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Hormonal regulation of $G\alpha_{i2}$ and mPRα in immortalized human oviductal cell line OE-E6/E7

Kati S. Mönkkönen[1,2], Reza Aflatoonian[2], Kai-Fai Lee[3], William S.B. Yeung[3], Sai-Wah Tsao[4], Jarmo T. Laitinen[5] and Alireza Fazeli[2,6]

[1]Department of Pharmacology and Toxicology, University of Kuopio, FIN-70211 Kuopio, Finland; [2]Academic Unit of Reproductive and Developmental Medicine, The University of Sheffield, Level 4, Jessop Wing, Tree Root Walk, Sheffield S10 2SF, UK; [3]Department of Obstetrics and Gynaecology, The University of Hong Kong, Pokfulam, Hong Kong; [4]Department of Anatomy, The University of Hong Kong, Pokfulam, Hong Kong; [5]Institute of Biomedicine, Department of Physiology, University of Kuopio, FIN-70211 Kuopio, Finland

[6]Correspondence address. Tel: +44-114-226-8195; Fax: +44-114-226-8538; E-mail: a.fazeli@sheffield.ac.uk

Heterotrimeric G proteins play a key role in membrane-mediated cell-signalling and hormonal regulation. Our earlier studies gave evidence of G protein subunit $G\alpha_{i2}$ being under hormonal regulation in human *in vivo*. In this study, we used immortalized human oviduct epithelial cell line OE-E6/E7 as a model to study the hormonal regulation of $G\alpha_{i2}$. We aimed at clarifying whether estradiol or progesterone could individually regulate the expression of $G\alpha_{i2}$ and its potential signalling partners. Furthermore, we aimed to investigate which sex hormone receptors could potentially mediate the gene regulation in OE-E6/E7 cell line. OE-E6/E7 cells were cultured for 5 days with different concentrations of estradiol or progesterone. Quantitative real-time polymerase chain reaction (Q-PCR) was performed using cDNA of the hormone-treated cells to reveal any changes in gene expression. The presence of potential receptor targets in these cells was studied using PCR. Our data clearly showed that low concentrations of estradiol up-regulated the expression of $G\alpha_{i2}$ gene and down-regulated the expression of membrane progesterone receptor mPRα gene in OE-E6/E7 cell line. Progesterone had no significant effect on $G\alpha_{i2}$ gene expression, but it caused up-regulation of mPRα gene expression. In conclusion, it appears that sex hormones regulate the expression of $G\alpha_{i2}$ and mPRα genes in a reverse manner in OE-E6/E7 cells. Our results suggest that estrogen receptor ERβ mediates the regulatory effects of estradiol in these cells.

Keywords: estradiol; fallopian tube; G protein; progesterone; regulation

Introduction

Fallopian tubes have a crucial role in early events of fertilization. They act as sperm reservoirs (Pacey *et al*., 1995; Baillie *et al*., 1997), enhancing motility and preserving viability of sperm (Murray and Smith, 1997). Furthermore, they play an important role in sperm capacitation (Kervancioglu *et al*., 1994, 2000). Epithelial cells on the luminal surface of Fallopian tubes are of special importance in fertility, as they are in direct cell-to-cell contact with gametes. Epithelial cells consist of two cell types, ciliated cells and non-ciliated secretory cells. Motility of the epithelial cilia is essential in gamete transport in association with tubal secretory flow and muscle contractility (Jansen, 1984). Epithelial secretory cells produce and secrete oviductins, oviduct-specific glycoproteins (Verhage *et al*., 1988; Wagh and Lippes, 1989; Arias *et al*., 1994), thus providing an optimal environment for fertilization and stimulating the early development of the embryo (Liu *et al*., 1995; Yeung *et al*., 1996).

Although the physiological roles of Fallopian tubes and the luminal epithelial cells are well established, knowledge about the signalling pathways involved remains limited. G protein-coupled receptors are the most widespread family of cell-surface receptors and their intracellular signalling partners, heterotrimeric G proteins, are recognized as crucial elements in various types of membrane-mediated cell-signalling. In reproductive endocrinology, G protein-coupled receptors have a regulatory role in gonadotrophin-releasing hormone (GnRH)-induced secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary gland (Tsutsumi *et al*., 1992; Chi *et al*., 1993). In gonads, G protein-coupled receptors regulate the synthesis and secretion of sex hormones by mediating gonadotrophin signalling (Loosfelt *et al*., 1989; McFarland *et al*., 1989; Minegishi *et al*., 1990, 1991; Sprengel *et al*., 1990). Heterotrimeric G proteins are classified into four families (G_s, G_i, G_q and G_{12}) (Hepler and Gilman, 1992). G_i family proteins are the most diverse and interact with a wide variety of G protein-coupled receptors, such as GnRH (Hawes *et al*., 1993; Stanislaus *et al*., 1998; Krsmanovic *et al*., 2001, 2003), FSH (Arey *et al*., 1997) and LH receptors (Herrlich *et al*., 1996). Recently, G_i family proteins have also been demonstrated to interact with cell-surface receptors for estrogen (Benten *et al*., 2001) and progesterone (Zhu *et al*., 2003b; Karteris *et al*., 2006), mediating the signal transduction of their rapid, non-genomic actions.

