G protein-coupled estrogen receptor 1 (GPER, GPR 30) in normal human endometrium and early pregnancy decidua†

Z. Kolkova¹*, V. Noskova¹, A. Ehinger², S. Hansson¹, and B. Cassléning

¹Department of Obstetrics and Gynecology, Skåne University Hospital, Lund SE-22185, Sweden ²Department of Pathology, Skåne University Hospital, Malmö SE-205 02, Sweden

*Correspondence address. E-mail: zuzana.kolkova@med.lu.se

Submitted on August 3, 2009; resubmitted on May 18, 2010; accepted on May 25, 2010

ABSTRACT: The recently identified trans-membrane G protein-coupled estrogen receptor 1 (GPER, GPR30) has been implicated in rapid non-genomic effects of estrogens. This focusses on expression and localization of GPER mRNA and protein in normal cyclic endometrium and early pregnancy decidua. Real-time PCR, western blotting, in situ hybridization and immuno-histochemistry were used. Endometrial expression of GPER mRNA was lower in the secretory phase than in the proliferative phase, and even lower in the decidua. The expression pattern was similar to that of ERα mRNA, but different from that of ERβ mRNA. Western blot detected GPER protein as a 54 kDa band in all endometrial and decidual samples. In contrast to the mRNA, GPER protein did not show cyclic variations. Apparently, a lower amount of mRNA is sufficient to maintain protein levels in the secretory phase. GPER mRNA was predominantly localized in the epithelium of mid- and late-proliferative phase endometrium, whereas expression in early proliferative and secretory glands could not be distinguished from the diffuse stromal signal, which was present throughout the cycle. Immuno-staining for GPER was stronger in glandular and luminal epithelium throughout the cycle. The cyclic variations of GPER mRNA obviously relate to strong epithelial expression in the proliferative phase, and the expression pattern suggests regulation by ovarian steroids. GPER protein is present in endometrial tissue throughout the cycle, and the epithelial localization suggests potential functions during sperm migration at mid-cycle, as well as decidualization and blastocyst implantation in the mid-secretory phase.

Key words: ERα / ERβ / in situ hybridization / membrane estrogen receptor / menstrual cycle / physiologic hormone concentrations

Introduction

Estrogens are crucial regulators of endometrial biology. Many of their effects are mediated by the classical estrogen receptors, ERα and ERβ, which function as estrogen-activated transcription factors in the nucleus, where they mediate genomic effects. In addition, it is well known that estrogens can induce immediate effects, which do not influence gene transcription, since responses occur within seconds or minutes (Szego et al., 1967; Pietras et al., 1977). These responses are generally referred to as non-genomic or membrane effects.

Depending on the cell type, the rapid responses to estrogen involve mobilization of various second messengers, e.g. cAMP, Ca²⁺, PI3 kinase (Morley et al., 1992; Lieberherr and Grosse, 1994; Gu and Moss, 1996; Le Mellay et al., 1997), interaction with membrane receptor, e.g. the epidermal growth factor (EGF) receptor, or activation of intracellular kinase pathways (Nelson et al., 1991; Migliaccio et al., 1996; Ho and Liao, 2002). Such observations suggested that a G protein-coupled receptor (GPR) mediated these fast effects. GPRs represent one of the largest and most diverse super-families of cell membrane receptors in mammals, and they respond to a great variety of ligands. Their serpentine structure passes through the membrane seven times from an extra-cellular amino-terminal end to an intra-cellular carboxy-terminal end. They signal via a hetero-trimeric G protein-complex that consists of an α-subunit with GTPase activity, and a βγ-subunit, which triggers protein kinase cascades, stimulates matrix metallo-proteinases and trans-activates surface receptors (Prenzel et al., 1999; Gether, 2000).

The G protein-coupled estrogen receptor 1 (GPER) was first cloned in 1996 and initially named GPR30 (Owman et al., 1996). It is widely distributed in the human body, i.e. brain, liver, heart, lung, pancreas, placenta, blood vessels, bone, lymphoid tissue, as well as endometrial, ovarian and breast cancer (Owman et al., 1996; Carmeci et al., 1997;
O'Dowd et al., 1998; Filardo et al., 2006; Haas et al., 2007; Smith et al., 2007; Hein et al., 2008; Zhang et al., 2008). The receptor has been localized both in the plasma membrane and in the endoplasmic reticulum (Pietras et al., 2001; Revankar et al., 2005; Thomas et al., 2005; Funakoshi et al., 2006; Otto et al., 2008).

