Lysophosphatidic acid and sphingosine 1-phosphate metabolic pathways and their receptors are differentially regulated during decidualization of human endometrial stromal cells

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ABSTRACT: In the luteal phase, human endometrial stromal cells (HESCs) undergo proliferation, migration and differentiation during the decidualization process under the control of the ovarian steroids progesterone and estrogen. Proper decidualization of stromal cells is required for blastocyst implantation and the development of pregnancy. The proliferation, migration and differentiation of HESCs in decidualization do not require the presence of a blastocyst but are greatly accelerated during implantation. Lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) are potent bioactive lysophospholipids that have critical roles in various physiological and pathophysiological processes, including inflammation, angiogenesis and cancer. The expression of the enzymes involved in LPA and S1P turnover and their receptors in HESCs during decidualization has not been characterized yet. We found that the LPAR1 and LPAR6 and S1PR3 receptors are highly expressed in HESCs. LPAR1, autotaxin (ATX), an LPA producing enzyme and lipid phosphate phosphatase 3 were up-regulated during decidualization. Interestingly, the expression of all S1P receptor subtypes and LPA receptors (LPAR2-6) mRNA was down-regulated after decidualization. We found that SPHK1 is highly expressed in HESCs, and is up-regulated during decidualization. S1P phosphatase SGPP1 and S1P lyase SGPL1 are highly expressed in HESCs. SGPP1 mRNA expression was significantly up-regulated in decidualized HESCs. In conclusion, this study shows the first time that specific LPA and S1P receptors and their metabolizing enzymes are highly regulated in HESCs during decidualization. Furthermore, we suggest that LPAR1 receptor-mediated signaling in HESCs may be crucial in decidualization process. SPHK1 activity and high turnover of S1P and LPA might be essential for precise regulation of their signaling during decidualization of human endometrium and implantation.

Key words: endometrium / decidualization / endometrial stromal cells / S1P / LPA

Introduction

Human endometrial stromal cells (HESCs) undergo proliferation and differentiation into decidual cells (decidualization) in the luteal phase of the menstrual cycle under the control of corpus luteum and this process is accelerated during implantation. Blastocyst implantation in the endometrium activates a complex of signaling pathways in decidualizing endometrial stromal cells of the mother and in invasive trophoblasts of the embryo (Cha et al., 2012). Various studies show that migration of decidualizing HESCs is essential for successful implantation, and facilitates the invasion of trophoblasts (Grewal et al., 2008; Gellersen et al., 2010). The molecular mechanism of activation of HESCs is poorly understood.

Lysophosphatidic acid (LPA), a potent bioactive lysophospholipid, acts as an autacoid and has a critical role in numerous physiological and...
pathophysiological processes, including cancer and pregnancy (Ye, 2008; Schober and Siess, 2012). In response to changes in the local tissue environment, LPA is synthesized and rapidly activates cells in the immediate vicinity (Tokumura, 1995). LPA is released from the endometrium of various animals, including sheep, cow and pig in early pregnancy. Thereby, endometrial epithelial cells were shown as a major source of LPA (Woclawek-Potocka et al., 2009a; See et al., 2012; Boruszewska et al., 2013). In the uterus, a higher expression of LPA producing enzymes including autotaxin (ATX) was shown compared with the trophectoderm (Liszewska et al., 2009). LPA binds and activates surface G protein-coupled receptors (GPCRs) of the EDG receptors family (LPAR1–3), and purinergic receptors family, including LPAR4 (GPR23), LPAR5 (GPR92) and LPAR6 (P2YS) (Chun et al., 2010; Schober and Siess, 2012). The role of LPA in pregnancy was first shown in LPAR1 knockout mice in which delayed implantation and defective embryo spacing was observed. Furthermore, exogenous prostaglandins rescued the defective implantation in these mice (Ye et al., 2005). LPA regulates the expression of factors that are involved in decidualization and vascularization of the mouse uterus (Beltrame et al., 2013).

In human, LPAR3 expression was increased during early and late secretory phase and down-regulated during mid-secretory phase in endometrial glandular epithelial and stromal cells (Guo et al., 2013).