During the menstrual cycle, Fallopian tubes undergo cyclic changes. Ovarian steroid hormones estrogen and progesterone change the morphology of luminal epithelial cells, alter the intraluminal environment and promote tubal activity and functions (Verhage *et al*., 1979). Estrogen is responsible for epithelial cell differentiation, hypertrophy and secretory activity as well as ciliogenesis. Progesterone induces

atrophy and deciliation (Verhage *et al*., 1979; Donnez *et al*., 1985). Largely due to limited access to Fallopian tube cells, the information on specific signalling components involved in their hormonal regulation is insufficient.

Our recent study showed specific localization of G protein subunit $G\alpha_{i2}$ in Fallopian tube epithelial cells and the cilia. Further, our data gave evidence of $G\alpha_{i2}$ being under hormonal regulation in female reproductive tract *in vivo* (Mönkkönen *et al*., 2007a). In the present study, we used immortalized human oviductal epithelial cell line OE-E6/E7 as a valuable model of Fallopian tube epithelium to study hormonal regulation of selected signalling proteins. We aimed at clarifying whether estradiol or progesterone could individually regulate the expression of G protein subunit $G\alpha_{i2}$ and its potential signalling partners such as membrane progesterone receptor mPRα. Additionally, we aimed at identifying the potential nuclear receptors mediating hormonal regulation in these cells.

Materials and Methods

Cell culture

Immortalized Human Fallopian tube cell line (OE-E6/E7) (Lee *et al*., 2001) was cultured at +37°C in DMEM (F12) culture media (Invitrogen, Paisley, UK) supplemented with 1% penicillin and streptomycin (Sigma-Aldrich, Poole, UK), 10% fetal calf serum (Invitrogen) and L-glutamine (Invitrogen) in 5% CO_2 atmosphere.

In the case of hormone treatment studies, OE-E6/E7 cells were cultured in triplicates at +37°C in DMEM (F12) culture media without serum and phenol red (Invitrogen) and supplemented with 1% penicillin and streptomycin (Sigma-Aldrich). Water-soluble estradiol (Sigma-Aldrich) was used at 0.1, 1, 10 and 100 nM concentrations. Water-soluble progesterone (Sigma-Aldrich) was used at concentrations of 1, 10, 100 and 1000 nM. In both experiments, control media was supplemented with cyclodextrin (Sigma-Aldrich), at a concentration equivalent to that present in hormone media. The cells were split into hormone media and cultured until confluency (for 5 days).

RNA isolation and cDNA synthesis

For genomic studies, OE-E6/E7 cells were washed with Ca^{2+} and Mg^{2+} free phosphate buffered saline (PBS), harvested using trypsin-EDTA (Invitrogen) and pelleted by centrifugation at 300*g* for 5 min. One milliliter of TRIreagent (Sigma-Aldrich) was added onto pellet (5×10^6 cells). Endometrial and Fallopian tube tissues used for control purposes in the present study were removed from RNAlater and homogenized in 3 ml of TRIreagent (Sigma-Aldrich) using an Ultra-Turrax homogenizer for 2 min. Total RNA from the tissues and pelleted cells stored in TRIreagent was extracted following standard protocol supplied by the manufacturer. Total RNA was treated with DNase I (DNA-free™, Ambion) to remove genomic DNA contamination from the samples. First-strand cDNA synthesis was performed using oligo dT primers (Metabion, Martinsried, Germany) and reverse transcription by SuperScript II (200 U/μl, Invitrogen). Negative controls were prepared without the enzyme (non-reverse transcribed controls, RT controls).

Polymerase chain reaction

PCR was performed with the constructed cDNAs, Platinum Blue PCR Super Mix (Invitrogen), and primers from Metabion. The primer pairs, amplified sequences and annealing temperatures used are described in detail in Table I. The primer sequences without reference were newly designed for this study. The amplification was run for 40 cycles under the following conditions: 95° 30 s, annealing temperature for each primer 30 s, 72° 30 s. All experiments included RT controls as well as negative controls (no cDNA). cDNA from endometrial and Fallopian tube tissues (Mönkkönen *et al*., 2007a) were used as control. PCR products were separated on 1.2% agarose gel.

