Prolactin Enhances the Proliferation of Proliferative Endometrial Glandular Cells and Endometrial Cancer Cells

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To elucidate the mechanism of endometrial cancer (EC) development in young hyperprolactinemic women, this study assessed the hormonal receptor expression, proliferation, and signaling induced by prolactin in endometrial glands (EG) and EC.

Prolactin receptor (PRLR) and estrogen receptor alpha (ER-α) in EG were evaluated during the menstrual cycle by immunohistochemistry. The following parameters were compared between EM-E6/E7/TERT cells, which originated from proliferative EG and Ishikawa cells. The expression levels of PRLR, pJAK2 (phosphorylated Janus Activating Kinase 2), its downstream pathways (MAPK, PI3K, and STAT), and ER-α were assessed after adding prolactin by Western blotting. U0126 was used as a MAPK inhibitor. The proliferation caused by estradiol was also examined by MTS assay after adding prolactin.

PRLR expression in the EG was significantly higher in the proliferative phase than in the secretory phase, and it was correlated with ER-α expression during the menstrual cycle. After adding prolactin, the expression of pJAK2, PRLR and ER-α was significantly increased in both cell lines, MAPK was activated after adding prolactin in both cell lines, and PI3K and STAT were activated only in EM-E6/E7/TERT cells. The increased proliferation induced by estradiol was enhanced after adding prolactin in both cell lines. All changes caused by prolactin were inhibited in Ishikawa cells pretreated with U0126.

Long-term effects of serum prolactin on persistent proliferative endometrium in the presence of estradiol may induce abnormal proliferation of EG in hyperprolactinemic women. Prolactin-PRLR signaling via MAPK may play a crucial role in the progression of EC in hyperprolactinemic women.

Endometrial proliferation varies substantially throughout the normal menstrual cycle. Estrogen receptor (ER) status shows a highly significant correlation with glandular proliferation rates [1]. The highest levels of ER in the endometrial glandular cells are expressed during the proliferative phase, whereas they decrease significantly during the secretory phase after ovulation [2]. If ovulation does not occur, the persistent proliferative effect caused by persistent expression of ER in the endometrial glandular cells and an inadequate effect of progesterone may lead to a higher risk of endometrial cancer (EC) [3, 4]. Hyperprolactinemia is a well-established cause of hypogonadotropic hypogonadism and

anovulatory infertility [5]. However, the involvement of hyperprolactinemia in the risk of EC remains unknown. In addition, the pattern of prolactin receptor (PRLR) expression in the endometrial glandular cells has not been determined in anovulatory women.

Elevated levels of serum prolactin were identified in patients with various types of cancer [6], and the highest levels of serum prolactin were shown in patients with EC after a comparison of those in various types of cancer [7]. A total of 17.1% of all-aged patients with type I EC showed hyperprolactinemia and were characterized by young age and low insulin resistance [6]. If confined to young women who had atypical endometrial hyperplasia (AEH) or early-stage EC aged 40 or younger, approximately 50% of them showed hyperprolactinemia [8]. Therefore, serum prolactin may potentially have a higher impact on the pathogenesis of particularly young women with EC.

Prolactin activates the signaling pathway via the phosphorylation of JAK2, including signal transducers and activators of transcription (STAT), protein kinase C and phosphatidylinositol 3-kinase (PI3K), and mitogen-activated protein kinase (MAPK) in cells expressing PRLR [9]. There are multiple isoforms of the PRLR in humans: the long form, intermediate form, and several short forms that are produced by splicing [10]. The long PRLR is the major form through which prolactin transmits its signals [11]. An immunohistochemical analysis revealed that the expression of PRLR and phosphorylated JAK2 in cancer cells was significantly increased in hyperprolactinemic patients with type I EC [6]. Prolactin increased the proliferative ability of some types of cancer cell lines that express PRLR, including EC cell lines [7, 12, 13]. Therefore, prolactin-PRLR signaling seems to be important for the progression of EC.

The present study aims to assess the hormonal receptor expression in the endometrial glands during the menstrual cycle and the differences between endometrial glandular cells and endometrial cancer cells in signaling pathways, the expression of hormonal receptors, and proliferative ability by prolactin to elucidate the progressive mechanism of EC in hyperprolactinemic women.

1. Materials and Methods

A. Tissue Collection

Endometrial tissues were obtained from 21 premenopausal and nonpregnant women who had undergone a hysterectomy for gynecological diseases at the Kumamoto University Hospital between 2008 and 2011. All women (range of age: 26 to 48 years) had a regular menstrual cycle (25–35 days) and did not receive exogenous hormonal therapy at least 3 months prior to the hysterectomy. The histological findings of the endometrium were classified into the early proliferative phase (EP) in 3 women, midproliferative phase (MP) in 2, late proliferative phase (LP) in 4, early secretory phase (ES) in 4, midsecretory phase (MD) in 3, and late secretory phase (LS) in 5, according to the dating criteria of the endometrium [14]. This study protocol was approved by the Institutional Review Board of Kumamoto University.

B. Immunohistochemical Evaluation

Immunohistochemical analysis was performed as previously described [8]. Anti-PRLR (B6.2) antibody (ab74608; Abcam) [15] and antiestrogen receptor alpha (ER-α) antibody (M7047; Agilent) [16] were used for staining the endometrium tissues. The immunoreactive score was calculated by multiplying the optical staining intensity (graded as 0 = no, 1 = weak, 2 = moderate, and 3 = strong staining) and the percentage of positive stained cells (0 = no staining, 1 = < 10% of the cells, 2 = 11–50% of the cells, 3 = 51–80% of the cells, and 4 = >81% of the cells) for an evaluation of the expression of PRLR and ER-α as previously described [17].

