Localization and variable expression of $G\alpha_{i2}$ in human endometrium and Fallopian tubes

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BACKGROUND: Heterotrimeric G proteins take part in membrane-mediated cell signalling and have a role in hormonal regulation. This study clarifies the expression and localization of the G protein subunit $G\alpha_{i2}$ in the human endometrium and Fallopian tube and changes in $G\alpha_{i2}$ expression in human endometrium during the menstrual cycle. METHODS: The expression of $G\alpha_{i2}$ was identified by Polymerase chain reaction (PCR), and localization confirmed by immunostaining. Cyclic changes in $G\alpha_{i2}$ expression during the menstrual cycle were evaluated by quantitative real-time PCR. RESULTS: We found $G\alpha_{i2}$ to be expressed in human endometrium, Fallopian tube tissue and in primary cultures of Fallopian tube epithelial cells. Our studies revealed enriched localization of $G\alpha_{i2}$ in Fallopian tube cilia and in endometrial glands. We showed that $G\alpha_{i2}$ expression in human endometrium changes significantly during the menstrual cycle, with a higher level in the secretory versus proliferative and menstrual phases ($P < 0.05$). CONCLUSIONS: $G\alpha_{i2}$ is specifically localized in human Fallopian tube epithelial cells, particularly in the cilia, and is likely to have a cilia-specific role in reproduction. Significantly variable expression of $G\alpha_{i2}$ during the menstrual cycle suggests $G\alpha_{i2}$ might be under hormonal regulation in the female reproductive tract *in vivo*.

Key words: cilia/endometrium/fallopian tube/G protein/menstrual cycle

Introduction

Among the cell-surface receptors, G protein-coupled receptors are the most widespread and diverse, playing an essential regulatory role in cell growth, hormonal regulation, sensory perception and neuronal activity (Hepler and Gilman, 1992). In reproduction, G protein-coupled receptors have a neuroendocrine regulatory role in GnRH-induced secretion of LH and FSH from the anterior pituitary gland (Tsutsumi *et al.*, 1992; Chi *et al.*, 1993). In gonads, G protein-coupled receptors mediate gonadotrophin signalling (Loosfelt *et al.*, 1989; McFarland *et al.*, 1989; Minegishi *et al.*, 1990, 1991; Sprengel *et al.*, 1990), thus regulating the synthesis and secretion of sex hormones.

G protein-coupled receptors communicate via heterotrimeric G proteins, which are recognized as crucial elements in various types of membrane-mediated cell-signalling. Heterotrimeric G proteins consist of α-, β- and γ-subunits. For the α-subunits, G proteins are divided into four classes (G_s, G_i, G_q and G_{12}) (Hepler and Gilman, 1992). Proteins of the G_i family are the most diverse and interact with a wide variety of G protein-coupled receptors. For example, they take part in hormonal regulation via interaction with 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). Moreover, the G_i family of proteins play a role in the signal transduction of rapid, nongenomic actions of estrogen (Benten *et al.*, 2001) and progesterone (Zhu *et al.*, 2003; Karteris *et al.*, 2006).

The dual balance between G_i and G_s signalling in the regulation of adenylyl cyclase has been well established. Proteins of G_i family can inhibit adenylyl cyclase and thus decrease intracellular cAMP concentration (Bokoch *et al.*, 1984; Katada *et al.*, 1984). Via this pathway, G_i-family protein $G\alpha_{i2}$ has been shown to take part in adrenergic signalling, controlling myometrium relaxation in the rat during pregnancy (Mhaouty *et al.*, 1995). In the human myometrium, the levels of $G\alpha_{i2}$ have been shown to decrease during pregnancy, suggesting that the consequent, altered balance between $G\alpha_{i2}$ and G_s could be responsible for maintaining the relaxation of uterus

during pregnancy (Europe-Finner *et al.*, 1993). Although the role of Gα_{i2} in myometrium has been thoroughly studied, the presence or the role of Gα_{i2} elsewhere in the human reproductive tract remains unclear.

Immunohistochemical studies in the rat have shown that Gα_{i2} is specifically localized in tissues having motile cilia with a characteristic 9+2 ultrastructure. Such a specific localization in rat oviductal, tracheal and brain ependymal cilia (Shinohara *et al.*, 1998) implies that Gα_{i2} may well serve a physiological function distinct from those of the other Gα subunits. It is probable that Gα_{i2} might play a cilia-specific physiological role. Interestingly, proteomic analysis has revealed Gα_{i2} as a resident axonemal protein of the human bronchial cilia (Ostrowski *et al.*, 2002). To date, however, there are no reports providing evidence of the localization of Gα_{i2} in any other human ciliated tissues such as Fallopian tubes. In this study, we identify the presence and localization of Gα_{i2} in tissues that are primarily in contact with gametes and provide the environment for fertilization, early development of the embryo as well as implantation, i.e. the human Fallopian tube and endometrium. We have also evaluated the potential changes in Gα_{i2} expression in the endometrium during the menstrual cycle to reveal any potential hormonal regulation of this G protein subunit in humans.