Sphingosine-1-phosphate (SIP) is another active lysophospholipid that regulates various physiological and pathophysiological processes, including cell growth, cellular trafficking, apoptosis, inflammation and cancer (Maceyka et al., 2012). SIP acts through five specific GPCRs of the EDG receptor family (S1PR1–5) that activate diverse downstream signaling pathways, and SIP is also an important intracellular messenger (Spiegel and Milstien, 2011; Maceyka et al., 2012). The expression of S1PR1 and S1PR3 was decreased in decidualizing stromal cells in mouse uterus (Skaznik-Wikiel et al., 2006). In human, S1PR3 protein expression was increased with gestational age (Yamamoto et al., 2010). SIP is produced via phosphorylation of sphingosine by sphingosine kinase-1 and 2 (SPHK1 and SPHK2) (Spiegel and Milstien, 2011). SPHK1 is up-regulated by progesterone (Jeng et al., 2007) and decidualization was defective in SPHK-deficient female mice (SPHK1/−/− SPHK2). It was found that prostaglandin signaling did not seem to be critical for the decidualization in these mice (Mizugishi et al., 2007).

Extracellular LPA and SIP are metabolized mainly by ecto-activities of lipid phosphate phosphatases (LPPs) (Brindley, 2004). In human, three genes, PPAP2a, PPAP2c and PPAP2b have been identified that encode LPP1, LPP2 and LPP3 proteins, respectively (Kai et al., 1997; Roberts et al., 1998). The role of LPPs in human decidualization is unknown. A study shows that mRNA of LPP1 and LPP3 was up-regulated during late pregnancy of women (Yamamoto et al., 2010). SIP is irreversibly degraded by a specific enzyme, SIP lyase (SGPL1) and its turnover is enhanced via dephosphorylation of SIP by two SIP phosphatases (SGPP1 and SGPP2) (Van Veldhoven et al., 2000; Mandal, 2001). The role of SGPP1, SGPP2 and SGPL1 in pregnancy is yet to be described. The expression of SGPL1 and SGPP2 mRNA was increased in endometrium of healthy women whereas only SGPL1 protein expression was increased in term compared with preterm (Yamamoto et al., 2010; Santulli et al., 2012).

It has been shown that LPA is present in human follicular fluids and has increased levels in IVF patients (Tokumura et al., 1999). SIP is also found in human follicular fluids where it mediates ovarian angiogenesis. Furthermore, it was shown that in pregnant rats, blood vessel density is increased and caspase-mediated apoptosis is decreased due to SIP treatment (Hernandez et al., 2009). These findings imply the importance for both SIP and LPA for fertility and function of human ovary.

The effect of ovarian hormones (progesterone and estrogen) on the expression of LPA and SIP receptors and their metabolic enzymes during decidualization of HESCs is not known yet. We hypothesized that specific LPA and SIP receptor subtypes and the turnover of these lysophospholipids are crucial for proper decidualization of HESCs. In the present study, we asked whether specific lysophospholipids (SIP and LPA) receptor subtype(s) and metabolic enzymes regulating the turnover of SIP and LPA are expressed in HESCs, if so, whether their expressions are regulated during decidualization of HESCs. This study shows the first time that specific LPA and SIP receptors and their metabolizing enzymes are highly regulated in HESCs during decidualization.

### Materials and Methods

#### Tissue collection and endometrial stromal cell isolation

Endometrial tissue samples were obtained with informed consent from premenopausal women (age 30–50 years) undergoing hysterectomy for benign reasons (fibroids). Women with endometriosis, endometrial hyperplasia or endometrial polyps were not included. Endometrial samples were taken from women either on Day 8 or on Days 21–25 of their menstrual cycle. The institutional ethical board of the University of Greifswald approved the study protocol.

Tissue samples were washed twice with phosphate-buffered saline (PBS) solution (GIBCO, Karlsruhe, Germany), minced in small pieces, and digested for 1 h at 37 °C in 0.5% collagenase (200 IU/mg; Biochrom, Berlin, Germany) in Delbecco’s Minimal Essential Medium (DMEM)/F-12 medium without phenol red (GIBCO). Digestion was stopped by an equal volume of DMEM/F-12 medium containing 10% charcoal-stripped fetal bovine serum (FBS; Biochrom) and 50 μg/ml gentamicin (Cambrex, Walkersville, MD, USA). Separation of HESCs was performed by filtration through a 40 μm nylon sieve (Falcon, Heidelberg, Germany). HESCs that passed the 40 μm nylon sieve were centrifuged at 300g for 10 min and then washed with PBS. Cells were seeded in 75 cm² culture flasks (Greiner, Frickenhausen, Germany), and incubated in DMEM/F-12 without phenol red containing 10% charcoal-stripped FBS and 50 μg/ml gentamicin. To reduce contamination by remaining non-adherent blood and epithelial cells, the culture medium was changed for the first time 45–60 min after plating. The purity of HESCs was >95%, as determined by immunofluorescent staining with stromal marker vimentin following standard procedures.

