well known[7-10]. We previously reported that PTHrP produces a similar response on distended gastric smooth muscle cells in the rat, and concluded that PTHrP might be an important gastrointestinal peptide which regulates gastric contractile activity[11]. Other physiological roles are the regulation of transepithelial calcium transport and the regulation of cell proliferation and differentiation[12-16]. A recent report suggested that PTHrP is involved in mucosal differentiation in the rat intestinal mucosa[17].

In the current study, we evaluated the expression of PTHrP and PTH/PTHrP receptor in the gastric mucosa and the physiological role of PTHrP on mucosal cell proliferation.

Materials

Animals

Male Wistar-Kyoto rats were purchased from Charles River Japan (Atsugi, Japan) and used at 20 - 24 weeks of age in this study. The animals were handled according to the guidelines of the NIH Animal Research Committee (Bethesda, MD). Rats were housed

in groups of 3 to 4 per cage in an air-conditioned room at 24℃ (lights on from 7 AM to 7 PM) at the Laboratory Animal Center of Nagasaki University. The rats were allowed free access to food (laboratory chow F2, Japan CLEA, Tokyo) and tap water. RWI stress was applied as described by Takagi and Okabe[18].

Localization of PTHrP and PTH/PTHrP receptor by immunohistochemistry and In situ hybridization

Localization of PTHrP and PTH/PTHrP receptor was performed by immunohistochemistry and in situ hybridization. The animals were deeply anesthetized with an overdose of ether after treatment and were perfusion-fixed with a suitable volume of 4% paraformaldehyde via the left ventricle. The stomachs were resected and the fundic portion of the glandular stomach was cut into 3mm thick slices and post-fixed overnight followed by paraffin embedding. The paraffin-embedded tissues were cut into 4 μm sections, deparaffinized with xylene, and rehydrated through a series of ethanols. Deparaffinized sections were preincubated with normal bovine serum to prevent nonspecific binding, and then were incubated overnight at 4℃ with an optimal dilution (5 μg/ml) of the primary polyclonal antibody against PTH/PTHrP receptor (Babco, Richmond, CA). Sections were washed in phosphate-buffered saline (PBS), and bound antibodies were localized by the avidin-biotin-peroxidase method using DAB or AEC as the chromogenic substrate. For PTHrP immunohistochemistry, monoclonal antibody was used (Oncogene Science, Inc., Uniondale, NY). The slides were sequentially incubated with alkaline phosphatase-conjugated goat anti-mouse immunoglobulin antibodies. The alkaline phosphatase reaction was revealed using a mixture of 5-bromo-4-chloro-3-indolylphosphate p-toluidine salt nitroblue tetrazolium chloride (BCIP/NBT). Negative controls were prepared in each case by replacing the primary antibody with nonimmune mouse serum. Rat kidney served as the positive control for PTH/PTHrP common receptor immunohistochemisty. Human PTHrP-producing lung cancer served as an internal positive control in immunostaining for PTHrP. To examine the topological relation to neck proliferating cells, double staining of PTH/PTHrP receptor and BrDU immunohistochemistry were performed. BrDU (100 mg/kg) was dissolved with phosphate-buffered saline and injected intraperitoneally two hours before sacrifice.

Using a digoxigenin RNA labeling kit (Boehringer Mannheim, Mannheim, Germany), antisense and sense RNA probes for rat PTH/PTHrP receptors were made. Hybridization was performed as described previously[19]. PTH/PTHrP receptor mRNA to be hybridized with digoxigenin-labeled riboprobe was detected by antibody to digoxigenin and stained with BCIP/NBT.