Materials and methods

Endometrial tissue collection and preparation for immunohistochemistry

The study was approved by the Local Ethics Committee and informed written consent was obtained prior to the collection of tissue samples. For immunohistochemical investigations, tissue samples were obtained from 6 fertile women, and for genomic studies, endometrial biopsies were obtained from 21 fertile women. All the women taking part in the investigation had regular cycles, showed no evidence of any pathological uterine disorder and had not used oral contraception or an intrauterine device during the previous 3 months. Biopsies were obtained in the operating theatre between 2 and 29 days after the last menstrual period (LMP). The mean age of the women taking part in the study was 35 (range 24–40) years and each had had at least one previous successful pregnancy.

Endometrial biopsies for immunohistochemistry were immediately snap-frozen and stored in liquid nitrogen until processed. Cryosections were cut at 5 μm and stored at −70°C until use. For genomic studies, endometrial biopsies were immediately placed in RNAlater (Ambion, Huntingdon, UK), followed by immersion in liquid nitrogen until processed.

Fallopian tube tissue collection and preparation for immunohistochemistry

Human Fallopian tube tissues were collected from nine patients undergoing total abdominal hysterectomy for benign gynaecological conditions. The mean age of the women taking part in the study was 42 (range 33–56) years.

Fallopian tube tissue samples for immunohistochemistry were immediately fixed in 10% formalin overnight and embedded in paraffin. Paraffin sections were cut at 5 μm. For genomic studies, Fallopian tube tissue samples were immediately placed in RNAlater (Ambion) and stored for 24 h at 4°C, followed by immersion and storage in liquid nitrogen until processed.

Cell culture

Fallopian tube tissue samples for primary culture of epithelial cells were obtained as follows: Fallopian tubes were placed in Hank's solution immediately after collection, cut open longitudinally and incubated 1 h with 0.25% collagenase (at 37°C, 95% O_2 and 5% CO_2). The cells were scraped gently using a sterile blade, washed with red blood cell lysing buffer (Sigma-Aldrich, Poole, UK) and then two to three times with Dulbecco's modified Eagle's medium (F12) (DMEM-F12, Invitrogen, Paisley, UK) culture media and plated into 75 ml flasks. Fallopian tube primary epithelial cells were cultured at 37°C in DMEM-F12 supplemented with 1% penicillin and streptomycin (Sigma-Aldrich), 10% fetal calf serum (Invitrogen) and 2 mM L-glutamine (Invitrogen) in 5% CO_2 atmosphere.

RNA isolation and cDNA synthesis

Tissues 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 (1–5 μg) was treated with Dnase I (DNA-free™, Ambion) to remove genomic DNA contamination from the samples. First strand complementary DNA (cDNA) synthesis was performed using oligo dT primers (Metabion, Martinsried, Germany) and RT by SuperScript II (200 U/μl, Invitrogen). RT controls were prepared without SuperScript II enzyme.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) was performed with the cDNAs, Platinum Blue PCR Super Mix (Invitrogen) and primers from Metabion. We used the following primer pairs: β-actin forward 5′-TGA CCC AGA TCA TGT TTG AGA CC-3′ and β-actin reverse 5′-GGA GGA GCA ATG ATC TTG ATC TTC-3′, Gα_{i2} forward 5′-CTT GTC TGA GAT GCT GGT AAT GG-3′ and Gα_{i2} reverse 5′-CTC CCT GTA AAC ATT TGG ACT TG-3′. The amplification was run for 35 cycles under the following conditions: 95°C 30 s, 58°C (β-Actin) or 65°C (Gα_{i2}) 30 s, 72°C 30 s. Amplified sequences were 643 and 212 base pairs for β-actin and Gα_{i2}, respectively. All experiments included RT controls as well as negative controls (no cDNA). PCR products were separated on 1.2% agarose gel.

Quantitative real-time PCR

Quantitative real-time PCR was performed with the cDNAs and the same primers that were used in PCR reactions. SYBR Green Jump Start Taq Readymix (S 4438, Sigma-Aldrich) master mix (containing 10 μl SYBR Green, 7 μl water, 1 μl of each primer (20 pmol) and 1 μl cDNA was added to each well of PCR plate and amplification was performed under the following conditions: 50 cycles (95° 30 s, 58° or 65° 30 s, 72° 30 s). All experiments included RT controls and negative controls (no cDNA) and were performed in triplicate.

Results were analysed using iCycler (Biorad laboratories Ltd., Hemel Hempstead, UK). To compare relative quantities of Gα_{i2} expression during the menstrual cycle, endometrial biopsies were divided into three groups: menstrual (LMP + 1–4; $n = 3$; LMP +1, +4 and +4), proliferative (LMP + 5–14; $n = 9$; early proliferative LMP +5, +5 and +7, mid-proliferative LMP +8, +9 and +10, late proliferative LMP +11, +12 and +13) and secretory (LMP + 15–29; n = 9; early secretory LMP +16, +16 and +17, mid-secretory LMP +20, +21 and +22, late secretory LMP +26, +28 and +29). Relative Gα_{i2} expression quantities were compared between these groups. The threshold cycle values were normalized against threshold value of human β-actin. The results were expressed as

mean $\pm$ SEM. Statistical analysis was performed by using one-way analysis of variance with Tukey's multiple comparison test. $P < 0.05$ was considered significant.

