

Parathyroid Hormone-related Peptide (PTH-rp) in Normal, Hyperplastic and Malignant Endometrial Tissue: an Immunohistochemical Analysis

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Abstract. Parathyroid hormone-related peptide (PTH-rp) is a peptide initially purified from tumors with hypercalcemia, which is also produced in human endometrium. The aim of this study was to determine the frequency and tissue distribution of PTH-rp in normal, hyperplastic and malignant endometrium. **Materials and Methods:** Paraffin-fixed endometrial tissue was obtained randomly from women in the proliferative (n=4), early secretory (n=5) and late secretory (n=5) phases, as well as glandular-cystic hyperplasia (n=5), adenomatous hyperplasia (AH) grade I (n=5), grade II (n=4), grade III (n=4) and endometrioid adenocarcinoma grade I (n=5). The PTH-rp expression was evaluated by immunohistochemical procedure. A semiquantitative analysis and a statistical evaluation was performed. **Results:** Immunohistochemical reaction with PTH-rp was primarily observed in glandular and luminal epithelial cells. Stromal and myometrial cells also expressed PTH-rp. The expression of PTH-rp in glands was significantly higher during the late secretory than in the proliferative phase. The highest expression was observed during AH grade III, while the lowest reaction was detected in the proliferative phase and adenocarcinoma. **Discussion:** PTH-rp was expressed in normal, hyperplastic and malignant endometrial tissue. A cyclical expression of PTH-rp in normal glandular epithelium was observed, being more prominent in the late secretory phase. AH grade I to III also expressed PTH-rp with higher immunostaining than adenocarcinoma. Since AH grade III can be considered as a

precursor of endometrial cancer, PTH-rp could be a marker of cell transformation. Endometrioid adenocarcinoma expressed the lowest PTH-rp immunostaining, indicating either a lower expression or a reflection of an increased PTH-rp shedding of malignant transformed endometrium. Further studies are required to establish the usefulness of PTH-rp as a marker in different endometrial pathologies.

Parathyroid hormone (PTH)-related protein (PTH-rp) was first isolated as a circulating factor responsible for malignancy-associated hypercalcemia (1). The tumor-produced PTH-rp binds at the amino-terminus to the common PTH/PTH-rp receptors in bone and kidney, causing hypercalcemia. PTH-rp exists in isoforms of 139, 141 and 173 amino acids with a 70% homology with PTH for the first 13 amino acids (2). Approximately 80% of hypercalcemic patients with solid tumors have detectable or increased plasma PTH-rp concentrations. (3).

PTH-rp mRNA or protein has been identified in the uterus, mammary tissue, ovary, placenta, testis and prostate and in the oviduct and shell gland of the chicken (4). PTH-rp is expressed and secreted by a variety of normal human tissues (5), including amnion, chorion, placenta, myometrium and decidua during pregnancy (6, 7). PTH-rp is also produced by normal endometrium with a cyclical expression pattern during the normal menstrual cycle (8).

High PTH-rp concentrations were demonstrated in rat myometrium during pregnancy, with the highest levels being in myometrial tissues of uterine horns occupied by the fetus (9). Additionally, estrogen treatment of nonpregnant rats caused an increase in PTH-rp mRNA levels in myometrial tissue (10). Previously, PTH-rp was demonstrated in human endometrial stromal cells in culture with an increase of PTH-rp after treatment with transforming growth factor-beta (11). PTH-rp is known to produce relaxation in smooth muscle cells (12, 13) and has also been shown to dilate placental blood vessels (14).

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Table I. *The German and WHO nomenclature for hyperplastic pathology of human endometrium.*

German classification	WHO classification
Glandular-cystic hyperplasia	Simple hyperplasia
Adenomatous hyperplasia grades I and II	Complex hyperplasia
Adenomatous hyperplasia grade III	Atypical hyperplasia

Although hypercalcemia is observed in cases of endometrial carcinomas, it is still a rare condition. There are limited data regarding the PTH-rp expression in hyperplastic and malignant endometrial tissue. Hypercalcemia was diagnosed in patients with endometrial clear cell adenocarcinoma (15), adenosquamous carcinoma (16) and papillary serous carcinoma (17). However, hyperplastic lesions and adenocarcinoma have not been studied to date.