GPER was at first considered an orphan receptor, i.e. with no identified ligand. In 2000, Filardo et al. reported that GPR30 was required for estrogen-induced trans-activation of EGF receptor and subsequent signaling via the Erk pathway (Filardo et al., 2000). They also noted that estrogen activation of GPER increased adenyl cyclase activity in ER negative, GPER positive breast cancer cells (Filardo, 2002; Filardo et al., 2000, 2002). Subsequently, GPER was demonstrated to have estrogen receptor properties with a single, high-affinity, saturable and a low capacity.

Materials and Methods

Endometrial and decidual tissue

Samples of endometrial tissue (n = 39) were obtained from patients 45 years or younger with regular menstruations operated with hysterectomy for benign conditions unrelated to endometrial dysfunction (e.g. leiomyoma, cervical dysplasia, uterine prolapse, etc.). Samples from patients using oral contraceptives or intrauterine devices and samples with abnormal histology were excluded. Tissue samples of early pregnancy decidua (n = 8) were obtained from surgical abortions of 7–10 weeks. The receptor was at first considered an orphan receptor, i.e. with no identified ligand. In 2000, Filardo et al. reported that GPR30 was required for estrogen-induced trans-activation of EGF receptor and subsequent signaling via the Erk pathway (Filardo et al., 2000). They also noted that estrogen activation of GPER increased adenyl cyclase activity in ER negative, GPER positive breast cancer cells (Filardo, 2002; Filardo et al., 2000, 2002). Subsequently, GPER was demonstrated to have estrogen receptor properties with a single, high-affinity, saturable and a low capacity estrogen-binding site (Revankar et al., 2005; Thomas et al., 2005). The reported Kd was 3–6 nmol/l, which is about 10 times higher than that of the nuclear ERs. Another interesting observation is that antagonists to nuclear ERs, e.g. tamoxifen and ICI 182780, as well as environmental estrogens, e.g. genistein induces agonistic effects via GPER, whereas the pregnancy estrogen, estradiol, is reportedly a GPER antagonist (Maggiolini et al., 2004; Lin et al., 2009; Lappano et al., 2010).

A number of studies have investigated GPER in female reproductive tissues, i.e. breast (Maggiolini et al., 2004; Filardo et al., 2006, 2008), uterus (Vivacqua et al., 2006; Smith et al., 2007; He et al., 2009; Lin et al., 2009) and ovaries (Wang et al., 2007, 2008; Albanito et al., 2008a, b; Henic et al., 2009). This is, however, the first report on expression levels and tissue localization of GPER mRNA and protein in cyclic human endometrium and early pregnancy decidua. Since estrogen responses are fundamental not only in endometrial biology, but also in female reproduction and gynecologic carcinogenesis, GPER may be involved in these processes.

Separated endometrial cells

Fresh endometrial tissue (n = 9) was separated in a stromal cell fraction and a glandular epithelial cell fraction as described (Cassleén et al., 1995). Briefly, the tissue was cut into 1 mm pieces and incubated for 40–60 min at 37°C on a shaker (BioSan ES-20) in dissociation solution i.e. PBS with crude collagenase 2.5 g/l, DNAse-I 0.25 g/l, tosyl-L-lysine-chloromethyl-ketone 0.2 μmol/l. After digestion, the suspension was filtered through a sterile 35 μm pore nylon mesh to remove undigested tissue fragments. The cell suspension was subsequently passed through a 35 μm pore mesh to collect the glands. Stromal cells were present in the flow-through and collected by centrifugation at 1200g for 5 min. Before the stromal cells and epithelial cell fractions were lyzed for RNA extraction, a sample was taken from each of them to estimate cell purity. These samples were processed with cytospin and immuno-histochemistry using monoclonal antibodies to human vimentin and cytokeratin 8 (Dako, Glostrup, Denmark), (see immuno-histochemistry). Only fractions with <10% contaminating cells were further analyzed with real-time PCR.