Immunohistochemistry

Cryosections of endometrium were thawed by immersion (15 min at 20 °C) into fixative containing 4% paraformaldehyde (Sigma-Aldrich) in 0.1 M phosphate-buffered saline (PBS), pH 7.4. The slides were then washed with PBS (2×5 min) and further fixed by immersion in -20°C methanol (4 min) followed immediately by treatment with -20°C acetone (2 min). After 2×5 min washes with PBS, endogenous peroxidase activity was blocked by 5% H_2O_2 (in distilled water) treatment (5 min). The slides were then washed with deionized water (2×5 min) and PBS (2×5 min). After this, the protocol follows the same blocking and staining as described subsequently for paraffin sections.

Fallopian tube paraffin sections were first dewaxed in xylene, rehydrated through a series of ethanols and finally washed with PBS. Endogenous peroxidase activity was quenched by a 20 min incubation with 3% H_2O_2 (v/v) in methanol. Antigen retrieval was performed by microwave irradiation in 10 mM citrate buffer, pH 6.0 (12 min). The slides were allowed to cool in the buffer and then washed with PBS (2×3 min).

Vectastain Elite ABC Kit (Vector Laboratories, Peterborough, UK) was used according to the manufacturers instructions for both cryosections and paraffin sections, with the following modifications. Slides were blocked in buffer containing 250 μl avidin D m/l (1 h room temperature). Mouse anti-$G_i\alpha$-2 monoclonal antibody, MAB3077 (Chemicon International, Temecula, CA, USA) was diluted (cryosections 1 : 1000, paraffin sections 1 : 500) in Dako antibody diluent (Dako UK Ltd., Cambridgeshire, UK) containing 250 μl biotin m/l and incubated overnight at 4°C. Primary antibody was omitted in negative controls. The slides were washed with PBS (5 min) and incubated with secondary antibody [1 : 200 biotinylated anti-mouse (Vector Laboratories)] for 30 min at 20°C. The slides were washed as before and incubated for 30 min with Vectastain ABC reagent (Vector Laboratories). After washing, binding was visualized by incubation with substrate 3,3′-diaminobenzidine (DAB) or DAB-Ni for 8 min (Vector Laboratories). The slides were rinsed with tap water (5 min) and PBS (3 min) and counterstained by using 10% haematoxylin (10 min). Following thorough rinse in tap water, slides were dehydrated through a series of ethanols, cleared in xylene and coverslipped with DePex mounting medium (VWR International, Lutterworth, UK).

The endometrial biopsy specimens were timed using LMP and morphology according to Noyes criteria (Noyes, 1950) and divided into three groups: menstrual, proliferative and secretory. The slides were viewed using a $\times 40$ objective on an Olympus CKX41 microscope. Digital images were captured with a Nikon Coolpix 5400 camera and cut to same size and shape keeping their original scales in Adobe Photoshop (Adobe Systems, Mountain View, CA, USA).

Results

PCR reveals the expression of $G\alpha_{i2}$ gene in human reproductive tissues

We used human Fallopian tube tissue and human endometrial biopsies to study the expression of $G\alpha_{i2}$ by PCR. Our data revealed that $G\alpha_{i2}$ is expressed in human Fallopian tube and human endometrium (Figure 1A and B). Our studies also confirmed that $G\alpha_{i2}$ is expressed in primary cultures of Fallopian tube epithelial cells (Figure 1C). Control experiments with non-reverse transcribed RNA of each sample confirmed that there was no contamination of human DNA in the samples.

Immunohistochemistry shows specific localization of $G\alpha_{i2}$ protein in Fallopian tube cilia and enrichment in endometrial glands

Immunostaining on human Fallopian tube paraffin sections showed specific localization of $G\alpha_{i2}$ protein in Fallopian tube epithelial cells and the cilia (Figure 2C). Positive staining was also seen in the cytoplasm of epithelial cells, surrounding the nuclei. In endometrial tissue, $G\alpha_{i2}$ staining was enriched in endometrial glands, but was present also in stroma (Figure 2A and B).

Quantitative real-time PCR shows alterations in $G\alpha_{i2}$ gene expression during the menstrual cycle

We carried out quantitative real-time PCR experiment on endometrial biopsies spanning the menstrual cycle (Figure 3). Based on the phase of the menstrual cycle of each patient, the biopsies were designated in three groups, namely menstrual (LMP + 1–4), proliferative (LMP + 5–14) and secretory (LMP + 15–29).

Our results demonstrated that endometrial expression of $G\alpha_{i2}$ gene changed during the cycle. The expression of $G\alpha_{i2}$ reached its peak in secretory phase and this was significantly higher ($P < 0.05$) compared with that of the menstrual and proliferative phases.