Quantitative real-time PCR

Quantitative real-time PCR (Q-PCR) was performed in triplicates with the constructed cDNAs and the same primers that were used in PCR reactions (Table I). SYBR Green Jump Start *Taq* Readymix (S 4438, Sigma-Aldrich) master mix [containing 10 μl SYBR Green, 7 μl of water, 1 μl of each primer (20 pmol) and 1 μl of cDNA] was added to each well of PCR plate and amplification was performed under the following conditions: 50 cycles (95° 30 s, annealing temperature of each primer used 30 s, 72° 30 s). All experiments included RT controls and negative controls (no cDNA).

Results were analyzed using iCycler (Biorad laboratories Ltd, Hemel Hempstead, UK). The threshold cycle values were normalized against threshold value of human β-actin. The results were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparison test. $P < 0.05$ was considered as statistically significant difference.

Western blot analysis

Immortalized Fallopian tube epithelial cells were washed with Ca^{2+} and Mg^{2+} free PBS, harvested using trypsin-EDTA (Invitrogen) and pelleted by centrifugation at 1000*g* for 5 min. The resulting pellet was snap frozen and stored −70°C. Pelleted cells were thawed by addition of ice-cold sonication buffer (1 mM EDTA, 20 mM Tris–HCl pH 7.5) containing 1% protease inhibitor cocktail (P8340, Sigma-Aldrich). The resulting cell suspension was lysed by sonication for 10 × 2 s on ice water bath. Protein content of the sonicates was measured using bicinchoninic acid protein assay by Pierce Biotechnology (Rockford, IL, USA).

Sonicates (50 μg protein/lane) were resolved in 10% SDS–PAGE and blotted onto PVDF membrane (Immobilon-P^{SQ} Transfer Membrane, 0.2 μM pore size, Millipore Corporation, Billerica, MA, USA). Non-specific binding was blocked by 5% non-fat dry milk in Tris-buffered saline containing 0.1% of Tween 20 (TTBS). Blots were incubated overnight in primary antibody [1:500 Mouse anti-$G\alpha_i$-2 monoclonal antibody, MAB3077 (Chemicon International, Temecula, CA, USA)] in 2.5% non-fat dry milk in TTBS at +4°C. After 4 × 10 min washes in TTBS, the blots were incubated in secondary antibody [1:2500 Goat anti-mouse IgG-HRP, A2554 (Sigma-Aldrich)] in 2.5%

Table I. Primer sequences used for the study.

Target	Product size (bp)	Forward 5′-3′	Reverse 5′-3′	Annealing temp (°C)	References
β-actin	643	TGA CCC AGA TCA TGT TTG AGA CC	GGA GGA GCA ATG ATC TTG ATC TTC	58	Mönkkönen *et al*. (2007a)
$G\alpha_{i2}$	212	CTT GTC TGA GAT GCT GGT AAT GG	CTC CCT GTA AAC ATT TGG ACT TG	65	Mönkkönen *et al*. (2007a)
ERα	288	GAA TCT GCC AAG GAG ACT CG	ATC TCT CTG GCG CTT GTG TT	64	—
ERβ	217	CCA GCA ATG TCA CTA ACT TGG A	TTC CCA CTA ACC TTC CTT TTC A	57	—
PR-A,B	221	GGA GAA CTC CCC GAG TTA GG	AGG GAG GAG AAA GTG GGT GT	61	—
PR-B	232	GAC TGA GCT GAA GGC AAA GG	CTG CTG GTC CTG CGT CTT TT	61	—
mPRα	200	GCG GCC CTG GTA CTG CTG C	CAC GGC CAC CCC CAC A	65	Karteris *et al*. (2006)
mPRβ	289	GCT GTT CAC TCA CAT CCC	TGG TGC AAC CCC CAG A	65	Karteris *et al*. (2006)

non-fat dry milk in TTBS for 1 h at 20°C. Blots were washed 4 × 10 min with TTBS and the chemiluminescent reaction was started with Western Lightning ECL substrate (Perkin-Elmer). The signal was detected on Hyperfilm ECL (Cat. no. RPN3103K, GE Healthcare UK Ltd, Buckinghamshire, UK). The films were scanned using ImageScanner (GE Healthcare UK Ltd).

Experimental design

PCR was used to study the expression of different target genes in OE-E6/E7 cell line. In order to clarify the effects of sex hormones on gene expression, OE-E6/E7 cell line was first cultured with different hormone concentrations for 5 days. Q-PCR was then performed using cDNA of the hormone-treated cells.