PTH/PTHrP receptor mRNA expression by RNase protection assay

Gene expressions of PTH/PTHrP receptor were examined in a steady state and at RWI stress condition by the RNase protection assay as described previously[19]. Rats were divided into the following two groups: group 1, non-stressed control; group 2, RWI stress for 4 h. In group 2, the rats were placed in a restraint cage and immersed up to the xyphoid process for 4 h in water at 23℃. All rats were given food and water ad libitum just before the treatment. Total RNA was extracted from the gastric fundus by the guanidinium thiocyanate method according to the manufacturer's instruction (RNAzol, Tel-Test, Friendswood, TX). Hybridization probes were labeled with [α-^{32}P] CTP using T3 RNA polymerase according to the supplier's guidelines (MAXI script kit, Ambion, Austin, TX). The RNA probes used were as follows: Rat PTH/PTHrP receptor, a 569-bp HindIII/Not I fragment of pTRI-GAPDH (Ambion). Total RNA (30 μg) prepared from rat stomach was hybridized overnight with $1x10^5$ cpm of riboprobe and 5 $x10^3$ cpm rat GAPDH probe, and then digested with RNase A and RNase T1 (RPAII ribonuclease protection kit, Ambion). The reaction products were resolved on 4% polyacrylamide/8M urea sequencing gels and analyzed after 72 h (PTH/PTHrP receptor) by autoradiography on XAR film with a single intensifying screen. Assays were performed in duplicate, and in each case GAPDH mRNA also was quantified.

Effects of PTHrP-(1-34) supplementation on cell proliferation

Effects of PTHrP-(1-34) (Cambridge Research Biochemicals, Cheshire, UK) pretreatment on cell proliferation were evaluated. PTHrP-(1-34) (10 μg ip) was administered 10 min before the beginning of RWI stress. To assess the cell proliferative state in the neck proliferative zone, BrDU incorporation was examined. BrDU (100 mg/kg) was dissolved with phosphate-buffered saline and injected intraperitoneally two hours before sacrifice in each group of rats. After perfusion-fixation with 4% paraformaldehyde, the stomachs were resected and the fundic portion of the glandular stomach was cut into 3 mm thick slices and post-fixed overnight followed by paraffin embedding. Deparaffinized sections were preincubated with normal

bovine serum to prevent nonspecific binding, and then were incubated overnight at 4℃ with an optimal dilution of the primary monoclonal against BrDU (Becton Dickinson, San Jose, CA). Immunohistochemistry of BrDU was performed as described previously[20]. Three arbitrary fields in each rat were scanned at random under 200 X magnification, and the average cell count was used as a representative value for each rat. BrDU incorporation by cells was analyzed with the image analyzer (MCID, Image Research Institute, Ontario, Canada), and the values were expressed as the number of positive cells per 1 mm length of mucosa.