The immunostained slides were evaluated independently by two of the authors who were not informed about the clinical backgrounds. An average score of two authors was used for comparison of PRLR and ER-α.

C. Cell Culture and Treatment with Estradiol (E2), Prolactin, and U0126

The immortalized human endometrial glandular cell line EM-E6/E7/TERT was derived from two women in the late proliferative phase who had regular menstrual cycles [18]. Ishikawa cells were obtained from the American Culture Collection (Manassas, VA). Ishikawa cells were derived from a 39-year-old woman diagnosed with endometrial carcinoma grade 1. Both cell lines were maintained in DMEM/Ham's nutrient mixture F-12 medium (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10% FBS at 37°C in a 5% CO_2-containing atmosphere. To examine the effects of prolactin and/or E2, cells were cultured in phenol red-free DMEM (Wako Pure Chemical Industries, Osaka, Japan) supplemented with 10% charcoal-stripped FBS (Gibco, Grand Island, NY) and 1% antibiotics/antimycotics (Gibco) as 10% charcoal-stripped serum (CSS), and recombinant human prolactin (#100–07, PeproTech, Rocky Hill, CT) or beta-E2 (E-8875; Sigma Chemical, St. Louis, MO) was added to the cell culture medium. To determine the inhibitory effect of MAPK signaling, cells were treated with U0126 (#9903, Cell Signaling Technology, Cell Signaling, Danvers, MA) for 2 hours prior to the addition of prolactin and/or E2. Phosphate-buffered saline was used as a vehicle for control cultures.

D. Immunoblot Analysis

Whole-cell lysates were prepared using Pierce RIPA buffer (#89901, Thermo Scientific, IL) containing 1% Halt Protease and Phosphatase Inhibitor Cocktail (#87786 Thermo Scientific, IL). Following quantification using a BCA Kit (#23227 Thermo Scientific, Rockford, IL), the lysates were subjected to polyacrylamide gel electrophoresis using 7.5–16% mini-gels (Mini-PROTEAN® TGX™ Precast Gel; Bio-Rad, CA) and transferred onto polyvinylidene difluoride membranes (#10600023, GE Healthcare, Darmstadt, Germany). After blocking in Blocking One (#03953-95, Nacalai Tesque, Kyoto, Japan), the membranes were incubated overnight at 4°C with primary antibodies, which included anti-PRLR (H300) antibody (sc-20992; Santa Cruz Biotechnology, Dallas, TX) [19], anti-ER-α antibody [E115] (ab32063; Abcam) [20], anti-phospho-JAK2 (Tyr1007/1008) (C80C3) rabbit mAb antibody (3776; Cell Signaling Technology, Danvers, MA) [21], anti-JAK2 (D2E12) XP rabbit mAb antibody (3230; Cell Signaling Technology, Danvers, MA) [22], anti-rabbit anti-STAT5, phospho (Tyr694) monoclonal antibody (ab32364; Abcam) [23], anti-STAT5 antibody (94205; Cell Signaling Technology, Danvers, MA) [24], anti-AKT1 + AKT2 + AKT3 (phospho S472 + S473 + S474) antibody (ab183758; Abcam) [25], anti-AKT1 + AKT2 + AKT3 antibody (ab184136; Abcam) [26], anti-phospho-mTOR (Ser2448) antibody (2971; Cell Signaling Technology, Danvers, MA) [27], anti-mTOR antibody (2972; Cell Signaling Technology, Danvers, MA) [28], anti-phospho-MEK1/2 (Ser217/221) (41G9) rabbit mAb antibody (9154; Cell Signaling Technology, Danvers, MA) [29], anti-MEK1/2 antibody (9122; Cell Signaling Technology, Danvers, MA) [30], anti-phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibody (4370; Cell Signaling Technology, Danvers, MA) [31], anti-p44/42 MAPK (Erk1/2) (137F5) rabbit mAb antibody (4695; Cell Signaling Technology, Danvers, MA) [32], and anti-alpha Tubulin antibody (ab4074; Abcam) [33]. Subsequently, the membranes were incubated with anti-goat anti-rabbit IgG, HRP-conjugated antibody (12–348; Millipore, Burlington, MA) [34] or anti-goat anti-mouse IgG, HRP conjugate antibody (12–349; Millipore, Burlington, MA) [35] conjugated to horseradish peroxidase for 1 hour at room temperature. Finally, the membranes were washed and visualized using PierceTM ECL Plus Western Blotting Substrate (#32132, Thermo Fisher Scientific, Rockford, IL).

E. Proliferation Assay

Cell viability was assessed with the MTS assay according to the manufacturer's protocol (CellTiter 96 Aqueous One Solution Cell Proliferation assay, Promega, Madison, WI). Cells (2×10^3/100 µL of 10% CSS per well) were seeded in 96-well flat-bottom plates. Ten percent CSS was replaced 24 hours after cell seeding, and the cells were cultured in the absence or presence of prolactin and/or E2. Forty-eight hours after the treatment, 20 µL MTS assay solution was added to each well for 2 hours. Absorbance was recorded at 490 nm on a SpectraMax 190 microplate reader (Molecular Devices, Sunnyvale, CA). Experiments were repeated 3 times and the percentage of cell survival was defined as the relative absorbance of untreated versus treated cells. The proliferation rate was calculated using the mean of triplicate experiments.

F. Statistical Analysis

Descriptive statistics were given as the mean ± SD. The mean values were compared between the 2 groups using the Mann-Whitney test or Student's t-tests. The Spearman's test was used to evaluate the correlation between the 2 groups. *P* values of less than 0.05 from two-sided tests were regarded as significant. All statistical analyses were performed using IBM SPSS Statistics for Windows (Version 24.0., IBM Corp., Armonk, NY).