After reaching confluence, HESCs were detached with trypsin-EDTA (GIBCO) and seeded in 24-well culture plates (Greiner) with a density of 1 × 10⁵ cells per well to perform experiments in quadruplicate. For all experiments in this study, cells were passaged only once and were used on passage 2 in the experiments.

#### In vitro decidualization

HESCs were decidualized in vitro by incubating the cells with 1 μM progesterone and 30 nM 17β-estradiol (Sigma-Aldrich, Taufkirchen, Germany) for 9 days. The medium was changed every 3 days. Decidualization was confirmed by measuring a significantly increased secretion of insulin-like growth factor-binding protein (IGFBP)-1 and prolactin as described previously (Fluhrl et al., 2007).
Real-time PCR

Total RNA was isolated using peqGOLD TriFast™ reagent (Peqlab, Erlangen, Germany) following the manufacturer’s protocol. First-strand cDNAs were synthesized with High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, Darmstadt, Germany) using random hexamer primers according to manufacturer’s protocol.

Gene-specific primers for real-time PCR were designed using Primer Express 3.0 (Applied Biosystems) and primer-target specificity was checked using nucleotide BLAST program (http://blast.ncbi.nlm.nih.gov). For real-time PCR, SYBR Green PCR master mix (Applied Biosystems) was used according to the manufacturer’s instructions. PCR was performed in duplicates on an ABI Prism 7300 (Applied Biosystems) with the following cycling program: 2 min at 50°C, 10 min at 95°C, 15 s at 95°C and 1 min at 60°C for a total of 40 cycles. PCR products were analyzed by thermal dissociation curve and by agarose gel electrophoresis to verify specific PCR product amplification. Data were normalized against the reference gene β-actin. The primer sequences of the genes used in this study are shown in Supplementary data, Table S1. The relative changes in gene expression were analyzed using 2-DDCt method as described before (Livak and Schmittgen, 2001).

Western blot

Western blots were performed as described before (Goyal et al., 2013). In brief, HESCs were directly lysed in an equal volume of 2× Laemmli buffer. Equal amounts of proteins in the samples were subjected to SDS–PAGE gel electrophoresis and then transferred to polyvinylidene fluoride membrane using the Trans-Blot Turbo System (Bio-Rad GmbH, Munich, Germany). Membranes were blocked with 5% (w/v) non-fat milk and incubated with the respective primary and secondary antibodies using 5% bovine serum albumin in Tris-buffered saline/T. The dilutions of the primary antibodies were: LPA1 (Cayman Chemicals, Biomol GmbH, Hamburg, Germany; dilution 1 : 800). Anti-P2RY5 antibody (abcam Inc., Cambridge, UK; 1 : 1000) and β-actin mouse monoclonal antibody (MBL Inc., Woburn, MA, USA; dilution 1 : 10 000). The dilution of the secondary goat-anti-rabbit 800 antibody and goat-anti-mouse 680 antibody (LI-COR Biosciences GmbH, Bad Homburg, Germany) was 1 : 10 000. Protein signal was detected using the Odyssey Infrared Imager with application Software 3.0.30 (LI-COR). For quantification of protein expression, densitometric analysis of the proteins was done using the same LI-COR software. LPAR1 protein expression was normalized against β-actin expression and compared with the control values. (Fold change).

Statistical analysis

A minimum of six independent experiments were performed each with a different human donor. Each experiment was performed in quadruplicate. Statistical analysis was carried out with multiple t-tests that were performed using GraphPad PRISM version 6 software (GraphPad, La Jolla, CA, USA). The results are calculated as mean ± SD, and data are shown as mean ± SD. The results having P-values of <0.05 were considered statistically significant.