Discussion

The present study demonstrates the existence and localization of $G\alpha_{i2}$ in human endometrium and Fallopian tube. Our data establish the specific localization of $G\alpha_{i2}$ in the Fallopian tube epithelial cells, particularly in the cilia of Fallopian tube
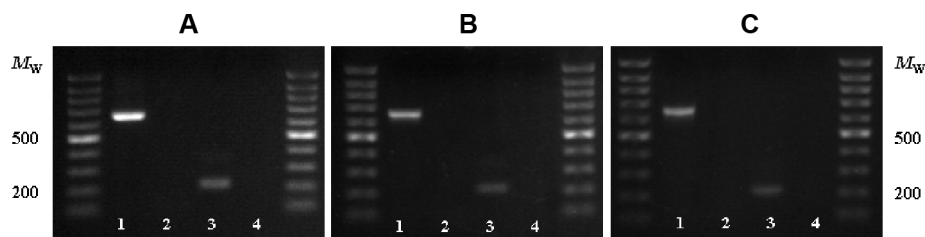
Figure 1. Polymerase chain reaction (PCR) showed $G\alpha_{i2}$ expression in Fallopian tube tissue (**A**), human endometrium tissue (**B**) and primary cultures of Fallopian tube epithelial cells (**C**). PCR products were separated on 1.2% agarose gel. 1, β-actin (643 base pairs); 2, β-actin RT control; 3, $G\alpha_{i2}$ (212 base pairs); 4, and $G\alpha_{i2}$ RT control: M_W, molecular weight (base pairs).
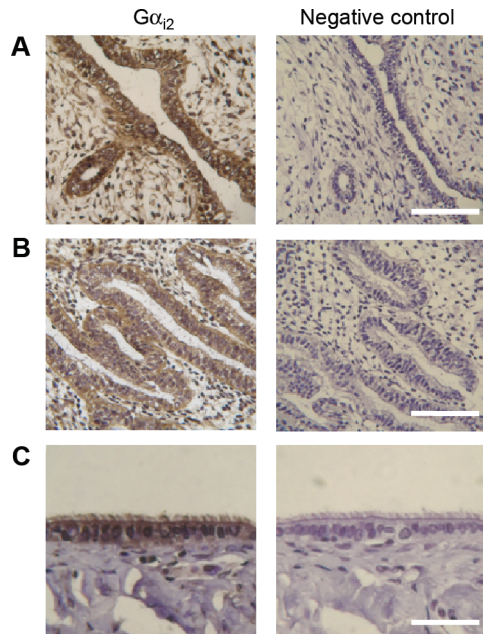
Figure 2. Immunostaining showing localization of $G\alpha_{i2}$ (brown) in human endometrial cryosections and Fallopian tube paraffin embedded sections. In the endometrium, $G\alpha_{i2}$ is enriched in endometrial glands of the proliferative phase (**A**) and secretory phase (**B**) Immunostaining of human Fallopian tube paraffin embedded sections (**C**) indicated specific localization of $G\alpha_{i2}$ in Fallopian tube epithelial cells and the cilia. Negative control slides were incubated with antibody diluent only. All the slides were counterstained with haematoxylin (blue). Scale bar: 100 µm (A, B), 40 µm (C).

epithelial cells. In human endometrium, we have demonstrated that localization of $G\alpha_{i2}$ is enriched in endometrial glands. We have also shown that $G\alpha_{i2}$ expression in human endometrium changes significantly during the menstrual cycle with maximum expression in the secretory phase, providing evidence that expression of this G_i subunit might be under hormonal regulation in the female reproductive tract *in vivo*.

The presence of G protein subunit $G\alpha_{i2}$ in rat myometrial membranes was first reported by Milligan *et al.* (1989), and the finding was later supported by a study suggesting differential regulation of $G\alpha_{i2}$ and $G\alpha_{i3}$ in rat myometrium during gestation (Tanfin *et al.*, 1991). In human myometrium, the levels of G protein subunits $G\alpha_{i1}$, $G\alpha_{i3}$, $G\alpha_q$ and $G\alpha_{11}$ have been shown to remain constant in pregnant and non-pregnant women, whereas levels of $G\alpha_{i2}$ decrease during pregnancy. The simultaneous, substantial increase in myometrial G_s suggested that the balance between $G\alpha_{i2}$ and G_s might be essential in regulating relaxation of the uterus during pregnancy (Europe-Finner *et al.*, 1993). Besides this, G_i family proteins have been suggested to be functionally linked to α_2 adrenergic signalling in human myometrium during pregnancy (Breuiller *et al.*, 1990). Later studies in the rat have confirmed the involvement of $G\alpha_{i2}$ and $G\alpha_{i3}$ in α_2/β_2 adrenergic signalling in the maintenance of uterus relaxation during rat pregnancy (Mhaouty *et al.*, 1995).

Unlike the thoroughly studied myometrium, the presence and role of $G\alpha_{i2}$ in other regions of the reproductive tract has remained largely obscure. Although the presence of G_i

family proteins has been described in human endometrium during artificial cycles of hormone replacement therapy, those studies rely solely on data from immunoblotting, using an antibody unable to discriminate between the closely related $G\alpha_{i1}$ and $G\alpha_{i2}$ (Bernardini *et al.*, 1995, 1999). Therefore, prior to our study, cyclical changes in $G\alpha_{i2}$ expression have not been reported in humans. Quantitative PCR showed that $G\alpha_{i2}$ expression in human endometrium *in vivo* significantly increased in the secretory phase of the menstrual cycle. This suggested that sex hormones, such as estrogen or progesterone, might regulate the expression of this G_i subunit in human endometrium. Furthermore, immunostaining clearly demonstrated the main localization of $G\alpha_{i2}$ in endometrial glands and partially in endometrial stroma. In the future, studies clarifying the presence of $G\alpha_{i2}$ in endometrial epithelial and stromal cells would be interesting to carry out in order to reveal whether $G\alpha_{i2}$ is expressed differently in these cells.