Therefore, the aims of this study were: a) the determination of the frequency and tissue distribution patterns of PTH-rp in normal, hyperplastic and malignant endometrium, and b) the assessment of PTH-rp expression as an immunohistochemical marker for premalignant endometrial lesions.

Materials and Methods

Paraffin-fixed endometrial tissue was randomly obtained from premenopausal women in the proliferative phase (n=5), early secretory phase (n=5) and late secretory phase (n=4), as previously described (18, 19). Additionally, endometrial samples diagnosed with glandular-cystic hyperplasia (n=5), benign endometrial polyps (n=5), endometrial polyps caused by tamoxifen (n=5), adenomatous hyperplasia (AH) grade I (n=4), grade II (n=5), grade III (n=5) and well-differentiated endometrioid adenocarcinoma (G1) (n=5) were obtained. Histopathological evaluation of the samples was performed according to Dallenbach and Poulson (20). The German and WHO nomenclatures for endometrial hyperplastic pathologies are listed in Table I. Recently, we have used the former classification to assess the cathepsin D (21) and CA-125 (22) immunohistochemical expression in human hyperplastic and malignant endometrial tissue, demonstrating a continuous significant increase of both parameters between AH grades I and III, while another research group, using the WHO classification, could not demonstrate such differences (23).

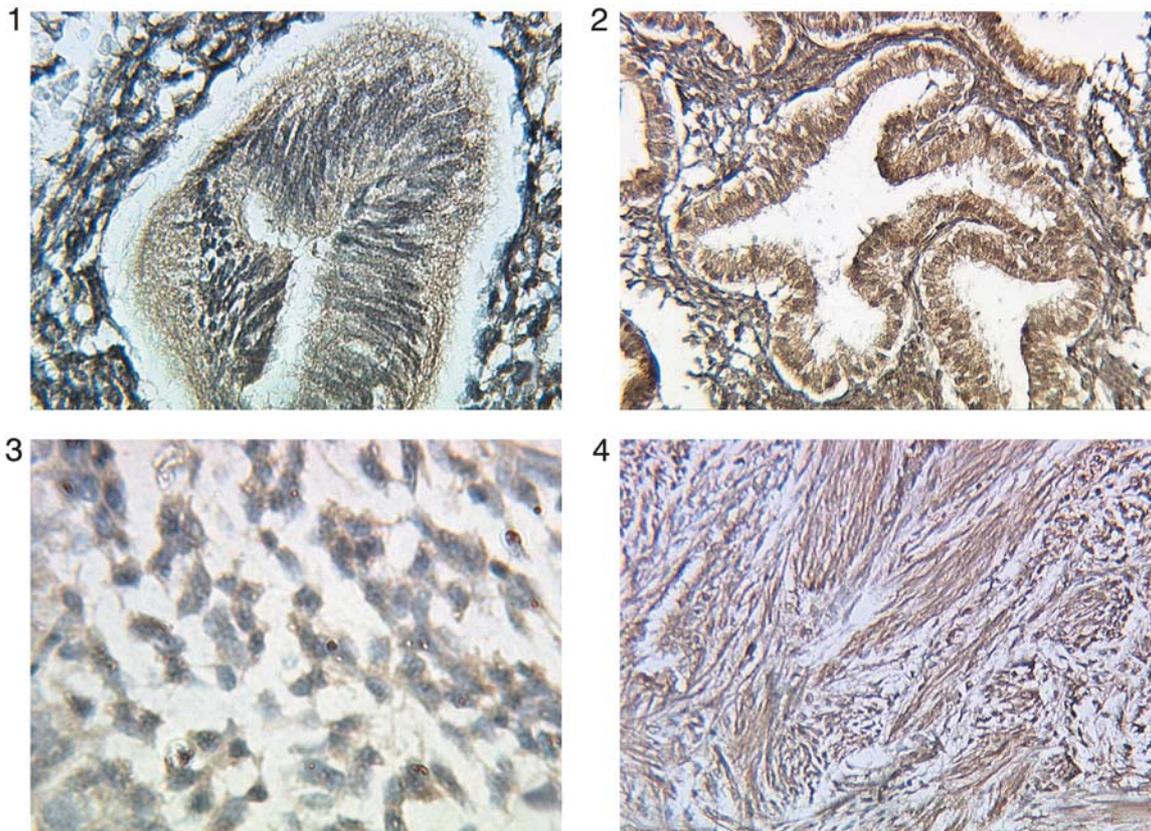
Immunohistochemical staining with PTH-rp antibody (parathyroid hormone related peptide (PTH-rp) 14-28, Human, N-terminal, rabbit IgG, dilution 1:100, Biotrend, Cologne, Germany) was performed with the Nexus®Autostainer and a DAB staining protocol with the use of a microwave (Ventana Medical System, Tuscon, AZ, USA), as previously described (19,