Results

LPA receptor 1 (LPAR1) is up-regulated during decidualization of HESCs

To examine the expression of known LPA receptor subtypes (LPAR1, 4) in proliferative HESCs, we measured the abundance of LPA receptors mRNAs by using real-time PCR. The expression of LPA receptor subtype mRNAs were found in an order LPAR6 > LPAR1 > LPAR3 > LPAR2 > LPAR4. LPAR5 mRNA was undetectable (Fig. 1A).

Previously, it was shown that LPAR1 is an important LPA receptor in mouse embryonic implantation and spacing (Ye et al., 2005). We asked whether the expression of LPA receptors is influenced during decidualization (mid luteal or secretory phase of endometrium). HESCs were decidualized in vitro using 17β-estradiol and/or progesterone for 9 days and the relative change in the expression pattern of LPA receptors mRNA were measured. We found that LPAR1 mRNA was up-regulated (~1.7-fold; P < 0.001) in decidualized HESCs (Fig. 1B) but not after treatment with any one of these hormones by itself. In contrast, the expression of other LPA receptor subtypes mRNA was significantly down-regulated after decidualization (Fig. 1B). Interestingly, after stimulation with 17β-estradiol, only LPAR4 expression was significantly down-regulated whereas stimulation of HESCs using progesterone led to a significant down-regulation of LPAR2, 3, 4, 6. To explore whether LPAR1 is also up-regulated at protein level, LPAR1 protein expression was analyzed by western blotting using an LPAR1 specific antibody. We could identify a specific band of ~75 kDa in HESCs and stimulation of cells with 17β-estradiol or progesterone alone resulted in a 1.5-fold higher LPAR1 protein expression. Indeed, a combination of both hormones even led to a 3-fold higher protein expression compared with unstimulated HESCs (Fig. 1C). Blots were also performed with a specific β-actin antibody and are shown as loading controls (Fig. 1C, right panel). Additionally, LPAR6 protein expression was also analyzed by western blotting using LPAR6 specific antibody. We observed more than two bands in HESCs and different cell lines (Supplementary data, Fig. S1). Multiple bands in different cells might be due to posttranslational modification or different splice variants of LPAR6.

These data suggest that LPAR1 might play a critical role in decidualization of human endometrium, and in contrast to mice, different LPA-mediated signaling is regulated in human decidualization process. Thereby, 17β-estradiol seems have a supportive role whereas progesterone seems to be the major hormone for decidualization and the combination of both hormones is necessary for successful decidualization.

Regulation of LPA metabolic enzymes in HESCs during decidualization

ATX plays a pivotal role in the generation of LPA by converting lysophosphatidylcholine into LPA (Tokumura et al., 2002). LPPs are integral membrane proteins with broad substrate specificity that dephosphorylate lipid substrates including LPA and S1P (Pyne et al., 2005; Yamamoto et al., 2010). We found that proliferative HESCs expressed the mRNA of all three LPPs (LPP1, LPP2 and LPP3) (Fig. 2A). LPP1 has a higher expression than LPP2 and LPP3. The expression of LPP2 and LPP3 gene was 2.5-fold lower than LPP1 (Fig. 2A). In contrast, mRNA expression of ATX was very low (Fig. 2A).

After decidualization of HESCs by treating cells with hormones, the expression of ATX gene was 7.2-fold up-regulated (P < 0.05, Fig. 2B). Interestingly, LPP3 gene was also highly (~4-fold) and significantly (P < 0.001) up-regulated (Fig. 2C). Additionally, after decidualization, LPP2 mRNA expression was decreased by 50% (P < 0.001), and the expression of LPP1 was 1.5-fold increased (P < 0.001; Fig. 2C). These data suggest a role of LPA in decidualization of human endometrium.

Regulation of SIP receptors expression during decidualization of HESCs

SIP1 is a lysosphospholipid that seems to play a crucial role in decidualization and placental development (Mizugishi et al., 2007; Goyal et al., 2013). We
investigated whether proliferative HESCs express the mRNA of known S1P receptors and if so, whether its expression is modulated during decidualization. We measured the abundance of known S1P receptor subtypes (S1PR1-5) mRNA by real-time PCR. S1PR3 is the only receptor that was highly expressed in HESCs (Fig. 3A). S1PR2 and S1PR1 were 15- and 60-fold less expressed in comparison to S1PR3 in these cells, respectively (Fig. 3A). S1PR4 and S1PR5 mRNA were undetectable (Fig. 3A).