2. Results

A. Immunohistochemistry Evaluation for PRLR and ER-α in the Endometrial Glandular Cells During the Menstrual Cycle

Immunostaining for PRLR and ER-α in the endometrial glandular cells during the menstrual cycle is presented in Figs. 1a–l. Comparisons of the mean immunoreactive scores of PRLR and ER-α in the endometrial glandular cells between the proliferative phase and the secretory phase are provided in Table 1. The expression of PRLR and ER-α in the endometrial glandular cells was significantly higher in the proliferative phase than in the secretory
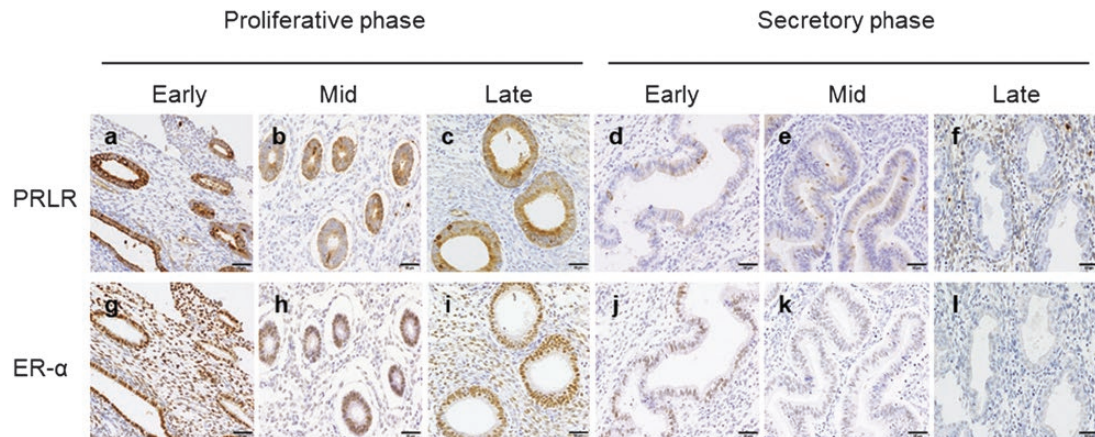

Figure 1. Immunostaining for PRLR and ER-α in the human endometrium during the menstrual cycle. Positive staining for PRLR in the cytoplasm of endometrial glandular cells during the proliferative phase (a, b, and c) and weak or negative staining for PRLR in the cytoplasm of endometrial glandular cells during the secretory phase (d, e, and f). Positive staining for ER-α in the nuclei of endometrial glandular cells (g, h, and i) during the proliferative phase and weak or negative staining for ER-α in the nuclei of endometrial glandular cells (j, k, and l) during the secretory phase. Hematoxylin counterstaining, magnification 200×.

phase (7.8 vs. 2.8, $P = 0.003$; 7.5 vs. 3.0, $P = 0.007$, respectively). The immunoreactive score of PRLR significantly correlated with that of ER-α in the endometrial glandular cells during the menstrual cycle (r = 0.585, $P = 0.005$) (Fig. 2).

Table 1.   Comparison of the Immunoreactive Scores of the Prolactin Receptor and Estrogen Receptor-Alpha in the Endometrial Glandular Cells Between the Proliferative Phase and the Secretory Phase

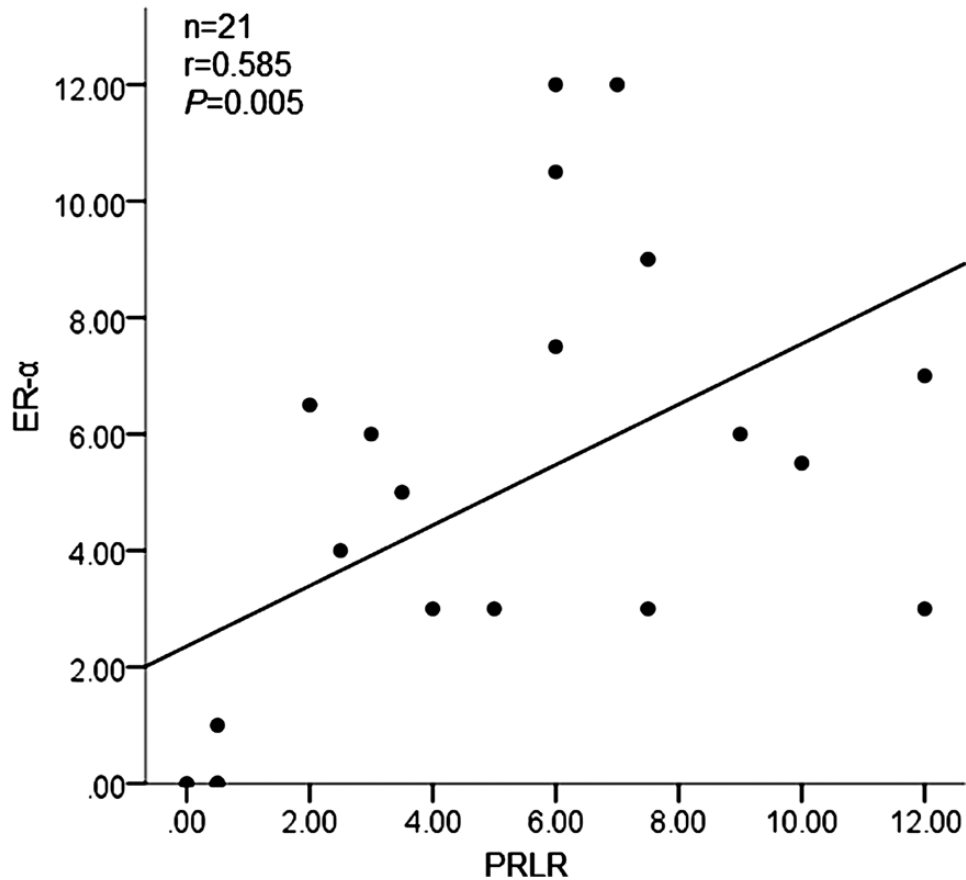|	Total (n = 21)		
	Proliferative phase (n = 9)	Secretory phase (n = 12)	*P* value
PRLR scoring (Mean ± SD, range)	7.8 ± 3.1 (2.5–12)	2.8 ± 2.7 (0–7.5)	0.003**
ER-α scoring (Mean ± SD, range)	7.5 ± 3.3 (3–12)	3.0 ± 3.0 (0–9)	0.007**