Hormone concentrations used in the present study were selected based on the reference serum levels for human females. For estradiol, normal physiological serum levels span from 0.1–1 nmol/l (during the menstrual cycle) to 10–100 nmol/l (during pregnancy). For progesterone, physiological serum concentrations during menstrual cycle vary from 1–10 nmol/l (follicular phase and at ovulation) to 10–100 nmol/l (luteal phase). Additionally, toward the end of pregnancy and parturition, up to 1000 nmol/l, progesterone concentrations are relevant, and were thus included in this study.

Results

PCR revealed the expression of $G\alpha_{i2}$ gene and Western blot the presence of the $G\alpha_{i2}$ protein in an immortalized human oviductal cell line

Our PCR studies confirmed that $G\alpha_{i2}$ gene is expressed in the immortalized human oviductal cell line OE-E6/E7 (Fig. 1A). Control experiments with non-reverse transcribed RNA of each sample confirmed that there was no contamination of human DNA in the samples. To verify the presence of $G\alpha_{i2}$ protein in OE-E6/E7 cells, Western blot analysis was carried out. Our results confirmed that $G\alpha_{i2}$ is present, although at a relatively low level, in these cells (Fig. 1B).

Hormone treatment revealed up-regulation of $G\alpha_{i2}$ gene by estradiol

In order to evaluate the effects of steroid hormones on expression of $G\alpha_{i2}$ gene, a hormone treatment experiment was carried out using OE-E6/E7 cell line. The cells were treated for 5 days with four concentrations of estradiol (0.1, 1, 10 and 100 nM) and progesterone (1, 10, 100 and 1000 nM), spanning the physiological concentration range of these hormones in human *in vivo*. Q-PCR revealed that $G\alpha_{i2}$ gene was up-regulated by low concentrations of estradiol (Fig. 2A). Cells treated with progesterone did not show any statistically significant changes in $G\alpha_{i2}$ expression (Fig. 2B).

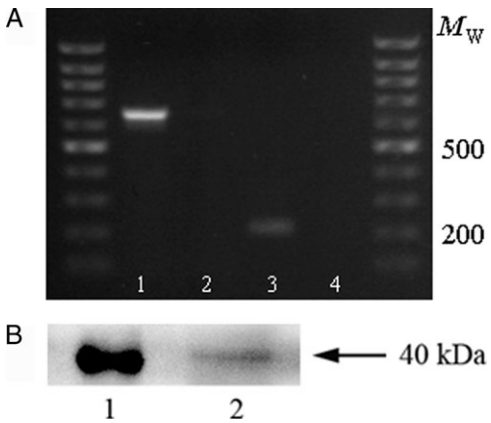

Figure 1: Expression of $G\alpha_{i2}$ gene and presence of $G\alpha_{i2}$ protein was confirmed in OE-E6/E7 cell line
(A) PCR showed $G\alpha_{i2}$ expression in an immortalized Fallopian tube epithelial cell line (OE-E6/E7). PCR products were separated on 1.2% agarose gel. 1, β-actin (643 bp); 2, β actin RT control; 3, $G\alpha_{i2}$ (212 bp); 4, $G\alpha_{i2}$ RT control, M_W, molecular weight (bp). (B) Western blot analysis confirmed the presence of $G\alpha_{i2}$ protein in immortalized Fallopian tube epithelial cell line (OE-E6/E7). 1, G protein standard, (2 μl / lane) bovine brain immunoblot standard (Calbiochem); 2, sonicate of Fallopian tube epithelial cells (50 μg/lane)

The potential nuclear target receptors for sex hormones were determined by PCR

In an attempt to clarify the potential receptor targets for sex hormones in OE-E6/E7 cells, we carried out PCR analysis using primers targeted against various sex hormone receptors (Table I). Our results show that in OE-E6/E7 cell line, only one nuclear receptor target for estrogen is present. Our data showed that ERβ receptor is expressed in these cells (Fig. 3B). No ERα receptor expression was detected (Fig. 3A). To verify this finding, the functionality of ERα primer used for PCR was also tested using cDNA of human Fallopian tube tissue and endometrium, and in both of these tissues ERα was expressed (Fig. 3A).

For progesterone, several receptor targets are expressed in OE-E6/E7 cell line (Fig. 4). Our studies showed that the novel membrane receptors for progesterone, namely mPRα and mPRβ, were both found to be expressed in OE-E6/E7 cell line. However, the expression level of mPRβ was very low. Additionally, nuclear PR-B receptor subtype was found to be expressed in these cells, as well as a PCR product common to PR-A and PR-B subtypes. The expression level of both PR-A,B and PR-B was very similar. For each of the receptors studied, control experiments with non-reverse transcribed RNA of each sample confirmed that there was no contamination of human DNA in the samples.