Total RNA extraction

The frozen tissue samples were weighed (55–70 mg) and disintegrated with a QIAGEN TissueLyser (Retsch Technology GmbH, Haan, Germany) in 70 μl of TRK Lysis Buffer E.Z.N.A.®. Then, the total RNA was extracted from disintegrated tissue as well as cultured cells (1–5 × 10^6) using the total RNA Kit (OMEGA Bio-tek, Doraville, GA, USA) according to the manufactures instruction. Possible genomic DNA contamination was removed from the total RNA preparation using DNA-free™ Kit (Applied Biosystems, Ambion, AM1906). The final content of RNA was assayed with NanoDrop Spectrophotometer ND-1000 (Saveen Werner, Limhamn, Sweden), and the quality was verified by electrophoresis in 1% agarose gel.

cDNA synthesis

Intact RNA was converted to cDNA by reverse transcription using TaqMan Reverse Transcription Regenets (Applied Biosystems, Foster City, CA, USA), according to protocols from the manufacturer. The final concentration of cDNA was 10 ng/μl verified by NanoDrop Spectrophotometer ND-1000. The samples were stored at −20°C until further used.

Real-time PCR amplification

The real-time PCR assay used ABI PRISM 7000 (Applied Biosystems, Foster City). The PCR reaction was run in duplicates, the following assays were used: GPR30 (assay Hs00173506_m1 and Hs01922715_s1, Applied Biosystems), ER-α (assay Hs00174860_m1, Applied Biosystems), ER-β (assay Hs00230957_m1, Applied Biosystems), and β-actin (assay Hs99999903_ml, Applied Biosystems). Assay ml for GPER detects transcript variants 2 (NM_001505.2) and 3 (NM_001039966.1). The probe spans an exon junction and does not detect genomic DNA. Assay 1 detects GPER transcript variants 2–4 (NM_001098201_2). The probes bind to a sequence within a single exon, and may thus detect genomic DNA. Therefore, genomic DNA was removed from the extracted total RNA before converting to cDNA and running the 1 assay. Two negative controls were included in each amplification and each reaction was carried out in duplicate. Quantification of the amplification products was done by comparison with the calibration curve obtained by 10-fold dilution of the template DNA (80–0.08 ng). All mRNA values were related to the level of β-actin mRNA in each sample.

In situ hybridization of GPER mRNA

Cryostat sections (14 mm thick) from each sample of frozen tissues were collected on siliconized glass slides. Prior to hybridization, tissue sections were pre-treated as described (Young, 1990). Sections were thawed and fixed directly in 4% buffered formaldehyde for 5 min, rinsed twice in...
Germany). The total concentration of protein was determined by the Ultrasonic processor UP50H (Hielscher Ultrasonics, GMbH, Teltow, with the sense probes showed no specific signal.

Sections hybridized (Kodak) and counterstained with 1% Giemsa. All slides were independently evaluated by two authors (S.H. and Z.K.). Sections hybridized with cRNA was performed as described (Cox et al., 1984; Whitfield et al., 1990). Briefly, 35S-cRNA probes were denatured at 65 °C for 5 min and placed in ice for 5 min. Hybridization was performed with 1 × 10^8 cpm of denatured 35S-cRNA probe per 50 μl hybridization buffer. The buffer consisted of 20 mmol/l Tris–HCl (pH 7.4), 1 mmol/l EDTA (pH 8), 300 mmol/l NaCl, 5% formamide, 10% dextran sulfate, 1 × Denhardt’s, 25 g/l yeast tRNA, 100 mg/l salmon sperm DNA, 250 mg/l total yeast RNA (fraction XI), 100 mmol/l diithiothreitol (DTT), 0.1% sodium thiosulfate and 0.1% sodium dodecyl sulfate (SDS). The hybridization solution (70 μl/15 cm²) was applied to the tissue sections, which were covered with untreated glass coverslips. Slides were then incubated at 56 °C in chambers humidified with 2 × SSC with 50% formamide for 24 h. After hybridization, slides were cooled to room temperature, and coverslips were floated off the slides with 4 × SSC.

Slides were then rinsed four times with 4 × SSC followed by immersion in 20 mg/l RNase A at 37 °C for 30 min. Sections were desalted in graded SSC solutions, washed twice in 0.1 × SSC at 65 °C for 30 min, dehydrated in graded ethanol solutions, and finally air-dried. The slides were exposed to Kodak Bio-Max MR film for 1–4 days, and subsequently coated with undiluted nuclear track emulsion (NTB, Kodak). After exposure for 4–5 weeks at 4 °C, slides were developed in D-19 solution. Sections were then rinsed in water and dehydrated in graded ethanol solutions, and air-dried. The slides were counterstained with 0.5% hematoxylin, dried, and mounted with Euparal.