Mann-Whitney test; ** indicate $P < 0.01$.

Figure 2. Correlation of the immunoreactive scores between PRLR and ER-α in the endometrium. Scatter plot shows that there was a positive correlation of the immunoreactive scores between PRLR and ER-α in 21 human endometrial tissues. Three women in the secretory phase showed the same score (PRLR: 0.5 and ER-α: 0.0). The correlation was evaluated by a Spearman's test. The number of women (n), coefficient of correlation (r), and *P* values (*P*) are indicated.

B. Activation of Downstream Signaling Pathways of PRLR and Altered Expression of PRLR and ER-α by Prolactin in EM-E6/E7/TERT Cells

The three isoforms of PRLR and the single isoform of ER-α were expressed in EM-E6/E7/TERT cells (Fig. 3). This characteristic was consistent with immunohistochemical findings of the endometrial glandular cells in the late proliferative phase. Then, 100 ng/ml of prolactin was added to the cell culture medium to determine the prolactin-mediated signaling pathways via PRLR. The phosphorylation of JAK2 was induced 1 hour after the addition of prolactin (Fig. 4a). The three major signaling pathways of PRLR following the phosphorylation of JAK2, including STAT, PI3K, and MAPK, were then examined. The phosphorylation of STAT5, AKT, mTOR, MEK1/2, and ERK1/2 was induced within 1 hour after adding prolactin (Fig. 4b– 4d). The expression of the longest isoform of PRLR and ER-α was increased 6 hours after adding prolactin (Fig. 4e).

C. Proliferative Effect of Prolactin and/or E2 on EM-E6/E7/TERT Cells

To assess the change in proliferative ability, 1 nM E2 and/or 100 ng/ml prolactin were added to the cell culture medium of EM-E6/E7/TERT cells. There was no significant change in cellular viability between the control and the group with prolactin ($P = 0.055$). The cellular viability was significantly increased after adding E2 ($P = 0.030$). There was also a significant increase in cellular viability after adding E2 and after adding both ($P = 0.036$) (Fig. 5).

D. Activation of Downstream Signaling Pathways of PRLR and Altered Expression of PRLR and ER-α by Prolactin in Ishikawa Cells

The three isoforms of PRLR and the single isoform of ER-α were also expressed in Ishikawa cells (Fig. 3). Then, 100 ng/ml of prolactin was added to the cell culture medium to determine the prolactin-mediated signaling pathways via PRLR. The phosphorylation of JAK2 was induced 1 hour after the addition of prolactin (Fig. 6a). The three major signaling pathways of PRLR following the phosphorylation of JAK2, including STAT, PI3K, and MAPK, were then examined. The phosphorylation of STAT5, AKT, and mTOR was not induced, and MEK1/2
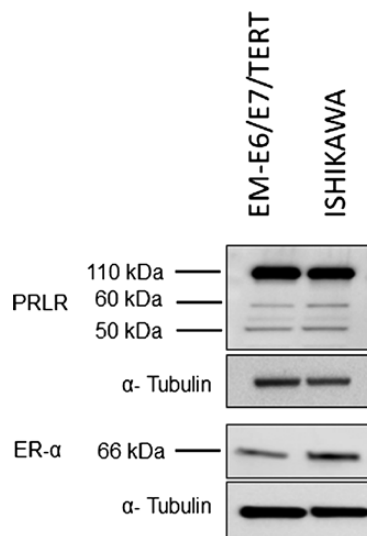