The novel, G protein-coupled membrane receptor mPRα was down-regulated by estradiol

Since we found estradiol to up-regulate $G\alpha_{i2}$, we aimed at investigating the potential effects of sex hormones on the novel, G protein-coupled membrane receptors for progesterone, which could represent

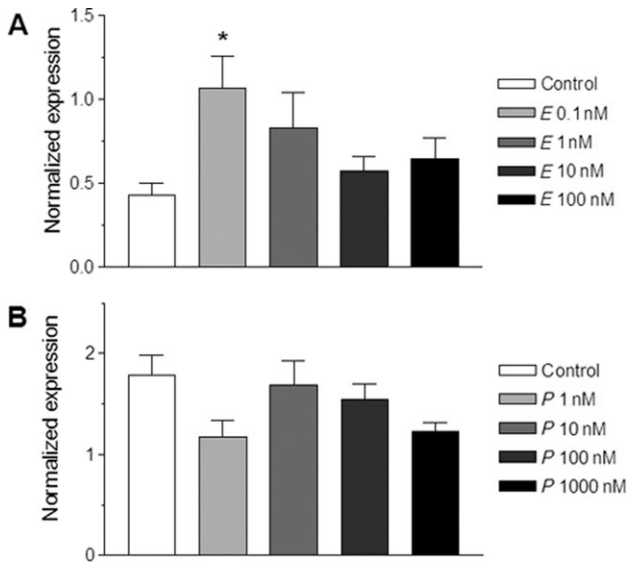

Figure 2: Q-PCR showed regulatory effects of estradiol and progesterone on expression of $G\alpha_{i2}$ gene
Immortalized Fallopian tube epithelial cell line (OE-E6/E7) was treated in triplicates with either estradiol (A) or progesterone (B) using concentrations of 0.1, 1, 10 and 100 nM or 1, 10, 100 and 1000 nM, respectively. The figure illustrates mean ± SEM of normalized $G\alpha_{i2}$ gene expression (n=9). Expression of $G\alpha_{i2}$ gene after estrogen treatment at 0.1 nM is significantly higher than after control treatment. *$P < 0.05$; One-way ANOVA with Tukey's multiple comparison test

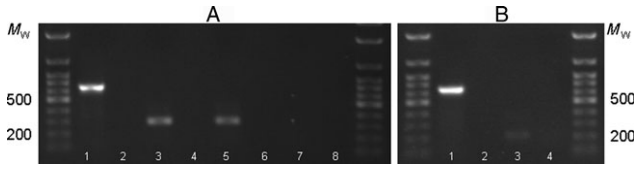

Figure 3: Expression of estrogen receptors was studied in an immortalized Fallopian tube epithelial cell line (OE-E6/E7)
For control purposes, we also used human Fallopian tube tissue and human endometrium tissue cDNA, since no ERα expression was seen in OE-E6/E7 cell line. ERα (A) 1, β-actin (643 bp) in OE-E6/E7 cell line; 2, RT control for β-actin in OE-E6/E7 cell line; 3, ERα (288 bp) in human Fallopian tube tissue; 4, RT control for ERα in human Fallopian tube; 5, ERα (288 bp) in human endometrium; 6, RT control for ERα in human endometrium; 7, ERα in OE-E6/E7 cell line; 8, RT control for ERα in OE-E6/E7 cell line. ERβ in OE-E6/E7 cell line (B), 1, β-actin (643 bp); 2, β-actin RT control; 3, ERβ (217 bp); 4, ERβ RT control. M_W, molecular weight (bp). PCR products were separated on 1.2% agarose gel