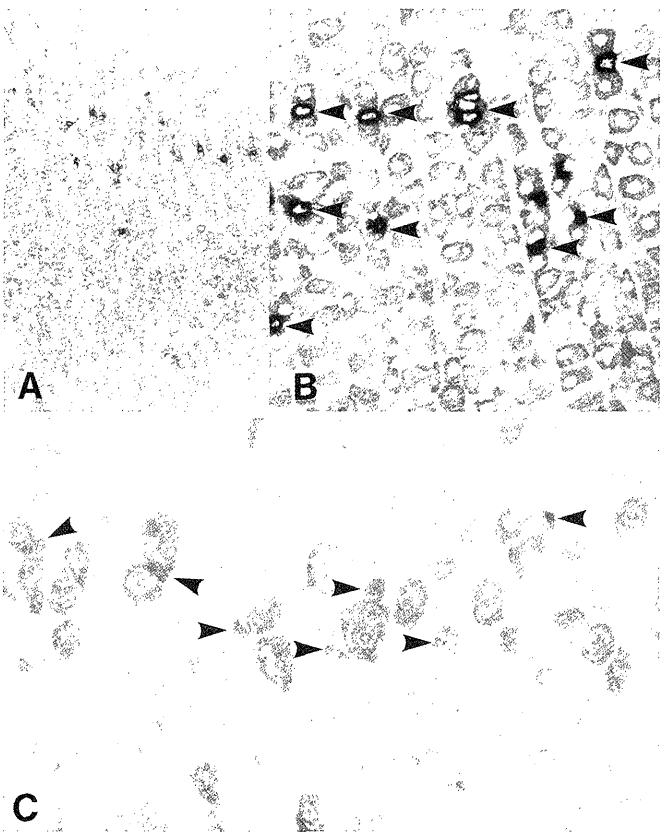

Figure 1. Double staining of PTHrP or PTH/PTHrP receptor with BrDU immunohistochemistry in the mucosal layer of the stomach. (A, B): PTH/PTHrP receptor (AEC colorization) was detected widely in the mucosal layer around the generative zone where BrDU-immunopositive cells (arrow heads, BCIP/NBT colorization) were present. PTH/PTHrP receptor expression was intense in and around the generative zone, and sparse or negative in the surface and basal layers. (A; x13, B; x66) (C): PTHrP immunopositive cells (BCIP/NBT colorization) and BrDU-positive cells (arrowheads, DAB colorization) were located close to each other, but existed independently. Nucleolar PTHrP immunoreactivities were partly detected. (x132)

Data analysis

All data are expressed as mean±SD. The ANOVA test was used to determine the statistical significance of the data, and $P<0.05$ was regarded as significant.

Results

Localization of PTHrP and PTH/PTHrP receptor

Immunoreactivity of PTH/PTHrP receptor was detected broadly in the gastric mucosa (Fig.1A, 1B), in contrast to it PTHrP expressed just vicinity of neck proliferative cells which incorporate BrDU (Fig.1C). Cells around the mucosal generative zone were particularly intensely positive to both antigens. The mucosal surface area and the deepest area were negative or barely positive. Double staining with BrDU demonstrated that BrDU positive cells were negative for both PTHrP and PTH/PTHrP receptor positive cells (Fig. 1B, 1C), although both cells were in close proximity to BrDU positive cells.

PTH/PTHrP receptor mRNA was detected in cells around the mucosal neck by in situ hybridization, localization similar to that with immunohistochemistry (Fig. 2).

Figure 2. In situ hybridization of PTH/PTHrP receptor mRNA. PTH/PTHrP receptor mRNA was expressed in the lower two-thirds of mucosal layer. Upper- and lower-most areas were negative for PTH/PTHrP mRNA. (x33)

PTH/PTHrP receptor gene expression

Gene expression of PTH/PTHrP receptor (Fig.3) was analyzed by ribonuclease protection assay in a steady state and at RWI stress. The PTHrP receptor mRNA transcript were detected in the gastric tissue of all groups. PTHrP receptor mRNA levels were weak at steady state. In contrast, the expression of PTH/PTHrP receptor was up-regulated at RWI stress.

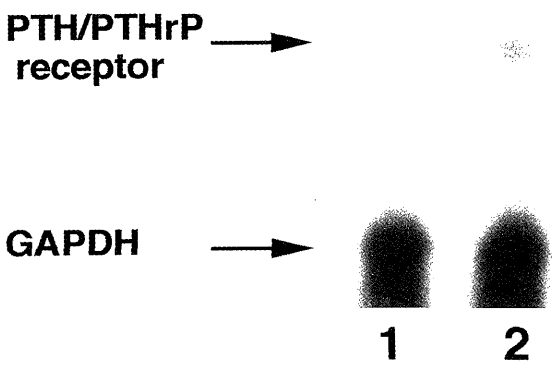

Figure 3. PTH/PTHrP receptor expression analyzed by ribonuclease protection assay. The labeled antisense probes used in these assays are PTH/PTHrP and rat GAPDH. Total RNA (30 μg) from the rat stomach (fundus) was used in the assay. PTH/PTHrP receptor expression was enhanced by RWI stress. Lane 1; control, Lane 2; RWI stress.