Western blot analysis of GPER

Frozen endometrial tissue (50–70 mg) was disintegrated in homogenizing buffer containing sucrose 1.25 mol/l, HEPES 200 mmol/l (pH 7.5), EGTA 10 mmol/l, DTT 100 mmol/l, soybean trypsin inhibitor 1 mg/ml, leupeptin 1 mg/ml and antipain 1 mg/ml at 4 °C using QIAGEN TissueLyser (Retsch Technology GmbH, Haan, Germany). Tissue debris and nuclei were removed by spinning the lysates at 1000 g for 10 min at 4 °C. Supernatants were filtered through one layer of gauze and subsequently centrifuged at 40 000 g for 45 min at 4 °C. The supernatant was stored at −20 °C (cytosol fraction). The pellet (membrane fraction) was washed and re-suspended in buffer containing Tris–HCl 500 mmol/l (pH 7.4), EGTA 10 mmol/l, PMSF (phenyl-methyl-sulfonyl-fluoride) 100 mmol/l and soybean trypsin inhibitor 1 mg/ml, and sonicated for 5 s with the Ultrasonic processor UP50H (Hiescher Ultrasonics, GMbH, Teltow, Germany). The total concentration of protein was determined by the BCA™ protein assay kit (Pierce Biotechnology, Rockford, IL, USA). Samples were stored at −20 °C until used.

Each sample of membrane fraction (20 μg total protein) was subjected to SDS-PAGE on a 12% NuPage™ Bis-Tris gel (Invitrogen, Carlsbad, CA, USA) using XCell SureLock™ MiniCell (Invitrogen). Proteins were transferred to membranes (iBlot™, Ethrog Biotechnologies Ltd., Invitrogen), which were blocked with Non-Fat Dry Milk (Bio-Rad) in TBS 20 mmol/l and Tween-20 0.1% at 4 °C overnight. The membranes were incubated for 1 h with 1 μg/ml anti-human GPER antibody (AF5534, R&D systems Inc., Minneapolis, MN, USA). The antibody was raised in goat and affinity-chromatography purified. The immunogen was a 62 amino acid Escherichia coli-derived peptide that constitutes the extra-cellular N-terminal of human GPER. After washings in TBS-Tween, the membranes were further incubated for 2 h with a secondary antibody against goat IgG labeled with horseradish peroxidase (Dako, Glostrup, Denmark) diluted 1:2000, and washed again in TBS-Tween supplemented with sodium chloride 0.2 mol/l. Immune-complexes were detected by the ECL Plus Western Blotting System (GE Healthcare, Amersham, Little Chalfont, UK) and membranes were exposed to Hyperfilm™ ECL (GE Healthcare) for 10 min.

Immu-no-histochemistry of GPER

Sections of formalin-fixed, paraffin-embedded tissues were de-paraffinized with Xylene, re-hydrated with 99 and 96% alcohol and finally washed in distilled water. Sections were subsequently treated with Buffer Dako S 1699, pH 6, for 5 min under pressure at 121 °C for antigen retrieval. After cooling and washing, endogenous peroxidase was blocked by Dako S 2023 solution for 5 min. The immuno-staining process employed Autostainer Plus (Dako A/S, Glostrup, Denmark). Sections were first incubated with the same primary antibody as used in the western blot (AF5534) diluted 1:50 for 1 h, and subsequently with the biotinylized anti-goat secondary antibody for 25 min. A streptavidin-peroxidase complex was used for detection. Hematoxylin was used for counterstaining. Peroxidase activity was visualized by the Dako Real™ Detection System (K5001, Dako). Non-immune goat IgG (Dako) replaced the primary antibody as negative control.

Statistical methods

All data are presented as scatter plots with median and percentiles. Mann–Whitney and Kruskal–Wallis tests were used to evaluate the significance of differences between groups, and the Jonckheere’s trend test evaluated the significance of trends between groups. All tests were two-sided, and a 5% level of significance was used. Spearman’s test was used to assess the rank correlation between different mRNA assays.