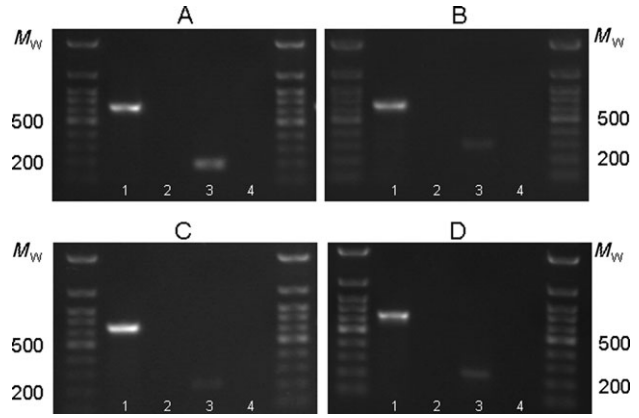

Figure 4: PCR showed expression of different progesterone receptors in an immortalized Fallopian tube epithelial cell line (OE-E6/E7)
mPRα (A), 1, β-actin (643 bp); 2, β-actin RT control; 3, mPRα (200 bp); 4, mPRα RT control. mPRβ (B), 1, β-actin (643 bp); 2, β-actin RT control; 3, mPRβ (289 bp); 4, mPRβ RT control. PR-A,B (C), 1, β-actin (643 bp); 2, β-actin RT control; 3, PR-A,B (221 bp); 4, PR-A,B RT control. PR-B (D), 1, β-actin (643 bp); 2, β-actin RT control; 3, PR-B (232 bp); 4, PR-B RT control. M_W: molecular weight (bp). PCR products were separated on 1.2% agarose gel

potential signalling partners for $G\alpha_{i2}$ in human reproductive tissues. We carried out Q-PCR on hormone-treated OE-E6/E7 cell line cDNA. Our data showed that mPRα expression is down-regulated at estradiol concentration of 1 nM whereas other concentrations show no changes compared with control (Fig. 5A). Down-regulation of mPRα roughly coincided with $G\alpha_{i2}$ up-regulation observed in this study. We also studied the effects of progesterone on mPRα gene expression and found that mPRα expression is up-regulated by 100 nM progesterone treatment (Fig. 5B). Owing to relatively low expression level of the other sex hormone receptors present in OE-E6/E7 cell line, we were unable to investigate the regulation of these genes following estradiol or progesterone treatment.

Discussion

The present study demonstrates that low concentrations of estradiol up-regulate the expression of $G\alpha_{i2}$ gene in immortalized human oviductal epithelial cell line OE-E6/E7, whereas progesterone has no significant effect. In contrast, our data show membrane progesterone receptor mPRα gene to be down-regulated by 1 nM estradiol and up-regulated by 100 nM progesterone. It seems sex hormones regulate the expression of $G\alpha_{i2}$ and mPRα genes in a reverse manner in

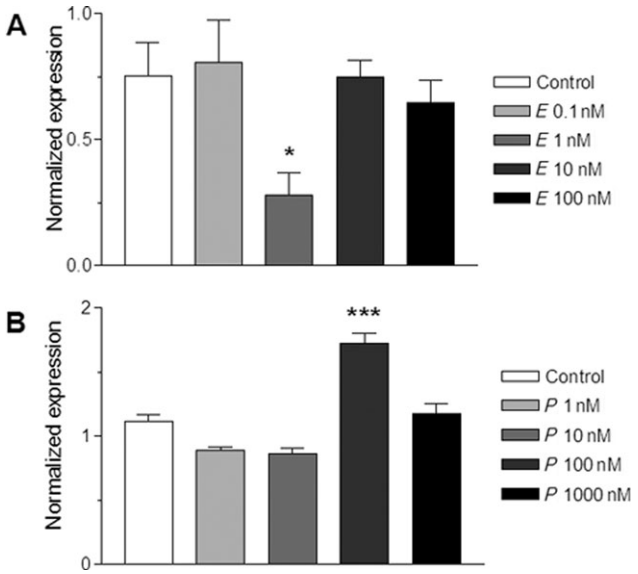

Figure 5: Q-PCR showed regulatory effects of estradiol and progesterone on expression of mPRα gene
Immortalized Fallopian tube epithelial cell line (OE-E6/E7) was treated in triplicates with either estradiol (A) or progesterone (B) using concentrations of 0.1, 1, 10 and 100 nM or 1, 10, 100 and 1000 nM, respectively. The figure illustrates mean ± SEM of normalized mPRα gene expression ($n = 9$). Expression of mPRα gene after 1 nM estradiol treatment is significantly lower and after 100 nM progesterone treatment significantly higher than after control treatments. $^*P < 0.05$; $^{***}P < 0.001$; One-way ANOVA with Tukey's multiple comparison test

OE-E6/E7 cells. Our results suggest that estrogen receptor ERβ mediates regulatory effects of estradiol in these cells.