Figure 3. Molecular weight and the expression of PRLR and ER-α in EM-E6/E7/TERT cells and Ishikawa cells. The three isoforms of PRLR, including a long isoform of 110 kDa, an intermediate isoform of 60 kDa, and a short isoform of 50 kDa, were expressed in both EM-E6/E7/TERT cells and Ishikawa cells. The single isoform of ER-α of 66 kDa was expressed in both EM-E6/E7/TERT cells and Ishikawa cells.
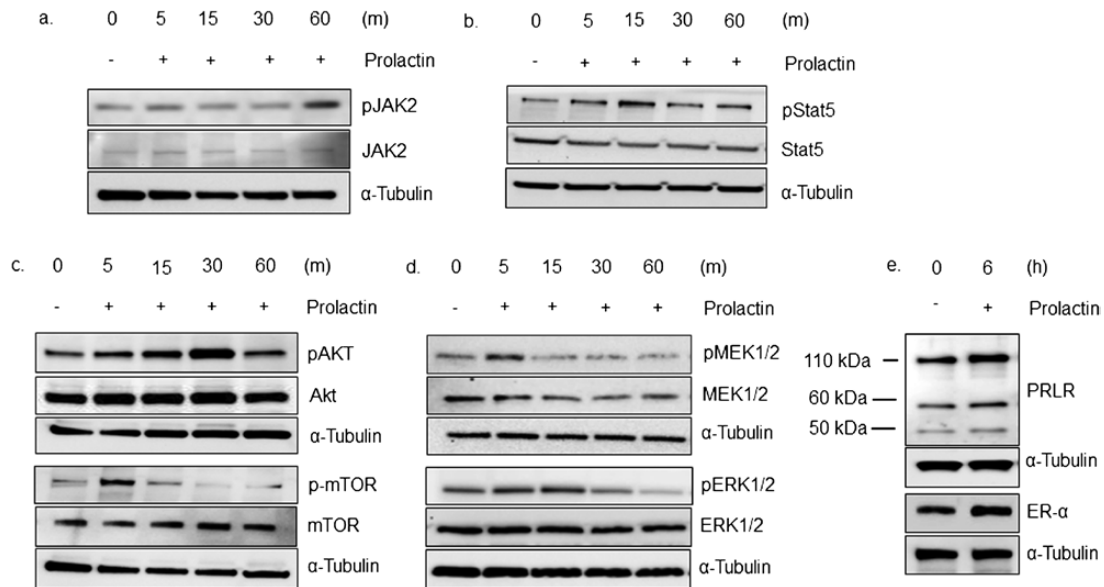
Figure 4. Time course of the phosphorylation of JAK2, STAT5, AKT, mTOR, MEK1/2, and ERK1/2 and the quantitative variation of PRLR and ER-α in EM-E6/E7/TERT cells after adding prolactin. (a) The maximal phosphorylation of JAK2 was observed in EM-E6/E7/TERT cells 1 hour after treatment with 100 ng/ml of prolactin. (b) The maximal phosphorylation of STAT5 was observed in EM-E6/E7/TERT cells 15 minutes after treatment with 100 ng/ml of prolactin. (c) The maximal phosphorylation of AKT and mTOR was observed in EM-E6/E7/TERT cells 30 minutes and 5 minutes after treatment with 100 ng/ml of prolactin, respectively. (d) The maximal phosphorylation of MEK1/2 and ERK1/2 was observed in EM-E6/E7/TERT cells 5 minutes and 15 minutes after treatment with 100 ng/ml of prolactin, respectively. (e) The increased expression of PRLR of 110 kDa and ER-α was observed in EM-E6/E7/TERT cells 6 hours after treatment with 100 ng/ml of prolactin
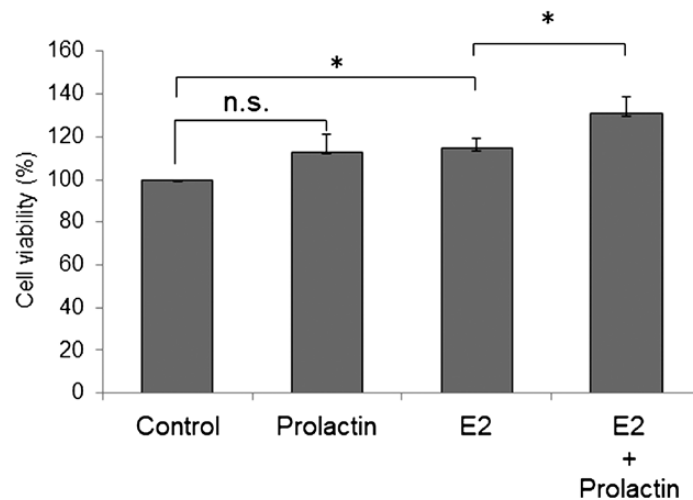

Figure 5. The effects of prolactin or/and E2 on the proliferation of EM-E6/E7/TERT cells. There was no significant difference in the number of viable cells before and after adding 100 ng/ml of prolactin ($P = 0.055$). The viable cell number was significantly increased after adding 1 nM E2 ($P = 0.030$). The viable cell number was significantly increased after adding both 100 ng/ml of prolactin and 1 nM of E2 compared to that after 1 nM of E2 ($P = 0.036$). Values are the mean ± SD of three experiments. Significant differences are shown by asterisks (*, <0.05); n.s. indicates no significant differences