It is likely that $G\alpha_{i2}$ is hormonally regulated in the human endometrium. Earlier studies on rat myometrium have shown that estradiol administration during rat pregnancy increases the levels of both $G\alpha_{i2}$ protein and $G\alpha_{i2}$ mRNA, whereas progesterone has no effect on $G\alpha_{i2}$ expression. Instead, progesterone was reported to cause a decrease in $G\alpha_q$ subunit expression (Cohen-Tannoudji *et al.*, 1995). Other studies in pregnant rat myometrium have suggested a regulatory role for progesterone in control of β_2 receptors (Maltier *et al.*, 1989) and G_s proteins (Elwardy-Merezak *et al.*, 1994), as well as in up regulation of β_2 receptor expression (Vivat *et al.*, 1992). Apart from the studies by Bernardini *et al.* (1995, 1999), the potential role for sex hormones in regulation of G proteins in the human has remained largely unexplored.

In the present study, we have reported for the first time the localization of $G\alpha_{i2}$ in Fallopian tube epithelial cilia. In Fallopian tubes, ciliary beat is essential for gamete transport in association with the tubal secretory flow and muscle contractility. Furthermore, Fallopian tubes have been proposed to act as sperm reservoirs, where the ciliated epithelial cells interact with sperm (Pacey *et al.*, 1995a,b; Baillie *et al.*, 1997; Reeve *et al.*, 2003). Fallopian tube epithelial cells have also been demonstrated to preserve the viability of sperm (Kervancioglu *et al.*, 1994; Murray and Smith, 1997; Kervancioglu *et al.*, 2000).

The mean age of patients included in the present study for Fallopian tube tissue collection was higher in comparison to those included for collection of endometrial biopsies. Difference of the mean ages between the groups was 7 years. Although the age difference might involve reduced fertility in the older patients, the expression or localization of $G\alpha_{i2}$ was not significantly altered, as all the individual patients showed similar results in immunostaining.

Given the fact that $G\alpha_{i2}$ is specifically localized in rat tissue motile cilia with a characteristic 9+2 ultrastructure, namely in rat oviductal, tracheal and brain ependymal cilia (Shinohara *et al.*, 1998), it seems evident that this G_i subunit might have a cilia-specific physiological role. Apart from proteomic analysis providing evidence of $G\alpha_{i2}$ as a resident axonemal protein of the human bronchial cilia (Ostrowski *et al.*, 2002), there are no reports describing $G\alpha_{i2}$ in any other human ciliated tissue.
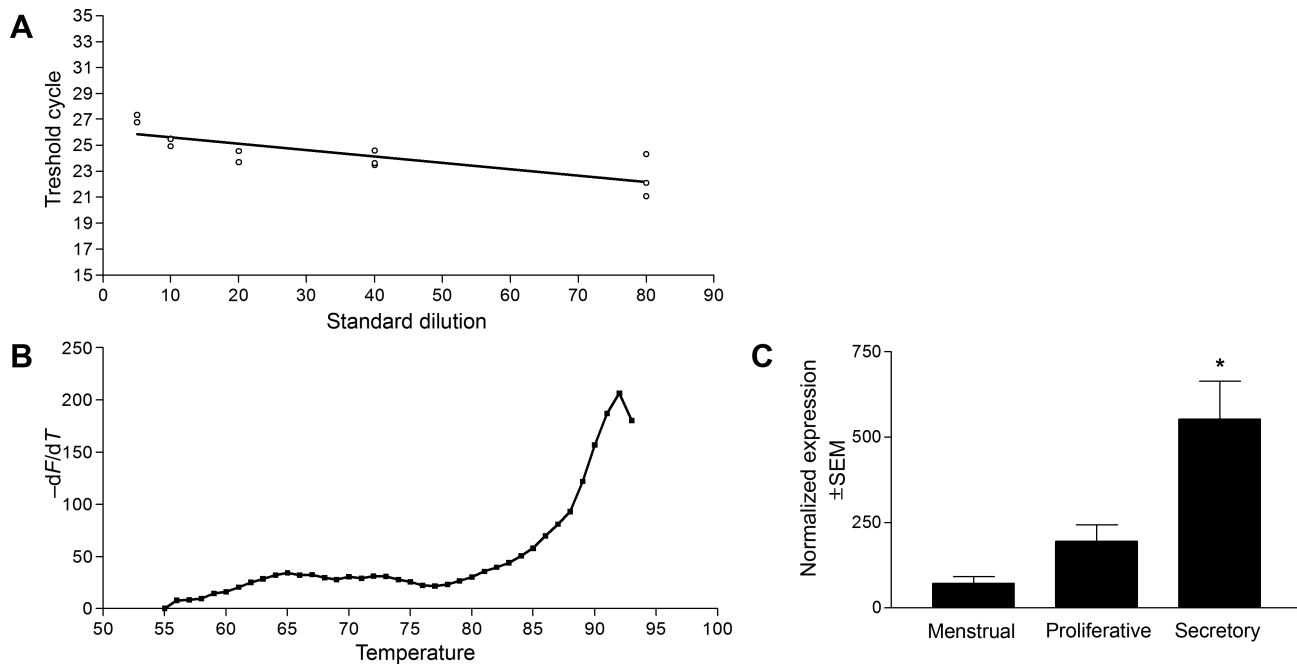
Figure 3. Quantitative real-time PCR uncovered variable expression of $G\alpha_{i2}$ gene in endometrium during the menstrual cycle. (**A**) Standard curve of the target gene. There was a significant negative correlation between the increasing dilution of standards and threshold cycle values ($r = -0.8127$, $P < 0.001$, $N = 3$ in each dilution). (**B**) Melting curve of the target gene PCR amplicon. There was only one product present in each reaction sample (dF/dT: ratio between the difference in fluorescence intensity and the difference in temperature). (**C**) Quantitative-real time PCR results during the menstrual cycle. Endometrial biopsies were assigned to three groups according to menstrual history of the patient (menstrual $n = 3$, proliferative and secretory $n = 9$). The figure illustrates mean $\pm$ SEM of normalized $G\alpha_{i2}$ gene expression. *Secretory phase was significantly different from the other phases, $P < 0.05$; One-way analysis of variance with Tukey's multiple comparison test.