Earlier, the presence and role of $G\alpha_{i2}$ in myometrium has been intensively studied (Milligan *et al*., 1989; Tanfin *et al*., 1991; Europe-Finner *et al*., 1993; Cohen-Tannoudji *et al*., 1995), and the role of $G\alpha_{i2}$ in the maintenance of myometrium relaxation during pregnancy has been established (Mhaouty *et al*., 1995). Apart from our recent study proposing hormonal regulation for $G\alpha_{i2}$ in human endometrium *in vivo* (Mönkkönen *et al*., 2007a), there are few reports indicative of the presence of G_i family proteins in human endometrium (Bernardini *et al*., 1995, 1999). Prior to the present study, however, the individual roles of estradiol and progesterone in regulation of $G\alpha_{i2}$ in female reproductive tract have remained unexplored. Since our previous study (Mönkkönen *et al*., 2007a) showed the increase in $G\alpha_{i2}$ gene expression under the changing physiological hormone combinations during the menstrual cycle, the present study aimed to clarify the potential individual roles of estradiol and progesterone in regulation of selected signalling components such as $G\alpha_{i2}$. Future studies should be targeted toward understanding the synergistic or inhibitory effect of different combinations of sex hormones on expression and function of $G\alpha_{i2}$.

Previous studies on rat myometrium have demonstrated that estradiol administration increases the levels of both $G\alpha_{i2}$ protein and $G\alpha_{i2}$ mRNA during pregnancy (Cohen-Tannoudji *et al*., 1995). These findings are in line with our present data on estradiol-induced up-regulation of $G\alpha_{i2}$ gene in immortalized human oviductal epithelial cell line. Regarding our recent studies showing significant increase in $G\alpha_{i2}$ gene expression in human endometrium during the menstrual cycle (Mönkkönen *et al*., 2007a), it seems likely that the increase observed then might be resulting from concentration peak of estrogen at late proliferative phase. It would be reasonable to confirm our present results of $G\alpha_{i2}$ gene up-regulation by studying the levels of $G\alpha_{i2}$ protein in OE-E6/E7 cells before and after hormone treatment.

However, since both the mRNA and protein levels of $G\alpha_{i2}$ are relatively modest in OE-E6/E7 cells, it is probable that immunoblotting would not be sensitive enough to detect the differences. Previously, progesterone treatment has been reported to have no effect on $G\alpha_{i2}$ expression in rat myometrium during pregnancy (Cohen-Tannoudji *et al*., 1995). This is in line with our data, since in the present study, progesterone treatment did not cause any significant differences in $G\alpha_{i2}$ expression.

Nuclear receptor for estrogen, ERα (Green *et al*., 1986), was long considered as the only estrogen receptor and thus responsible for all estrogen actions. Since the discovery of the second nuclear estrogen receptor ERβ (Kuiper *et al*., 1996; Mosselman *et al*., 1996), it has become evident that ERα and ERβ not only have separate genes to encode them, but also distinct functions and transcriptional activity (Byers *et al*., 1997; Weihua *et al*., 2000; Critchley *et al*., 2001). In the rat oviduct epithelium, only ERα subtype is expressed (Fitzpatrick *et al*., 1999; Sar and Welsch, 1999; Pelletier *et al*., 2000), whereas immunolocalization studies in human have demonstrated both estrogen receptors ERα and ERβ to be present in Fallopian tube epithelial cells (Taylor and Al-Azzawi, 2000). Our present data indicate that ERβ is the only nuclear receptor for estrogen expressed in OE-E6/E7 cell line. In our previous studies, we also noted limited ERα gene expression, but were unable to detect ERα protein presence (Lee *et al*., 2001). Probably ERα transcript has been lost during the passage of these cells, since presently only ERβ is expressed in OE-E6/E7 cell line. This finding suggests that ERβ is likely to mediate the hormonal regulation of $G\alpha_{i2}$ and mPRα, which was observed in our study. However, given the complexity of estrogen signalling and its modulation *in vivo*, direct conclusions regarding ERβ-mediated regulation of $G\alpha_{i2}$ and mPRα genes in human *in vivo* cannot be drawn based on our data. During the recent years, evaluation of estrogen signalling pathways has become more complicated than ever, as completely novel aspects on estrogen signalling have emerged. A number of recent reports have provided evidence of functional G protein-coupled receptors for estrogen (Benten *et al*., 2001; Qiu *et al*., 2003; Maggiolini *et al*., 2004; Revankar *et al*., 2005; Thomas *et al*., 2005; Vivacqua *et al*., 2006). Some of these report receptor localization on plasma membrane (Thomas *et al*., 2005) and some, unexpectedly, in endoplasmic reticulum (Revankar *et al*., 2005). Clearly, more studies are needed to clarify the role of these novel classes of estrogen receptors in reproductive physiology.

In this study, we investigated which of the known progesterone receptors are expressed in OE-E6/E7 cell line and found that both membrane receptors mPRα and mPRβ are expressed, although the expression level of mPRβ was relatively low. Additionally, we detected expression for nuclear progesterone receptors PR-A,B and PR-B, at similar expression level for both. Both PR-A and PCR-B are products of a single gene, and the only difference between them is the additional 165 amino acids present solely in the N-terminus of PR-B receptor. Since the expression level of PR-B was similar to that obtained with the primer pair recognizing both PR-A and PR-B receptor subtypes, we concluded that PR-B is the main nuclear PR receptor in these cells, and thus, might be responsible for the progesterone-induced genomic functions, namely mPRα up-regulation, observed in this study.