Results

Quantification of mRNA for GPER, ERα and ERβ in endometrial tissue

Membrane fractions were analyzed for GPER mRNA with assay m1, which detects transcript variants 2 and 3 (Fig. 1A), and with assay s1, which detects transcript variants 2–4 (Fig. 1B). A lower number of samples were available for assay s1, since samples had to be pooled in order to obtain enough total RNA to allow removal of genomic DNA. Both assays demonstrated significantly higher GPER mRNA expression in the proliferative than in the secretory and menstrual phases. Decidual tissue samples had also low levels in both assays. The level of mRNA dropped rapidly in the early secretory phase and stayed low till the end of the cycle. In addition, the s1 assay showed a significant peak in the mid-proliferative phase, and significantly higher levels in secretory endometrial samples than in decidual samples (Fig. 1B). The differences in the expression pattern between the two assays suggest that transcript variant 4 is expressed in the endometrium both in the proliferative and secretory phases. Five out of thirteen proliferative phase samples were notably high, while the majority of proliferative samples and all secretory and menstrual phase samples were lower.


downloaded from https://academic.oup.com/molehr/article-abstract/16/10/743/1063550 by guest on 11 February 2019
For comparison, ERα and ERβ mRNA were quantified in the same set of samples. ERα mRNA had a similar cyclic pattern as GPER mRNA, but did not drop to low levels until the mid-secretory phase (Fig. 2A). ERβ mRNA did not vary significantly over the menstrual cycle, except for a peak in the late secretory phase (Fig. 2B).

**In situ hybridization for GPER mRNA**

GPER mRNA was localized in tissue sections from samples taken from the same set of endometrial samples as used for real-time PCR. Distinctive expression of the mRNA was localized in glandular epithelial cells in the mid- and late proliferative phase (Fig. 3). Altogether five proliferative samples, that expressed the highest amount of GPER mRNA in qPCR, showed a distinct glandular staining. The hybridization signal was evenly distributed within positive glands. In the remaining proliferative samples together with all secretory and menstrual phase samples, glandular mRNA expression could not be distinguished from the diffuse stromal signal that was present without variation throughout the cycle. Sections hybridized with the sense probe were negative.
Quantification of GPER mRNA in separated endometrial cells

To further verify our observation that epithelial cells are the main source of GPER mRNA, we separated proliferative endometrial tissues into glandular epithelial cells and stromal cells, and assayed GPER mRNA with real-time PCR. Epithelial cells contained more GPER mRNA than corresponding stromal cells (Fig. 4). More specifically, three out of eight epithelial samples expressed noticeably higher levels of GPER mRNA than the rest of the epithelial samples and all stromal cell samples.

Western blot

Membrane protein fractions, prepared from homogenates of endometrial tissue \( (n = 28) \) and decidual tissue \( (n = 7) \), were subject to western blotting using a purified goat anti-human GPER antibody. All endometrial and decidual tissue samples had a single band at 54 kDa (Fig. 5). Densitometric scanning of the 54 kDa band did not show significant variation over the menstrual cycle. Membrane proteins prepared from HEK-293 cells served as negative control, and had no detectable band. Placental tissue and MCF-7 breast cancer cell were used as positive controls.

Immuno-histochemistry

Tissue distribution of GPER protein was analyzed with immuno-histochemistry in 29 endometrial and 4 decidual tissue samples. GPER staining was localized in both glandular and luminal epithelial cells, as well as in stromal cells. GPER staining was more intense in the glands than in the stroma. Epithelial cells stained both in the apical and the basal part in proliferative glands, whereas mainly in the basal part in secretory glands. Stromal staining was predominantly detected in the functional layer of the endometrium. Staining intensity differed more between individual samples than between phases. Generally, staining was not seen in vessel walls or myometrium. Furthermore, staining was not increased in perivascular stroma. Particularly in the secretory phase, staining was not evenly distributed but rather regional within the tissue. Also in decidual tissue, GPER immuno-staining was present in both glandular and luminal epithelium, and in the stroma. Breast cancer tissue was used as positive control.