and ERK1/2 were phosphorylated within 1 hour after adding prolactin (Fig. 6b–6d). The expression of the longest isoform of PRLR and ER-α was also increased in Ishikawa cells 6 hours after adding prolactin (Fig. 6e).
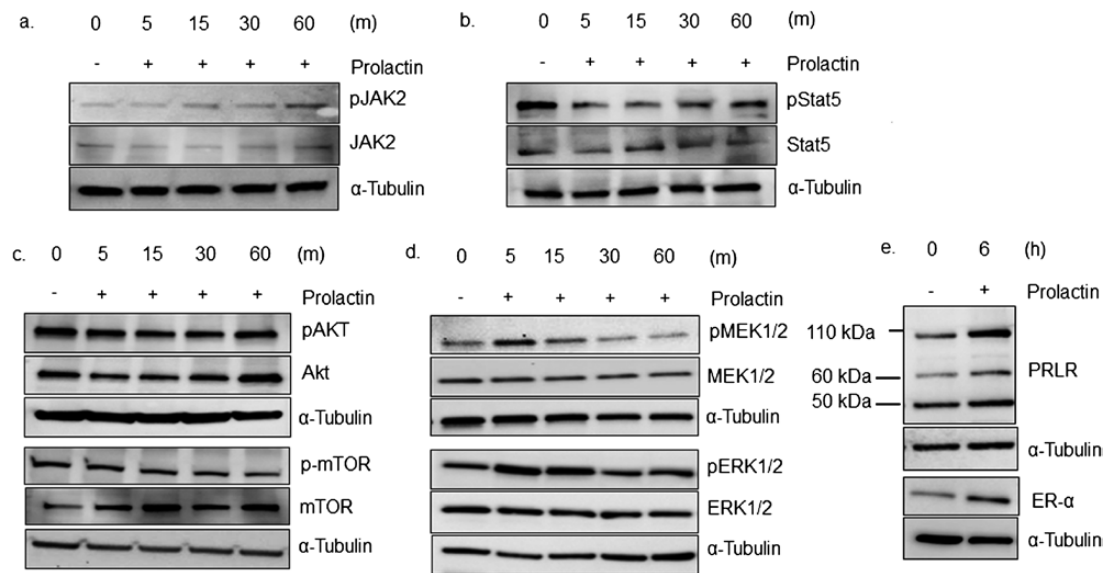
Figure 6. Time course of the phosphorylation of JAK2, STAT5, AKT, mTOR, MEK1/2, and ERK1/2 and the quantitative variation of PRLR and ER-α in Ishikawa cells after adding prolactin. (a) The maximal phosphorylation of JAK2 was observed in Ishikawa cells 1 hour after treatment with 100 ng/ml of prolactin. (b) The phosphorylation of STAT5 was not changed in Ishikawa cells after treatment with 100 ng/ml of prolactin. (c) The phosphorylation of AKT and mTOR was not changed in Ishikawa cells after treatment with 100 ng/ml of prolactin. (d) The maximal phosphorylation of MEK1/2 and ERK1/2 was observed in Ishikawa cells 5 minutes and 15 minutes after treatment with 100 ng/ml of prolactin, respectively. (e) The increased expression of PRLR of 110 kDa and ER-α was observed in Ishikawa cells 6 hours after treatment with 100 ng/ml of prolactin (f) The maximal phosphorylation of ERK1/2 was observed in Ishikawa cells 5 minutes and 15 minutes after treatment with 100 ng/ml of prolactin, and the phosphorylation of ERK1/2 was completely inhibited in Ishikawa cells pretreated with 10 μM of U0126 after treatment with 100 ng/ml of prolactin. (g) The increased expression of PRLR of 110 kDa and ER-α was observed in Ishikawa cells 6 hours after treatment with 100 ng/ml of prolactin, and the decreased expression of PRLR and ER-α was observed in Ishikawa cells pretreated with 10 μM of U0126 6 hours after treatment with 100 ng/ml of prolactin.

Only MAPK was activated in Ishikawa cells after adding prolactin, and this finding was different from that in EM-E6/E7/TERT cells. To examine the inhibitory effect of MAPK, Ishikawa cells were treated with 10 μM of U0126, which is an inhibitor of MEK1/2, for 2 hours prior to the addition of prolactin. The phosphorylation of ERK1/2 was completely inhibited in Ishikawa cells treated with U0126 after adding prolactin (Fig. 6f). The expression of the longest isoform of PRLR and ER-α was decreased in Ishikawa cells treated with U0126 6 hours after adding prolactin compared to Ishikawa cells without U0126 6 hours after adding prolactin (Fig. 6g).

E. Proliferative Effect of Prolactin or/and E2 on Ishikawa Cells Without or With U0126

To assess the change in proliferative ability, 1 nM E2 or/and 100 ng/ml prolactin were added to the cell culture medium of Ishikawa cells. The cellular viability was significantly increased after adding prolactin or E2 compared to the control group ($P = 0.001$ and $P < 0.001$, respectively). There was also a significant increase in cellular viability after adding E2 and after adding both ($P = 0.012$) (Fig. 7a). The cellular viability was not changed in Ishikawa cells without U0126 compared to Ishikawa cells pretreated with U0126 ($P = 0.474$), and the increased viability by prolactin was inhibited in Ishikawa cells pretreated with U0126 ($P = 0.008$) (Fig. 7b). The increased viability by prolactin and E2 was also inhibited in Ishikawa cells pretreated with U0126 ($P = 0.039$) (Fig. 7c).
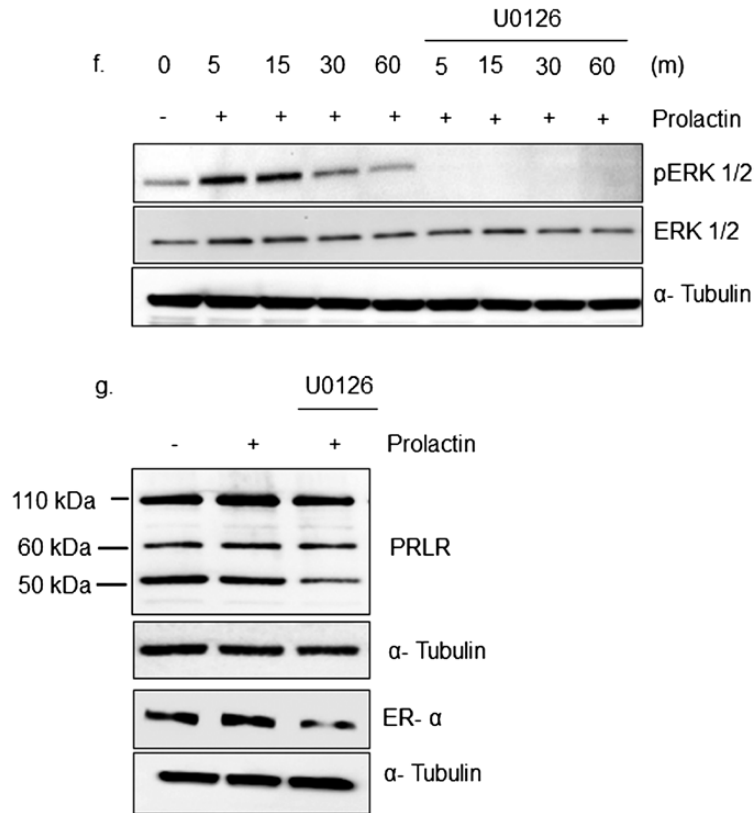
Figure 6. Continued.