In addition to positive immunostaining of Fallopian tube cilia, we have reported here positive immunostaining surrounding the nuclei. This presumably represents pre-stage $G\alpha_{i2}$, which is still undergoing synthesis, or alternatively, $G\alpha_{i2}$, which is ready for transport into cilia by intraflagellar transport mechanisms. This intracellular machinery is vital for assembly and maintenance of the cilia, as it transports essential particles, such as proteins synthesised in the cytoplasm of cell, into the cilia and returns the turnover products to the cytoplasm (Rosenbaum and Witman, 2002).

Studies with $G\alpha_{i2}$-knockout mice have established a crucial regulatory role for the $G\alpha_{i2}$ subunit in immunological processes (Rudolph *et al.*, 1995a,b; Jiang *et al.*, 1997; Dalwadi *et al.*, 2003; Fan *et al.*, 2005; Han *et al.*, 2005; Zhang *et al.*, 2005). $G\alpha_{i2}$ has been revealed to control regulation of T-cell proliferation (Zhang *et al.*, 2005) and B-cell development (Dalwadi *et al.*, 2003). Furthermore, $G\alpha_{i2}$ has been suggested to mediate chemokine signalling (Han *et al.*, 2005). However, reports of $G\alpha_{i2}$-knockout studies have not provided any information on potential involvement of this G_i subunit in modulation of mice fertility. Interestingly, a recent study on $G\alpha_{i2}$-knockout mice showed $G\alpha_{i2}$ to differentially regulate inflammatory mediator production in response to microbial stimuli and proposed a Toll-like receptor (TLR)-signalling regulating, anti-inflammatory role for $G\alpha_{i2}$ by an yet unknown mechanism (Fan *et al.*, 2005). Regarding the potential link between TLR-signalling and $G\alpha_{i2}$ in female reproductive tract, it is noteworthy that our previous studies showing the localization pattern of several TLRs (Fazeli *et al.*, 2005)

showed a pattern of localization similar to that we now report for $G\alpha_{i2}$. Future studies should be directed towards understanding whether $G\alpha_{i2}$ might share signalling pathways with TLRs and potentially have a TLR-signalling regulating role in human reproductive tract.

In conclusion, our studies reveal the presence of $G\alpha_{i2}$ in human endometrium and Fallopian tube epithelium, especially the cilia of fallopian tube epithelial cells. To the best of our knowledge, this is the first report of the localization of $G\alpha_{i2}$ in ciliated reproductive tissue in the human. We also report here, for the first time, the alterations in $G\alpha_{i2}$ expression during human menstrual cycle. Our data imply this G_i family subunit might be under hormonal regulation in the female reproductive tract *in vivo*. Further studies are required to clarify the physiological role of $G\alpha_{i2}$ in the female reproductive tract.

References

Arey BJ, Stevis PE, Deecher DC, Shen ES, Frail DE, Negro-Vilar A and Lopez FJ (1997) Induction of promiscuous G protein coupling of the follicle-stimulating hormone (FSH) receptor: a novel mechanism for transducing pleiotropic actions of FSH isoforms. Mol Endocrinol 11,517–526.

Baillie HS, Pacey AA, Warren MA, Scudamore IW and Barratt CL (1997) Greater numbers of human spermatozoa associate with endosalpingeal cells derived from the isthmus compared with those from the ampulla. Hum Reprod 12,1985–1992.

Benten WP, Stephan C, Lieberherr M and Wunderlich F (2001) Estradiol signaling via sequestrable surface receptors. Endocrinology 142,1669–1677.

Bernardini L, Moretti-Rojas I, Brush M, Rojas FJ and Balmaceda JP (1995) Status of hCG/LH receptor and G proteins in human endometrium during artificial cycles of hormone replacement therapy. J Soc Gynecol Investig 2,630–635.

Bernardini L, Moretti-Rojas I, Brush M, Rojas FJ and Balmaceda JP (1999) Changes in expression of adenyl cyclase activity in human endometrium during hormone replacement therapy and ovarian stimulation. Mol Hum Reprod 5,955–960.

Bokoch GM, Katada T, Northup JK, Ui M and Gilman AG (1984) Purification and properties of the inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. J Biol Chem 259,3560–3567.