To explore, whether the expression of S1P receptors is regulated during decidualization of endometrium, HESCs were decidualized in vitro for 9 days. Interestingly, the expression of all S1P receptor subtypes was modulated during decidualization. S1PR3 expression was significantly decreased, while S1PR2 and S1PR1 expression was increased. S1PR4 and S1PR5 expression remained undetectable (Fig. 3B).
mRNA was down-regulated after decidualization (Fig. 3B). S1PR1 and S1PR3 receptors were down-regulated by \( \approx 40\% \) \((P, 0.02, \text{Fig. 3B})\). In contrast, S1PR2 receptor was slightly but significantly down-regulated by \( \approx 15\% \) \((P, 0.05, \text{Fig. 3B})\). The highest expression of S1PR3 in undifferentiated cells and down-regulation S1PR1 and S1PR3 receptors during decidualization suggest a role of S1P-mediated regulation of decidualization process during pregnancy.

**SPHK1 is up-regulated during decidualization of HESCs**

Decidualization was impaired in sphk1\(^{-/-}\)-sphk2\(^{+/-}\) knockout mice \((\text{Mizugishi et al., 2007})\). In human decidualization, the regulation of SPHKs expression is completely unknown. Two isoforms of SPHK (SPHK1 and SPHK2) are known in mammals \((\text{Liu et al., 2000; Nava et al., 2000})\). To explore the expression of SPHKs in proliferative phase of HESCs, we measured the mRNA expression of both SPHK isoforms. We found that SPHK1 is highly expressed in proliferative HESCs. The mRNA expression was \( \approx 10\)-fold higher than SPHK2 mRNA \((\text{Fig. 4A})\).

To explore the effect of decidualization process on SPHKs isoform, HESCs were decidualized in vitro for 9 days and the relative changes in the expression pattern of SPHK mRNA were measured. We found that SPHK1 but not SPHK2 was up-regulated \((\approx 2\)-fold\) in differentiated HESCs \((\text{Fig. 4B})\). These data suggest that SPHK1 might also have a role in decidualization process in human.

![Figure 2](https://academic.oup.com/molehr/article-abstract/20/10/1016/1026517/Lysophosphatidic-acid-and-sphingosine-1-phosphate/1020Bruennert et al.)

**Figure 2** mRNA expression of LPPs in HESCs. The total RNA of HESCs was isolated and then the expression of autotaxin (ATX) and LPPs transcripts was measured by real-time PCR. **(A)** Bar diagram shows the relative mRNA expression of LPPs in HESCs during proliferative phase. LPP1 is the highest expressing gene among LPPs, whereas ATX is expressed at very low level. **(B)** and **(C)** HESCs were decidualized by hormonal treatment and then the relative mRNA expression of ATX and LPPs was measured. ATX expression is increased by 7.2-fold \((\text{B})\) and LPP3 mRNA expression is increased by 4.2-fold \((\text{C})\) in decidualized HESCs compared with untreated HESCs. Data were normalized against the \(\beta\)-actin gene. Results are means \(\pm\) SD of seven independent experiments with different HESCs derived from different patients. *\(P < 0.05\). **\(P < 0.001\).
Regulation of S1P metabolic enzymes in HESCs during decidualization

The expression and regulation of lysophospholipids (S1P and LPA) metabolic enzymes are not known in human uterus. SGPP1 and SGPP2 catalyze the dephosphorylation of S1P (Mandala, 2001). SGPL1 catalyzes the essentially irreversible cleavage of S1P (Van Veldhoven et al., 2000).

We found that SGPP1 and SGPL1 are highly expressed in proliferative HESCs whereas SGPP2 is expressed on a very low level (Fig. 5A). After decidualization of HESCs for 9 days in vitro, the expression of SGPP1 mRNA was significantly up-regulated by 1.4- to 1.5-fold (P < 0.05, Fig. 5B). The expression of SGPL1 did not significantly change during decidualization (Fig. 5B). These data suggest that the S1P turnover may increase during the decidualization in human.