Figure 3  G protein-coupled estrogen receptor 1 (GPER) mRNA was detected in endometrial tissue sections hybridized with \(^{35}\)S-labelled antisense riboprobe. The panel shows bright field (A) and dark field (B) images of a mid proliferative phase endometrial tissue section. Glandular epithelial cells have distinct signal as compared with the surrounding stroma. Immuno-histochemical staining with the GPER antibody of endometrial tissue obtained in the early proliferative (D, E), mid secretory phases (F), as well as decidual tissue (G). As negative control in late proliferative sample non-immune goat IgG replaced the antibody IgG (C). Staining was generally more prominent in epithelial cells than in stromal cells, and it appeared stronger in proliferative than in secretory glands. Staining had often a regional distribution, particularly in the secretory phase. Myometrium and vessel walls were generally without staining. Stromal staining was more distinct in zona functionalis (D) than in zona basalis (E). Decidual tissue staining was distributed both in glandular and luminal epithelial cells, and also in stroma. GPER positive cells in the stroma had similar localization as cells expressing vimentin (H), whereas no HCG-positive cells were detected in consecutive sections (I). Scale bar for A, B = 100 \( \mu \)m; for C, D, F, G, H, I = 50 \( \mu \)m; and for E = 120 \( \mu \)m.
Discussion

Quantitative PCR detected GPER mRNA in all endometrial and decidual tissue samples. The levels were higher in proliferative endometrium and lower in secretory and menstrual endometrium. Lowest levels were, however, found in decidual tissue. The cyclical variation included a significant peak in the mid–late proliferative phase, as well as a quick drop to low levels already in the early secretory phase. High levels in the proliferative phase suggest up-regulation in response to estradiol. ERα or ERβ, functioning as activated transcription factors, may mediate this effect of estradiol. Alternatively, up-regulation may be mediated by EGF receptor signaling via the MAPK/ERK pathway. Both EGF and its receptor are up-regulated in the proliferative phase (Imai et al., 1995), and EGF receptor signaling has major estrogenic effects in uterine tissue (Nelson et al., 1991). Actually, Albanito and Vivacqua et al. reported up-regulation of GPER expression by EGF and the transforming growth factor (TGF)α in breast cancer cells (Albanito et al., 2008a, b; Vivacqua et al., 2009). In contrast, however, we have found that GPER mRNA was up-regulated by estradiol in only one, and by EGF in another out of seven ovarian cancer cell lines (Kolkova et al., unpublished work).

The drop of GPER mRNA in the early secretory phase suggests a prompt response to raising levels of progesterone. This can either result from down-regulated transcription or from decreased stability of the mRNA, although progesterone is known as a stabilizer of mRNA (Sandberg et al., 1997). Ahola et al. reported that GPER mRNA was up-regulated by progesterone in breast cancer cells (Ahola et al., 2002). The difference may partly relate to the fact that progesterone stimulates cell proliferation in the breast, but is anti-proliferative in the endometrium.

The expression profile of ERα mRNA was similar to that of GPER mRNA, but did not drop to low levels until the mid-secretory phase. Our data are in agreement with previously described cyclic variation of ERα in endometrial epithelial cells (Koji and Brenner, 1993; Matsuzaki et al., 1999; Witek et al., 2001). We found a positive correlation between the levels of GPER mRNA and ERα mRNA. A positive, but incomplete, association between ERα and GPER was also reported in an immuno-histochemical study of breast cancer (Filardo et al., 2006).

Our in situ hybridization study found a distinct GPER mRNA signal in the epithelium of five mid/late proliferative phase samples, and the same five samples, had prominent content of GPER mRNA in the qPCR assay. This indicates good agreement between the two methods. The other samples in the proliferative phase together with all samples in the secretory and menstrual phases had lower levels in the qPCR, and epithelial expression could not be distinguished from the diffuse stromal signal by in situ hybridization. Expression in the stroma was weak and appeared stable throughout the cycle.

The experiment with separated endometrial cells also showed a prominent expression in the fraction of proliferative epithelial cells. In contrast, neither in situ hybridization nor separated endometrial cells showed prominent expression in proliferative stromal cells.
These data taken together strongly suggest that the cyclic variation of GPER mRNA in the endometrium mainly results from increased epithelial expression in the mid/late proliferative phase, whereas stromal expression seems stable over the cycle. In this respect, GPER appears similar to ERα which also shows cyclic variation in the epithelial cells in the functional zone (Matsuzaki et al., 2000; Taylor et al., 2005).

This study of fertile endometria found stronger immuno-staining for GPER protein in the epithelium than in the stroma. A previous immuno-histochemical study of post-menopausal benign endometrium found GPER in the stroma only (Smith et al., 2007). Since our mRNA data suggest that epithelial expression is responsible for the cyclic variation and that increased expression is driven by estrogen, it is likely that absence of epithelial expression in their study results from lack of estrogen stimulation in post-menopausal women. Alternatively, the use of a different antibody may bring different results.