21). Briefly, the slides were air-dried, then incubated with inhibitor serum and protease solution. This protease solution (protease reagent 1; Ventana Medical System) contains an alkaline protease, an endopeptidase of the serine protease family. Incubations with biotinylated antibodies avidin-peroxidase were subsequently performed, followed by several washing steps with PBS after each incubation step, as described by the manufacturer. Visualization of peroxidase activity was performed with DAB and H₂O₂ and counterstaining was performed with hematoxylin/blueing reagent. Positive cells showed a brownish color and negative controls as well as unstained cells were blue. The standardization, dilution and optimization of this protocol were primarily tested on endometrial samples from the secretory phase.

The intensity and distribution patterns of the specific PTH-rp immunohistochemical staining reaction were evaluated using the semiquantitative score (graded as 0=no, 1=weak, 2=moderate and 3=strong staining). Sections were examined using an Olympus (Tokyo, Japan) photomicroscope. Digital images were obtained with a digital camera system (Olympus) and were saved on computer. The results were evaluated using the ANOVA analysis for comparison and assessment of significant differences of the means (SPSS, Chicago, IL, USA). Significance was assumed at $p < 0.05$.

Results

The immunohistochemical reaction with the PTH-rp antibody was observed in glandular epithelial, stromal and myometrial cells. In glandular epithelial cells, PTH-rp was distributed primarily on the basal cell surface with a lower intensity on the apical cell side in the proliferative phase (Figure 1), while during late secretory phase, a diffuse immunostaining was observed (Figure 2). Stromal cells also expressed the PTH-rp antigen, but with an undifferentiated pattern (Figure 3). Interestingly, myometrial cells expressed PTH-rp with an undifferentiated pattern throughout the menstrual cycle (Figure 4). The staining reaction became more intense in glandular epithelial cells during the menstrual cycle, being significantly higher during the late secretory phase than the proliferative phase (Figure 5). Endometrial hyperplasia and endometrioid adenocarcinomas were also positive for PTH-rp antigen. In hyperplastic endometrial tissue, the highest expression of PTH-rp was observed in AH III, which was statistically significantly higher when compared to AH I and II (Figure 6). The lowest expression of PTH-rp was observed in adenocarcinoma. The immunohistochemical expression was heterogeneous within the tumors, whereas differences in the staining intensity between different glands in individual tumor sections resulted in a focal immunohistochemical-positive reaction. AH grades I-III had a significantly higher PTH-rp expression than endometrioid adenocarcinoma, which was statistically significantly lower compared to all groups (Figure 6).



Figures 1-4. Immunohistochemical localization of PTH-rp in human endometrium. A weak positive immunohistochemical staining, primarily in glandular epithelial cells during the proliferative phase is present. PTH-rp is present at the apical and basal cell surface (Fig. 1, x250). Staining intensity becomes more intense during progression of the menstrual cycle, being significantly higher during the late secretory phase (Fig. 2, x125). Stromal cells (Fig. 3, x400) and myometrial cells (Fig. 4, x250) also expressed PTH-rp, but in a undifferentiated distribution pattern.