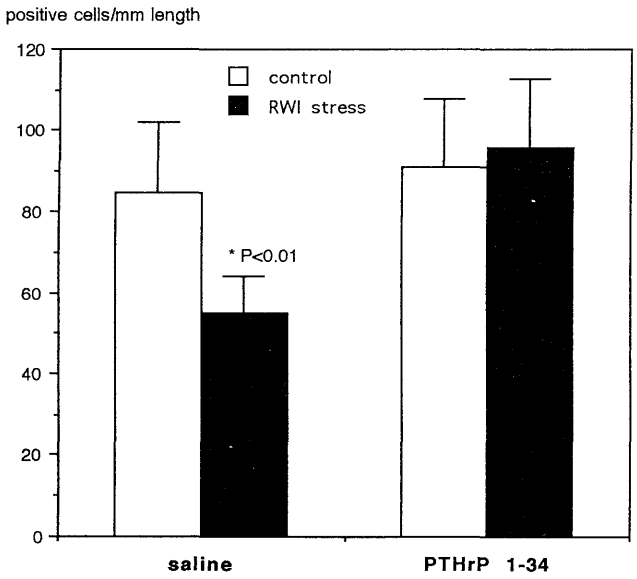

Figure 5. Effect of PTHrP-(1-34) on BrDU incorporation at RWI stress. BrDU incorporation was significantly decreased by RWI stress in non-treated rats. In contrast, PTHrP-(1-34) pretreatment prevented decrease of BrDU incorporation at stress, although cell proliferative effect was not observed by PTHrP-(1-34) pretreatment in the non-stress condition.

External PTHrP-(1-34) effects on cell proliferation

Results of BrDU incorporation study were shown in Fig.4 and 5. Fig.4 shows representative finding of BrDU immunohistochemistry. Fig.5 shows the cell number of BrDU incorporation per mm. Cells with BrDU incorporation were encountered in the proliferative zone (Fig.4). In non-stressed rats, no significant differences were present with or without PTHrP-(1-34) pretreatment. BrDU incorporation was significantly decreased in RWI stressed animals. In contrast, PTHrP-(1-34) pretreatment prevented the suppression of BrDU incorporation at stress and this value tended to be higher than that of steady state control.

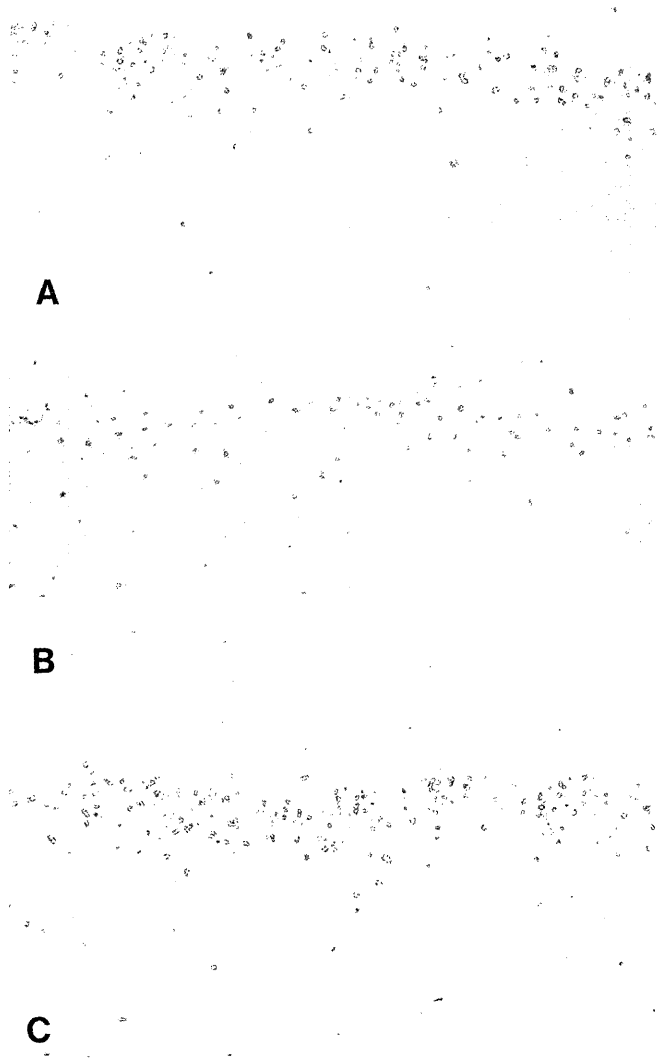

Figure 4. BrDU incorporation. Cells with BrDU incorporation were encountered in the proliferative zone (A; steady state control). BrDU incorporation was significantly decreased in RWI stressed animals (B). PTHrP-(1-34) pretreatment prevented the suppression of BrDU incorporation at stress (C). (x13)