3. Discussion

The present study revealed that the expression of PRLR in the endometrial glandular cells was significantly increased in the proliferative phase, and this finding was positively correlated with that of ER-α during the menstrual cycle. The expression pattern of ER in the glandular cells was different from that in the stromal cells in the endometrium, indicating that autocrine and paracrine mechanisms are involved in embryo implantation and early pregnancy [2, 36]. However, the involvement of serum prolactin in the proliferation of endometrial glandular cells has not been fully elucidated. The expression pattern of PRLR limited to the glandular cells had not been previously examined during the menstrual cycle. One study assessed the expression of PRLR in the endometrium, including glandular cells and stromal cells, to elucidate the role of the temporal expression of extrapituitary prolactin in implantation and placentation by using in situ hybridization and immunohistochemistry [37]. This report demonstrated that PRLR expression was high in the mid to late secretory phase in the whole lesion of the endometrium and concluded that extrapituitary prolactin plays an important role via PRLR expressed in the decidualization of both stromal and glandular cells of the endometrium. Another study revealed that the positive rate of prolactin mRNA extracted from the endometrial tissue was high in the mid to late proliferative phase of the menstrual cycle [38]. Therefore, the present study is the first time that PRLR expression limited to the endometrial glandular cells was evaluated during the menstrual cycle with the purpose of focusing on the carcinogenesis of EC, and significantly high expression of PRLR was detected in the glandular cells in the proliferative phase.

The positive correlation between the expression pattern of PRLR and that of ER-α was also shown during the menstrual cycle in the present study. In healthy women with a regular menstrual cycle, serum levels of prolactin were significantly increased in the late
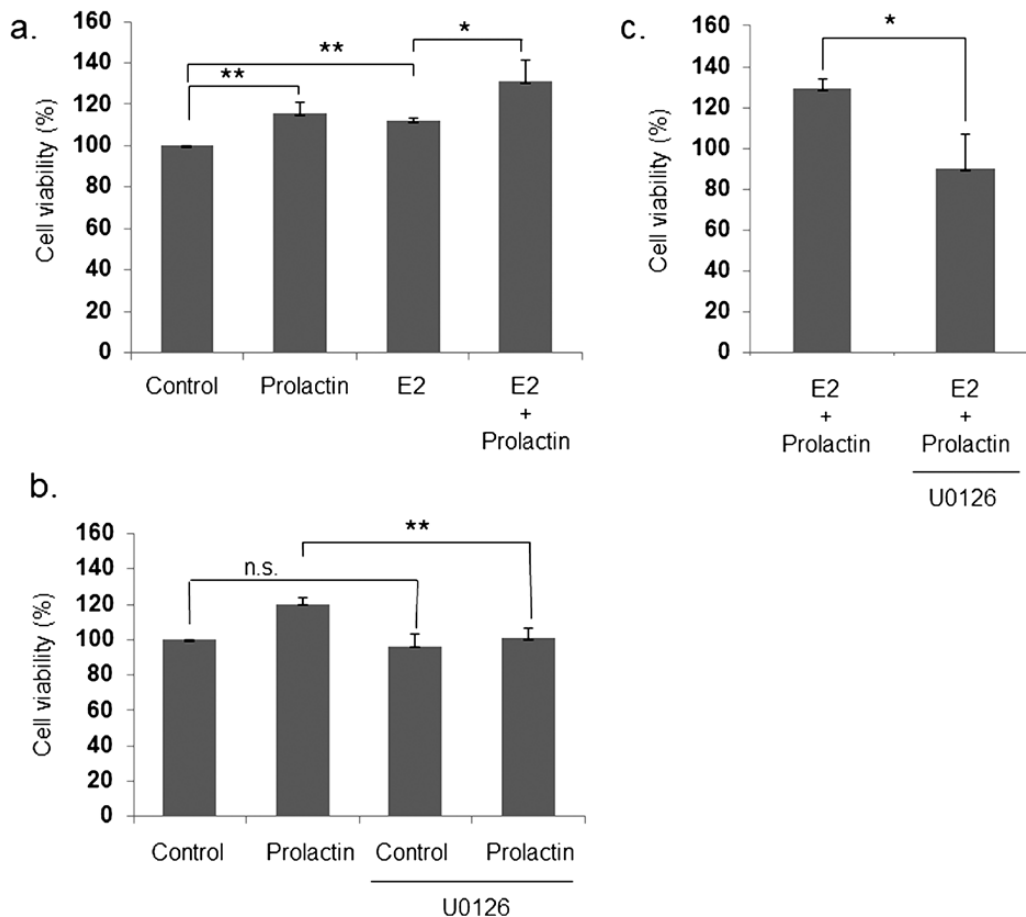
Figure 7. The effects of prolactin or/and E2 on the proliferation of Ishikawa cells with or without U0126. (a) The viable cell number was significantly increased after adding 100 ng/ml of prolactin or 1 nM of E2 compared to the control group ($P = 0.001$, $P < 0.001$, respectively). There was a significant increase in the number of viable cells number after adding 1 nM E2 with or without prolactin ($P = 0.012$). (b) The viable cell number was not changed in Ishikawa cells with or without U0126 ($P = 0.474$). After adding 100 ng/ml of prolactin, the viable cell number of Ishikawa cells pretreated with U0126 was significantly decreased compared to that of Ishikawa cells without U0126 ($P = 0.008$). (c) After adding both 100 ng/ml of prolactin and 1 nM of E2, the viable cell number was significantly decreased in Ishikawa cells pretreated with U0126 compared to that in Ishikawa cells without U0126 ($P = 0.039$). Values are the mean ± SD of three experiments. Significant differences are shown by asterisks (*, <0.05, **, <0.01); n.s. indicates no significant differences.