Discussion

PTH-rp is expressed and secreted in a variety of normal tissue (5), including human placenta (6, 7) and normal endometrium (8). We demonstrated, by immunohistochemical methods, that human normal, hyperplastic and malignant endometrial tissue also expressed PTH-rp in a differential way.

PTH-rp expression was observed in endometrial glandular epithelial cells, stromal cells and endometrial smooth muscle cells, confirming previous results (8, 11, 24, 25). A cyclical expression pattern of PTH-rp was demonstrated in normal human endometrial glandular epithelial cell tissue. Recently, PTH-rp and PTH-rp receptor mRNA was also detected in normal human endometrium (8). Competitive RT-PCR revealed that the expression of PTH-rP mRNA was higher during the proliferative phase than in the secretory phase, with no statistical differences in the PTH/PTH-rP receptor mRNA expressions (8). However, we identified a significant rise

between the proliferative and the secretory phase of immunoreactive PTH-rp in glandular epithelial cells. This PTH-rp distribution pattern in normal endometrium suggests that this peptide is a secretory product of human endometrial cells. However, the precise function in normal human endometrial tissue remains to be elucidated. Marked induction of PTH-rp expression by mechanical stretch and vasoconstrictor agents, together with its ability to relax smooth muscle, indicate an autocrine or paracrine role in the control of blood flow or response to contractile stimuli in the human uterus. PTH-rp expression in glandular endometrial epithelium was related to the menstrual cycle, being more prominent in the late secretory phase. Therefore, it might function in an autocrine or paracrine manner to control blood flow, especially during the late secretory phase. The significantly higher PTH-rp expression in the late secretory phase could also reflect an increased shedding of the transformed secretory endometrium and a paracrine role in endometrial function and maturation.

□ proliferative phase ■ early secretory phase ■ late secretory phase

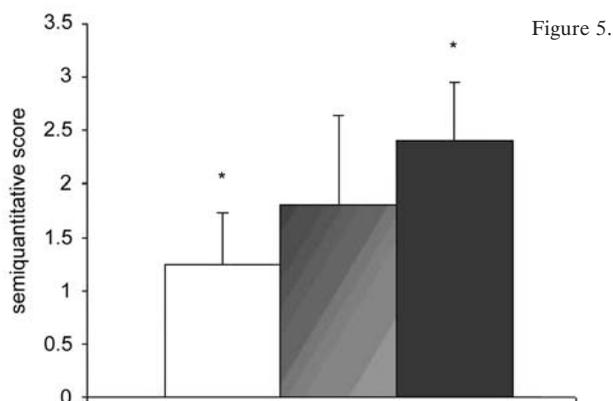
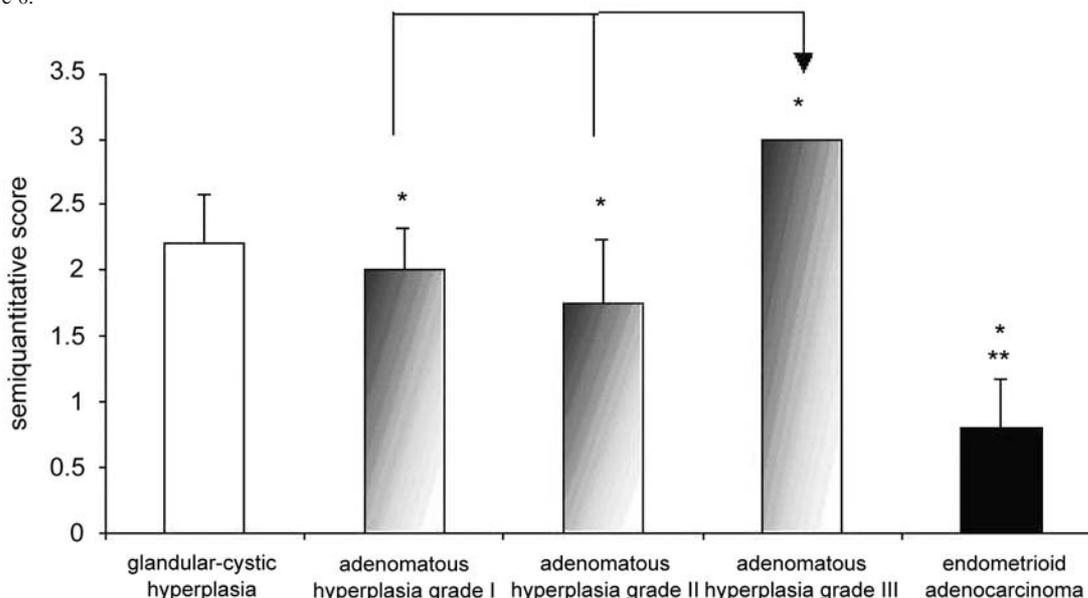


Figure 5.