To date, membrane progesterone receptors have been characterized in various species, including human (Zhu *et al*., 2003a,b). A recent study established the presence and functionality of mPRα and mPRβ in human myometrium and used human myometrial cells to study their hormonal regulation. Both mPRα and mPRβ were reported to be up-regulated with 100 nM estradiol, while mPRα was also up-regulated with 100 nM progesterone and estradiol-progesterone combination (Karteris *et al*., 2006). Regarding our present results in the light of these previous findings, our data on 100 nM progesterone-induced mPRα up-regulation are in line with Karteris *et al*. results. However, with estradiol we observed down-regulation of mPRα at 1 nM, and no effect on mPRα expression at 100 nM, the concentration at which Karteris *et al*. reported up-regulation. Potential factors behind these differences could be either the different cell types used and thus variable repertoire of transcriptional factors or, alternatively, differences in estradiol concentrations and treatment times. Namely, Karteris *et al*. used acute, 4–16 h treatments, whereas our cell line was cultured with hormones for 5 days. In the present study, we investigated only hormonal regulation of mPRα, since the expression level of mPRβ in OE-E6/E7 cell line was too low for delicate quantitative genomic analyses.

Earlier studies have shown that G_i family proteins have a role in the signal transduction of rapid, non-genomic actions of estrogen (Zhu *et al*., 2003b) and progesterone (Karteris *et al*., 2006). Given the established relationship between G_i family proteins and the G protein-coupled membrane receptors for progesterone, it is relevant to assume that $G\alpha_{i2}$ and progesterone receptor mPRα might have a common signalling pathway in Fallopian tube epithelial cells. However, establishing the direct signalling relationship between the two would require future experimentation. Progesterone is known to decrease ciliary motility in Fallopian tube (Mahmood *et al*., 1998), and thus have a regulatory role in gamete transport. Recent studies have shown the specific localization of nuclear progesterone receptors (Teilmann *et al*., 2006) and the novel mPRγ receptors (Nutu *et al*., 2007) in oviductal luminal epithelial cells and the ciliary membrane in rat and human, and proposed a role for those receptors in ciliary beat regulation and gamete transport. Earlier, a rapid, non-genomic signalling pathway for progesterone has been proposed in regulation of ciliary motility (Wessel *et al*., 2004).

Immortalized human oviductal cell line OE-E6/E7 used in our study has been shown to retain morphological features characteristic of secretory epithelial cells (Ling *et al*., 2005). Although many signalling events in Fallopian tube epithelial cells are likely to require contribution of secretory cells, lack of ciliated cells in this cell line is naturally limiting the interpretation of the results. Since $G\alpha_{i2}$ is, in various tissues, localized specifically in cells which have motile cilia (Shinohara *et al*., 1998; Ostrowski *et al*., 2002; Mönkkönen *et al*., 2007a), it is likely to have a general, cilia-specific role. As a first proof of this, we recently showed $G\alpha_{i2}$ to control ciliary motility and cerebrospinal fluid homeostasis in rat brain (Mönkkönen *et al*., 2007b).

Regarding other potential roles for $G\alpha_{i2}$ in Fallopian tube epithelial cells, it is noteworthy that recently, a Toll-like receptor (TLR) signalling regulating, anti-inflammatory role has been proposed for $G\alpha_{i2}$ by an yet unknown mechanism (Fan *et al*., 2005). According to our previous studies, localization pattern of $G\alpha_{i2}$ in human reproductive tract (Mönkkönen *et al*., 2007a) is similar to that of several TLRs in female reproductive tract (Fazeli *et al*., 2005). Interestingly, various TLRs also seem to have hormonal regulation similar to $G\alpha_{i2}$ in human endometrium *in vivo* (Aflatoonian *et al*., 2007).

In conclusion, our study demonstrates for the first time the hormonal regulation of $G\alpha_{i2}$ expression in human oviductal epithelial cell line OE-E6/E7 by estradiol. Additionally, the present study provides valuable information on hormonal regulation of the novel, G protein-coupled membrane receptor for progesterone, mPRα, which was shown to be up-regulated by progesterone and down-regulated by estradiol in OE-E6/E7 cell line. Furthermore, our study suggested ERβ receptor to mediate the estradiol-induced regulation of $G\alpha_{i2}$ and mPRα expression.

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