Endometrial content of GPER protein did not follow the cyclic variation of GPER mRNA, since protein levels did not drop in the secretory phase. The protein levels showed substantial individual variation, but no significant cyclic variation. Apparently, lower levels of mRNA are required to maintain GPER protein levels in the secretory phase. A similar relation between GPER mRNA and protein levels was also reported in a study of hamster ovary (Wang et al., 2007).

Recent data show that a significant amount of GPER is located intracellularly and slowly matures to the plasma membrane (Sandén and Leeb-Lundberg, unpublished observations). Furthermore, plasma membrane GPER is apparently subject to rapid and constitutive endocytosis. Since GPER protein levels are maintained with lower levels of GPER mRNA in the secretory phase, it is possible that either of these processes, i.e. internalization, recycling or maturation, is modified by progesterone to decrease turnover of the protein. Theoretically, a portion of the receptor pool could be differentially distributed between these membrane compartments in the proliferative as compared with the secretory phase. However, our techniques did not allow identification of such differences in intracellular localization of the GPER protein.

Like ERα GPER is widely distributed in the body (Owman et al., 1996), and these two receptors are furthermore co-expressed in many cell types. Numerous reports describe various interactions between these two receptors, and in certain cell types they are mutually dependent. Albanito et al. reported that GPER and ERα were both required, together with intact EGFR signaling, for estrogen-stimulated proliferation of ovarian cancer cells (Albanito et al., 2008a, b). However, other types they seem to antagonize each other. In ERα-positive cancer cell of breast, urothel and prostate origin, ERα stimulates whereas GPER antagonizes cell proliferation (Teng et al., 2008; Ariazi et al., 2010; Chan et al., 2010). Also, in pheochromocytoma cells ERα reportedly stimulates while GPER inhibits the dopamine transporter mechanism (Alyea et al., 2008). In these situations, the contrasting effects of these two estrogen receptors appear to balance each other. Such observations suggest an emerging concept of yet another regulatory level of the peripheral response to estrogen.

Szego et al. reported more than 40 years ago that estrogen stimulation caused an immediate increase of intracellular concentration of cAMP (Szego and Davis, 1967). Identification of GPER as a specific membrane bound estrogen receptor opened new possibilities to explain such immediate non-genomic membrane effects. In fact, stimulation of membranes, which express GPER, increases the release of cAMP (Thomas et al., 2005). Also, other second messengers like Ca2+, PI3K and NO increase their intracellular concentrations following activation of GPER (Revankar et al., 2005; Martensson et al., 2009). Finally, connective tissue growth factor is a down-stream mediator of GPER-induced migration in breast cancer cells (Pandey et al., 2009).

Our data on GPER together with the literature on ERα suggest that these two receptors are co-expressed in both epithelium and stroma in normal human endometrium, particularly in the proliferative phase. It is therefore likely that GPER plays a role in endometrial biology, possibly in collaboration with ERα. As a matter of fact, the specific GPER antagonist G-15 inhibits estrogen stimulated epithelial proliferation in mice, and such stimulation was further augmented by the specific GPER agonist G-1 (Dennis et al., 2009).

Little is known about possible interactions between GPER and ERβ. However, biochemical similarities between ERα and ERβ, as well as reports that ERβ is expressed in both epithelial and stromal cells of the endometrium, suggest that such interactions are possible.

Discovery of the new membrane bound estrogen receptor GPER may allow understanding of the previously reported non-genomic membrane effects of estrogen. Moreover, endometrial expression of GPER suggests a function in the reproductive process and may extend to new approaches in development of contraceptives, hormonal replacement therapies and even hormone targeted cancer treatment. The observation that tamoxifen treatment of patients with breast cancer in some cases results in endometrial proliferation and even malignant development (van Leeuwen et al., 1994) may actually find an alternative explanation in studies of endometrial GPER.

Authors’ roles

Z.K., performed most experiments and wrote the manuscript along with BC; while V.N. performed the tissue culture and A.E., was the pathologist. S.H. was the molecular biology advisor – and B.C., the mentor was in charge of planning.

Funding

Swedish Research Council, Swedish Cancer Fund, Crafoord and Nilsson Foundation, Skåne University Hospital and Region Skåne funds.

References


Matsuoka S, Uehara S, Murakami T, Fujikura J, Funato T, Okamura K. Quantitative analysis of estrogen receptor alpha and beta messenger ribonucleic acid levels in normal endometrium and ovarian...