proliferative phase, and the overall pattern of serum prolactin was similar to that of 17β-E2 during the menstrual cycle, suggesting that E2 and prolactin may play an important role via PRLR and ER-α in the late proliferative phase [39]. The present study also showed that prolactin positively regulated the expression of both PRLR and ER-α and enhanced the proliferation induced by E2 in EM-E6/E7/TERT cells. A previous study showed that prolactin may show synergistic action with E2 via the regulation of the ER expression in the corpora lutea of pregnant rats [40]. The mechanism of high expression of both PRLR and ER-α may be favorable for the proliferation of endometrial glandular cells in women with regular menstrual cycles. Both serum E2 and prolactin may play an important coordinated role, particularly in the proliferative endometrial glands.

Risk factors of EC vary according to patients' body mass index (BMI). The analysis of women with EC under the age of 50, divided according to BMI, revealed that diabetes and irregular menstrual cycles were noted in obese women, while normal-weight women had a

high rate of nulliparous, irregular menstrual cycles and a history of infertility, indicating that hormonal factors, possibly polycystic ovarian syndrome (PCOS), may contribute to the development of EC in young normal-weight women [41]. Our previous study demonstrated that young age and low insulin resistance were noted in women with type I EC who had shown hyperprolactinemia [6]. Both PCOS and hyperprolactinemia are responsible for anovulation or oligo-ovulation. In such women, the proliferative endometrium that shows significantly increased expression of ER and PRLR in the glandular cells may persist longer, as shown in the present study. The persistent secretion of E2 and prolactin may induce the abnormal proliferation of the endometrium via the positive regulation of PRLR and ER, and the regulatory roles of progesterone and progesterone withdrawal in the endometrium are suboptimal or absent in women with hyperprolactinemia. Therefore, hyperprolactinemia may increase the risk of EC.

The present study also demonstrated that prolactin regulates PRLR and ER-α and enhances proliferation in EC cells as well as in endometrial glandular cells. In breast cancer cell lines and hepatocellular cancer cell lines that express PRLR, the proliferative ability was significantly increased with the increased expression of PRLR by the addition of prolactin [12, 13]. The increased immunohistochemical expression of PRLR in EC tissues was noted in EC patients with high levels of serum prolactin, indicating that the increased expression of PRLR may be relevant to the progression of EC in hyperprolactinemic women [6]. Moreover, prolactin possibly regulates the functions of ER-α as well as those of PRLR in cancer cells. Prolactin activated ER-α in breast cancer cell lines, suggesting that its action is crucial for the proliferative and transcriptional actions of breast cancer [42]. One epidemiological study demonstrated that the increased risk of breast cancer in postmenopausal women who showed high levels of serum prolactin was confined to those taking hormone replacement therapy [43]. The involvement of prolactin in the progression of EC cannot be explained without referring to its interaction with E2 and ER-α, and the progression of EC may be associated with both direct effects of prolactin and changes in endocrine and hormonal receptor expression caused by increased levels of serum prolactin.

The activated pathways via the phosphorylation of JAK2 after adding prolactin were different between the 2 cell lines used in the study. All three major signaling pathways of the PRLR were activated after adding prolactin in EM-E6/E7/TERT cells, suggesting that prolactin may mediate multiple functions other than proliferation in the endometrial glands under physiological conditions. On the other hand, only MAPK was activated after adding prolactin in Ishikawa cells, suggesting that prolactin-mediated activation of MAPK was important for both the activation of E2-dependent proliferation and the regulation of PRLR and ER-α in endometrial cancer cells. This finding is supported by the evidence that chronic exposure of immortalized endometrial fibroblasts to prolactin activated Ras, which is upstream of MAPK, and resulted in malignant transformation [7]. Compared to the prolactin-mediated activation of MAPK in endometrial cancer, that of STAT5 is crucial for the progression of prostate cancer, and evidence for the efficacy of pharmacological targeting of STAT5 to inhibit castrate-resistant growth of prostate cancer has been reported [44]. Similarly, strategies to suppress the actions of prolactin in breast cancer and prostate cancer are being developed. A phase I trial to evaluate the clinical utility of a potent anti-PRLR neutralizing antibody, LFA102, in patients with breast or prostate cancer is underway [45].

Unlike these cancers, prolactin does not seem to be closely associated with all types of EC. Hyperprolactinemia can be newly suggested as a risk factor for young women with type I EC [6]. Therefore, most patients can be cured after the standard treatment. The necessity of fertility-sparing therapy, including medroxyprogesterone acetate, is now increasing, as there is an increased prevalence of EC in young women. However, the risk of disease relapse and exacerbation during the observational period is high. Our retrospective observational study revealed that cabergoline, which is a selective and long-acting dopamine agonist, contributed to preserving the uterus in young hyperprolactinemic patients with AEH

or early-stage EC treated with medroxyprogesterone acetate by reducing serum prolactin levels [8]. Further studies are necessary to establish the efficacy of this therapy.

In conclusion, the persistent effect of the high levels of serum prolactin on the endometrium at the proliferative phase in the presence of E2 may induce abnormal proliferation of the endometrial glandular cells via enhanced ER and PRLR in hyperprolactinemic women. Prolactin-PRLR signaling via MAPK may play a crucial role in the progression of hyperprolactinemic women with EC. Antiprolactin drugs may contribute to enhancing the effect of fertility-sparing treatment and suppressing recurrences in young hyperprolactinemic women with early-stage EC.

Acknowledgments

Additional Information

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  Disclosure Summary: The authors declare no conflicts of interest.

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