Discussion

At present, at least three major secretory forms of the peptide have been shown to exist: an amino-terminal species, a mid-region species, and a carboxyl-terminal species. It is clear that multiple receptors for PTHrP must exist, and these can be divided into two categories: 1) an NH2-terminal PTHrP receptor that binds the PTH-like region of PTHrP, and 2) receptors for midregion and COOH-terminal PTHrP secretory forms. The most well established effects of PTHrP are mediated via an NH2-terminal PTHrP receptor. NH2-terminal PTHrP binds to the classical PTH receptor and activates both the adenyl cyclase/protein kinase A pathway as well as the cytosolic calcium/inositol phosphate/protein kinase C pathway[21]. However, the role of PTH/PTHrP receptor on mucosal growth and differentiation remains poorly understood now. In this study we focused on PTH/PTHrP receptor expression and its regulation of gastric mucosal proliferation.

Recent reports suggest that PTHrP plays an important role in cell growth and differentiation in many organs or tissues by an autocrine/paracrine mechanism[12-16,22], and the effect of PTHrP on growth or differentiation depends on the tissue and cell type. In the development of neonatal tissues, PTHrP and PTH/PTHrP receptor are essential. PTHrP in breast milk might have a local role in the neonatal gut. In the rat intestine, immunoreactive PTHrP was observed in jejunal epithelial cells all along the villus but not in crypt cells, suggesting a role for PTHrP in differentiating intestinal epithelial cells[17]. Our results suggested that PTHrP and PTH/PTHrP receptor plays some roles in gastric mucosal cell growth and differentiation.

Double staining of BrDU clearly demonstrated that cells with BrDU incorporation were negative for both PTHrP and PTH/PTHrP receptor, although they were closely located in the mucosal generative zone. Furthermore PTH/PTHrP receptor expression and BrDU incorporation fluctuated reciprocally under different conditions (RWI stress and steady state). These findings indicate that S phase cells do not express PTHrP and PTH/PTHrP receptor. The similar pattern was observed in PTH/PTHrP receptor and PCNA expression in benign soft tissue tumor[23]. There have been a limited number of studies on cell cycle and PTHrP. Okano et al indicated a role for PTHrP in the process of smooth muscle cell division[24]. They reported that constitutive immunreactive levels of PTHrP are low in normally cycling vascular smooth muscle cell and PTHrP-immunoreactive cells were enriched in G2+M.

Immunoreactivity of PTHrP in gastric tissue has been previously reported[11]. Smooth muscle cells in the proper muscle layer and small arteries showed immunoreactivity. PTH/PTHrP receptor expression was reduced abruptly in the most differentiated cells of the gastric mucosa. Similar expression pattern has been reported in the enchondral bone formation[25].

Cell proliferative effect of PTHrP-(1-34) might be elicited directly through PTH/PTHrP receptor and followed via signal transduction pathways. But there is a possibility to elicit PTHrP effect through cooperation of other cytokines, for instance EGF. PTHrP (1-34) increases the synthesis of EGF receptors during differentiation process of placenta[26]. In addition, EGF activating the PKC pathway are involved in the up regulation of PTHrP expression in mammary epithelial cells[27]. TGF β is one of up-regulatory factors for PTHrP, and is known to be an apoptotic factor and involved in intestinal mucosal differentiation.

PTH/PTHrP receptor mRNA increased at RWI stress. This up-regulation was induced by hypercorticosteronemia. A previous study demonstrated that dexamethasone dramatically increased steady state levels of PTH/PTHrP receptor mRNA in a time- and dose-dependent manner[28]. In this study serum corticosterone level increased about three times higher than non-stressed control, and the steroid inhibitor (metyrapone, 11b-hydroxylation inhibitor) pretreatment abolished up-regulation of PTH/PTHrP receptor mRNA was abolished by (data not shown). This up-regulation of PTH/PTHrP receptor at RWI stress explains the effective cell proliferative activation by the external PTHrP-(1-34) compared with steady state control. In contrast, PTHrP-(1-34) did not increase BrDU incorporation in a steady state when PTH/PTHrP receptor expression was in a basal level. These findings suggest that PTHrP and its cognate receptor play an important role on maintaining the mucosal integrity rather than simple cell proliferation or differentiation.