Breuiller M, Rouot B, Litime MH, Leroy MJ and Ferre F (1990) Functional coupling of the alpha 2-adrenergic receptor-adenylate cyclase complex in the pregnant human myometrium. J Clin Endocrinol Metab 70,1299–1304.

Chi L, Zhou W, Prikhozhan A, Flanagan C, Davidson JS, Golembo M, Illing N, Millar RP and Sealfon SC (1993) Cloning and characterization of the human GnRH receptor. Mol Cell Endocrinol 91,R1–R6.

Cohen-Tannoudji J, Mhaouty S, Elwardy-Merezak J, Lecrivain JL, Robin MT, Legrand C and Maltier JP (1995) Regulation of myometrial Gi2, Gi3, and Gq expression during pregnancy. Effects of progesterone and estradiol. Biol Reprod 53,55–64.

Dalwadi H, Wei B, Schrage M, Spicher K, Su T T, Birnbaumer L, Rawlings DJ and Braun J (2003) B cell developmental requirement for the G alpha i2 gene. J Immunol 170,1707–1715.

Elwardy-Merezak J, Maltier JP, Cohen-Tannoudji J, Lecrivain JL, Vivat V and Legrand C. (1994) Pregnancy-related modifications of rat myometrial Gs proteins: ADP ribosylation, immunoreactivity and gene expression studies. J Mol Endocrinol 13,23–37.

Europe-Finner GN, Phaneuf S, Watson SP and Lopez Bernal A (1993) Identification and expression of G-proteins in human myometrium: up-regulation of G alpha s in pregnancy. Endocrinology 132,2484–2490.

Fan H, Zingarelli B, Peck OM, Teti G, Tempel GE, Halushka PV, Spicher K, Boulay G, Birnbaumer L and Cook JA (2005) Lipopolysaccharide- and gram-positive bacteria-induced cellular inflammatory responses: role of heterotrimeric Galpha(i) proteins. Am J Physiol Cell Physiol 289,C293–C301.

Fazeli A, Bruce C and Anumba DO (2005) Characterization of Toll-like receptors in the female reproductive tract in humans. Hum Reprod 20,1372–1378.

Han SB, Moratz C, Huang NN, Kelsall B, Cho H, Shi CS, Schwartz O and Kehrl JH (2005) Rgs1 and Gnai2 regulate the entrance of B lymphocytes into lymph nodes and B cell motility within lymph node follicles. Immunity 22,343–354.

Hawes BE, Barnes S and Conn PM (1993) Cholera toxin and pertussis toxin provoke differential effects on luteinizing hormone release, inositol phosphate production, and gonadotropin-releasing hormone (GnRH) receptor binding in the gonadotrope: evidence for multiple guanyl nucleotide binding proteins in GnRH action. Endocrinology 132,2124–2130.

Hepler JR and Gilman AG (1992) G proteins. Trends Biochem Sci 17, 383–387.

Herrlich A, Kuhn B, Grosse R, Schmid A, Schultz G and Gudermann T (1996) Involvement of Gs and Gi proteins in dual coupling of the luteinizing hormone receptor to adenylyl cyclase and phospholipase C. J Biol Chem 271,16764–16772.

Jiang M, Boulay G, Spicher K, Peyton MJ, Brabet P, Birnbaumer L and Rudolph U (1997) Inactivation of the G alpha i2 and G alpha o genes by homologous recombination. Receptor Channel 5,187–192.

Karteris E, Zervou S, Pang Y, Dong J, Hillhouse EW, Randeva HS and Thomas P (2006) Progesterone signaling in human myometrium through two novel membrane G protein-coupled receptors: potential role in functional progesterone withdrawal at term. Mol Endocrinol 20,1519–1534.

Katada T, Northup JK, Bokoch GM, Ui M and Gilman AG (1984) The inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. Subunit dissociation and guanine nucleotide-dependent hormonal inhibition. J Biol Chem 259,3578–3585.

Kervancioglu ME, Djahanbakhch O and Aitken RJ (1994) Epithelial cell coculture and the induction of sperm capacitation. Fertil Steril 61, 1103–1108.

Kervancioglu ME, Saridogan E, Aitken RJ and Djahanbakhch O (2000) Importance of sperm-to-epithelial cell contact for the capacitation of human spermatozoa in fallopian tube epithelial cell cocultures. Fertil Steril 74,780–784.

Krsmanovic LZ, Mores N, Navarro CE, Tomic M and Catt KJ (2001) Regulation of Ca2 + -sensitive adenylyl cyclase in gonadotropin-releasing hormone neurons. Mol Endocrinol 15,429–440.

Krsmanovic LZ, Mores N, Navarro CE, Arora KK and Catt KJ (2003) An agonist-induced switch in G protein coupling of the gonadotropin-releasing hormone receptor regulates pulsatile neuropeptide secretion. Proc Natl Acad Sci USA 100,2969–2974.

Loosfelt H, Misrahi M, Atger M, Salesse R, Vu Hai-Luu Thi MT, Jolivet A, Guiochon-Mantel A, Sar S, Jallal B, Garnier J, et al. (1989) Cloning and sequencing of porcine LH-hCG receptor cDNA: variants lacking transmembrane domain. Science 245,525–528.

Maltier JP, Benghan-Eyene Y and Legrand C (1989) Regulation of myometrial beta 2-adrenergic receptors by progesterone and estradiol-17 beta in late pregnant rats. Biol Reprod 40,531–540.