Figures 5-6. Immunoreactive score of the PTH-rp expression in normal, hyperplastic and malignant endometrium. Expression of PTH-rp was significantly higher during the late secretory phase than the proliferative phase (Fig. 5, *). A continuous increase in PTH-rp expression was found in AH, with significant differences between AH grades I and II compared to AH grade III (Fig. 6, * arrow). The PTH-rp expression in endometrial cancer was the lowest observed, being significantly lower than in all groups (Fig. 6, **). Values represent means \pm SEM. Significance was taken at $p < 0.05$ (asterisks).

Figure 6.



Probably, PTH-rp might have at least two distinct roles in the uterus: a relaxing action on the muscle and a novel effect either within the uterine lumen or the endometrial layer, as previously suggested for rat endometrial tissue (13).

We also demonstrated immunohistochemical staining reactions of PTH-rp in hyperplastic and malignant endometrial tissue. Interestingly, diffuse cytoplasmic staining was found in hyperplasia, a clinico-pathological result of unopposed estrogen effects. Estrogen might act directly in endometrial normal stroma cells to increase PTH-rP mRNA levels and PTH-rP protein production (11). Therefore, an association of PTH-rp with the endometrial

estrogen metabolism might be possible in hyperplastic tissue, although such data are still missing. In adenomatous hyperplasia, a high PTH-rp immunostaining was observed. This might suggest a functional role in endometrial pathogenesis, either by influencing the cells or by regulating the uterine blood flow. Since AH grade III can be considered a precursor of endometrial cancer, PTH-rp could be a marker of cell transformation. Further, because adenomatous hyperplasia, and especially AH grade III, can be considered as a precursor lesion of endometrial cancer, PTH-rp could be a possible marker in assessing malignant transformation

Endometrial carcinomas can arise from hyperplastic precursor lesions, probably due to unopposed stimulation by estrogen. The estrogen-dependent type of endometrial carcinomas consist of histopathologically well-differentiated endometrioid carcinomas (G1-G2), although some types of endometrial cancer might be estrogen-independent (26). In human endometrial cancer cells, the precise expression and regulation of PTH-rp is still unknown. The significantly lower PTH-rp expression in well-differentiated estrogen-dependent endometrioid carcinomas may also reflect increased shedding of the transformed endometrium, although serological data are still missing. Interestingly, the potent vasorelaxant PTH-rp expression is modulated by transforming growth factor-beta (TGF- β) in normal endometrial stromal cells (27). Although TGF- β plays a substantial role in endometrial carcinogenesis (28), a possible relationship between these two substances in endometrial cancer has not yet been elucidated. However, in breast cancer, PTH-rp has been associated with enhanced metastasis to the bone (29, 30). The lower expression of PTH-rp might explain the low bone metastatic potential of endometrial adenocarcinomas. Although PTH-rp was expressed in endometrial adenocarcinomas, it might be a more useful marker in the follow-up of patients with non-endometrioid tumors.

In conclusion, we showed an expression of PTH-rp in normal and hyperplastic endometrial tissue with highest expression in the late secretory menstrual phase and hyperplastic lesions. However, the prognostic value of PTH-rp expression in pathological endometrial conditions still remains uncertain. An increase in PTH-rp immunostaining was observed in AH. Since AH grade III can be considered as a precursor of endometrial cancer, this cytokine could be a possible parameter for assessing malignant transformation. In summary, this initial study suggests a widespread but complex role for PTH-rp in hyperplastic and malignant endometrial growth regulation.

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