References

1. Suva L, Winslow GA, Wettenhall REH, Hammonds RG, Moseley JM, Diefenbach-Jagger H, Rodda OP, Kemp BE, Rodriguez H, Chen EY, Hudson PJ, Martin TJ, Wood WI. A parathyroid hormone-related protein implicated malignant hypercalcemia. *Science* 237: 893-6, 1987
2. Mangin M, Ikeda K, Dreyer BE, Broadus AE. Isolation and characterization of the human parathyroid hormone-like peptide gene. *Proc Natl Acad Sci USA* 86: 2408-12, 1989
3. Yasuda T, Banville D, Hendy GN, Goltzman D. Characterization of the human parathyroid hormone-like peptide gene. *J Biol Chem* 264: 7720-5, 1989
4. Ikeda K, Weir EC, Mangin N, Dannies PS, Kinder B, Deftos LJ, Brown EM, Broadus AE. Expression of messenger ribonucleic acids encoding a parathyroid hormone-like peptide in normal human and animal tissues with abnormal expression in human

parathyroid adenomas. *Mol Endocrinol* 2: 1230-6, 1988
5. Moseley JM, Hayman JA, Danks JA, Alcorn D, Grill V, Southby J, Horton MA. Immunohistochemical detection of parathyroid hormone-related protein in human fetal epithelia. *J Clin Endocrinol Metab* 73: 478-84, 1991
6. Abou-Samra AB, Juppner H, Force T, Freeman MW, Kong XF, Schipani E, Urena P, Richards J, Bonventre JV, Potts JT, Kronenberg HM, Segre GV. Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells: A single receptor stimulates intracellular accumulation of both cAMP and inositol triphosphates and increases intracellular free calcium. *Proc Natl Acad Sci USA* 89: 2732-6, 1992
7. Thiede MA, Harm SC, McKee RL, Grasser WA, Doung LT, Leach RMJr. Expression of the parathyroid hormone-related protein gene in the avian oviduct: potential role as a local modulator of vascular smooth muscle tension and shell gland motility during the egg-laying cycle *Endocrinology* 129: 1958-66, 1991
8. Thiede MA, Weir AGEC, Brines ML, BurtisWJ, Ikeda K, Dreyer BE, Garfield RE, Broadus AE. Intrauterine occupancy controls expression of the parathyroid hormone-related peptide gene in preterm rat myometrium. *Proc Natl Acad Sci USA* 87: 6969-73, 1990
9. Daifotis AG, Weir EC, Dreyer BE, Broadus AE. Stretch-induced parathyroid hormone-related peptide gene expression in the rat uterus. *J Biol Chem* 267: 23455-8, 1992
10. Yamamoto M, Harm SC, Grasser WA, Thiede MA. Parathyroid hormone-related protein in the rat urinary bladder: a smooth muscle relaxant produced locally in response to mechanical stretch. *Proc Natl Acad Sci USA* 89: 5326-30, 1992
11. Ito M, Ohtsuru A, Enomoto H, Nakashima M, Nakayama T, Shichijo K, Sekine I, Yamashita S. Expression of parathyroid hormone-related peptide in perturbation of rat gastric motility. *Endocrinology* 134:1936-42, 1994
12. Kaiser SM, Laneuville P, Bernier SM, Rhim JS, Kremer R, Goltzman D. Enhanced growth of a human keratinocyte cell line induced by antisense RNA for parathyroid hormone-related peptide. *J Biological Chemistry* 267:13623-8, 1992
13. Holick MF, Ray S, Chen TC, Tian X, Persons KS. A parathyroid hormone antagonist stimulates epidermal proliferation and hair growth in mice. *Proc Natl Acad Sci USA* 91 :8014-6, 1994
14 Tsukazaki T, Ohtsuru A, Enomoto H, Yano H, Motomura K, Ito M, Namba H, Iwasaki K, Yamashita S. Expression of parathyroid hormone-related protein in rat articular cartilage. *Calcified Tissue International* 57 :196-200, 1995