McFarland KC, Sprengel R, Phillips HS, Kohler M, Rosemblit N, Nikolics K, Segaloff DL and Seeburg PH (1989) Lutropin-choriogonadotropin receptor: an unusual member of the G protein-coupled receptor family. Science 245,494–9.

Mhaouty S, Cohen-Tannoudji J, Bouet-Alard R, Limon-Boulez I, Maltier JP and Legrand C (1995) Characteristics of the alpha 2/beta 2-adrenergic receptor-coupled adenylyl cyclase system in rat myometrium during pregnancy. J Biol Chem 270,11012–11016.

Milligan G, Tanfin Z, Goureau O, Unson C and Harbon S (1989) Identification of both Gi2 and a novel, immunologically distinct, form of Go in rat myometrial membranes. FEBS Lett 244,411–416.

Minegishi T, Nakamura K, Takakura Y, Miyamoto K, Hasegawa Y, Ibuki Y, Igarashi M and Minegish T (1990) Cloning and sequencing of human LH/hCG receptor cDNA. Biochem Biophys Res Commun 172,1049–1054.

Minegishi T, Nakamura K, Takakura Y, Ibuki Y, Igarashi M and Minegish T (1991) Cloning and sequencing of human FSH receptor cDNA. Biochem Biophys Res Commun 175,1125–1130.

Murray SC and Smith TT (1997) Sperm interaction with fallopian tube apical membrane enhances sperm motility and delays capacitation. Fertil Steril 68,351–357.

Noyes RW, Hertig AT and Rock J (1950) Dating the endometrial biopsy. Fertil Steril 1,3–825.

Ostrowski LE, Blackburn K, Radde KM, Moyer MB, Schlatzer DM, Moseley A and Boucher RC (2002) A proteomic analysis of human cilia: identification of novel components. Mol Cell Proteomics 1,451–465.

Pacey AA, Davies N, Warren MA, Barratt CL and Cooke ID (1995a) Hyperactivation may assist human spermatozoa to detach from intimate association with the endosalpinx. Hum Reprod 10,2603–2609.

Pacey AA, Hill CJ, Scudamore IW, Warren MA, Barratt CL and Cooke ID (1995b) The interaction in vitro of human spermatozoa with epithelial cells from the human uterine (fallopian) tube. Hum Reprod 10,360–366.

Reeve L, Ledger WL and Pacey AA (2003) Does the Arg-Gly-Asp (RGD) adhesion sequence play a role in mediating sperm interaction with the human endosalpinx? Hum Reprod 18,1461–1468.

Rosenbaum J.L. and Witman G. B. (2002) Intraflagellar transport. Nat Rev Mol Cell Biol 3,813–825.

Rudolph U, Finegold MJ, Rich SS, Harriman GR, Srinivasan Y, Brabet P, Boulay G, Bradley A and Birnbaumer L (1995a) Ulcerative colitis and adenocarcinoma of the colon in G alpha i2-deficient mice. Nat Genet 10,143–150.

Rudolph U, Finegold MJ, Rich SS, Harriman GR, Srinivasan Y, Brabet P, Bradley A and Birnbaumer L (1995b) Gi2 alpha protein deficiency: a model of inflammatory bowel disease. J Clin Immunol 15, 101S–105S.

Shinohara H, Asano T, Kato K, Kameshima T and Semba R (1998) Localization of a G protein Gi2 in the cilia of rat ependyma, oviduct and trachea. Eur J Neurosci 10,699–707.

Sprengel R, Braun T, Nikolics K, Segaloff DL and Seeburg PH (1990) The testicular receptor for follicle stimulating hormone: structure and functional expression of cloned cDNA. Mol Endocrinol 4,525–530.

Stanislaus D, Ponder S, Ji TH and Conn PM (1998) Gonadotropin-releasing hormone receptor couples to multiple G proteins in rat gonadotrophs and in GGH3 cells: evidence from palmitoylation and overexpression of G proteins. Biol Reprod 59,579–586.

Tanfin Z, Goureau O, Milligan G and Harbon S (1991) Characterization of G proteins in rat myometrium. A differential modulation of Gi2 alpha and Gi3 alpha during gestation. FEBS Lett 278,4–8.

Tsutsumi M, Zhou W, Millar RP, Mellon PL, Roberts JL, Flanagan CA, Dong K, Gillo B and Sealfon SC (1992) Cloning and functional expression of a mouse gonadotropin-releasing hormone receptor. Mol Endocrinol 6,1163–1169.

Vivat V, Cohen-Tannoudji J, Revelli JP, Muzzin P, Giacobino JP, Maltier JP and Legrand C (1992) Progesterone transcriptionally regulates the beta 2-adrenergic receptor gene in pregnant rat myometrium. J Biol Chem 267,7975–7978.

Zhang Y, Finegold MJ, Jin Y and Wu MX (2005) Accelerated transition from the double-positive to single-positive thymocytes in G alpha i2-deficient mice. Int Immunol 17,233–243.

Zhu Y, Rice CD, Pang Y, Pace M and Thomas P (2003) Cloning, expression, and characterization of a membrane progestin receptor and evidence it is an intermediary in meiotic maturation of fish oocytes. Proc Natl Acad Sci USA 100,2231–2236.