15. McCauley LK, Koh AJ, Beecher CA, Cui Y, Decker JD, Franceschi RT. Effects of differentiation and transforming growth factor beta 1 on PTH/PTHrP receptor mRNA levels in MC3T3-E1 cells. *Journal of Bone & Mineral Research* 10:1243-55, 1995
16. Hastings RH, Duong H, Burton DW, Deftos LJ. Alveolar epithelial cells express and secrete parathyroid hormone-related protein. *American Journal of Respiratory Cell & Molecular Biology* 11:701-6, 1994
17. Li H, Seitz PK, Thomas ML, Selvanayagam P, Rajaraman S, Cooper CW. Widespread expression of the parathyroid hormone-related peptide and PTH/PTHrP receptor genes in intestinal epithelial cells. *Lab Invest* 73:864-70, 1995
18. Takagi K, Okabe S. The effects of the production and recovery processes of the stress ulcer. *Jpn J Pharmacol* 18: 9-18, 1968
19. Ozeki S, Ohtsuru A, Seto S, Takeshita S, Yano H, Nakayama T, Ito M, Yokota T, Nobuyoshi M, Segre GV, Yamashita S, Yano K. Evidence that implicates the parathyroid hormone-related peptide in vascular stenosis. Increased gene expression in the intima of injured carotid arteries and human restenotic coronary lesions. *Arterioscler Thromb Vasc Biol.* 16:565-75, 1996
20. Hashiguchi J, Ito M, Sekine I. The effect of the autonomic nervous system on cell proliferation of the gastric mucosa in stress ulcer formation. *J Auton Nerve Syst* 43: 179-88,1993
21. Philbrick WM, Wysolmerski JJ, Galbraith S, Holt E, Orloff JJ, Yang KH, Vasavada RC, Weir EC, Broadus AE, Stewart AF. Defining the roles of parathyroid hormone-related protein in normal physiology. *Phyiol Rev* 76:127-73, 1996
22. Li H, Seitz PK, Selvanayagam P, Rajaraman S, Cooper CW. Effect of endogenously produced parathyroid hormone-related peptide on growth of a human hepatoma cell line (Hep G2). *Endocrinology* 137:2367-74,1996
23. Nakashima M, Ito M, Ohtsuru A, Alipov GK, Matsuzaki S, Nakayama T, Yamashita S, Sekine I. Expression of parathyroid hormone (PTH)-related peptide (PTHrP) and PTH/PTHrP receptor in giant cell tumor of tendon sheath. *J Pathol* 180:80-4, 1996
24. Okano K. Pirola CJ. Wang HM. Forrester JS. Fagin JA. Clemens TL. Involvement of cell cycle and mitogen-activated pathways in induction of parathyroid hormone-related protein gene expression in rat aortic smooth muscle cells. *Endocrinology* 136:1782-9, 1995
25. Lee K, Lanske B, Karaplis AC, Deeds JD, Kohno H, Nissenson RA, Kronenberg HM, Segre GV. Parathyroid hormone-related peptide delays terminal differentiation of chondrocytes during endochondral bone development. *Endocrinology* 137:5109-18, 1996
26. Alsat E, Haziza J, Scippo ML, Frankenne F, Evain-Brion D. Increase in epidermal growth factor receptor and its mRNA levels by parathyroid hormone (1-34) and parathyroid hormone-related protein (1-34) during differentiation of human trophoblast cells in culture. *J Cell Biochem* 53:32-42, 1993
27. Ferrari SL, Rizzoli R, Bonjour JP. Effects of epidermal growth factor on parathyroid hormone-related protein production by mammary epithelial cells. *J Bone Min Res* 9:639-44, 1994
28. Urena P. Iida-Klein A. Kong XF. Juppner H. Kronenberg HM. Abou-Samra AB. Segre GV. Regulation of parathyroid hormone (PTH)/PTH-related peptide receptor messenger ribonucleic acid by glucocorticoids and PTH in ROS 17/2.8 and OK cells. *Endocrinology* 134:451-6, 1994