Discussion

The roles of LPA and S1P in reproduction have been investigated using various animal models, but their role in decidualization of human endometrium has not been described yet (Ye, 2008; Ye and Chun, 2010). To our knowledge, this is the first report showing that LPA and S1P metabolic pathways and its receptors are differentially regulated...
during decidualization of HESCs. Our data suggest that LPAR1 receptor-mediated signaling SPHK1 and LPP3 might play a crucial role in the decidualization of human endometrium. Furthermore, a defined orchestration of estradiol and progesterone is mandatory for a successful in vitro decidualization of HESCs.

Presently, six well-characterized and three putative LPA receptors are known (Chun et al., 2010; Schober and Sessi, 2012). In the past, most of the studies that were performed to analyze the expression profile of LPA receptors in the uterus of human and animal models such as mouse, bovine, sheep and pig were mainly focused on EDG family of receptors (LPAR1-3) (Chen et al., 2008; Chun et al., 2010). A previous study showed that only LPAR1 receptor mRNA was expressed in HESCs of the proliferative and secretory phase in vitro (Chen et al., 2008). In contrast, we found that the expression of LPAR1 and LPAR6 are higher than LPAR2 and LPAR3 as measured by quantitative real-time PCR. Interestingly, our data show that LPAR1, but not other LPA receptors was up-regulated after in vitro decidualization, suggesting a specific role of LPAR1 in decidualized stromal cell function. Notably, other LPA receptors (LPAR2-6) were significantly down-regulated during HESCs decidualization. The effects of progesterone on LPA receptor subtype mRNA expression is much higher than those of estradiol. These data suggest that progesterone might be the major regulator of the decidualization process whereas estradiol may have a supportive role in the process. An orchestration of both hormones leads to a successful in vitro decidualization and has the strongest effect on gene expression of LPA receptors.

Recently, one study showed that LPAR1 protein was mainly expressed in stromal cells of the bovine uterus (Boruszewska et al., 2013). In mouse, LPAR3, that has a crucial role in embryo implantation, is mainly localized in epithelial cells of the uterus and is up-regulated after progesterone treatment (Ye et al., 2005; Hama et al., 2006). In human, it was recently shown that LPAR3 expression was significantly increased during the early (15–18 days) and late secretory phase (24–28 days) and down-regulated in glandular epithelial cells and stromal cells in mid-secretory phase (19–23 days; the implantation window; Guo et al., 2013). Our in vitro experiment conditions mimic the mid-secretary phase of women’s menstrual cycle, and in agreement with this study, we found that the expression of LPAR3 is down-regulated after hormonal treatment. A defect in embryo spacing and embryo implantation but not in decidualization was observed in LPAR3 knockout mice (Ye et al., 2005).

Notably, exogenous prostaglandins such as PGE2 and PGI2 rescued the defective implantation but not an embryo crowding in LPAR3 deficient female mice. These data suggest that LPAR3 receptor might regulate the synthesis of prostaglandins and as a consequence, embryo implantation (Ye et al., 2005).

LPA stimulates the production of PGE2 in bovine endometrial stromal cells possibly mediated via LPAR1 receptor (Woclawek-Potocka et al., 2009b). In co-culture of mouse embryos with human decidual cells, LPA was able to increase embryo outgrowth and also induced actin stress fiber formation via Rho-dependent pathway (Shiokawa et al., 2000). LPA induced IL-8 expression via activation of LPAR1-dependent NF-kB-pathway in HESCs suggesting a role in angiogenesis of endometrium (Chen et al., 2008). LPA enhanced the expression of factors that are essential for uterine decidualization and vascularization in mouse (Beltrame et al., 2013). We suggest LPAR1 might have an essential role in decidualization and embryo implantation. Therefore, it can be a potential biomarker for human endometrium receptivity.

LPA is released from bovine, sheep and porcine endometrium, both during the estrous cycle and early pregnancy (Woclawek-Potocka et al., 2009a; Seo et al., 2012; Boruszewska et al., 2013). LPA concentration was significantly higher in epithelial than in stromal cells from bovine endometrium (Boruszewska et al., 2013). ATX/ENPP2 belongs to the ENPP family of ecto- and exo-enzymes and plays a pivotal role in the generation of LPA by converting lysophosphatidylcholine into LPA (Tokuura et al., 2002). It was found that ATX was expressed at a higher level in epithelial cells than in stromal cells from bovine endometrium (Boruszewska et al., 2013). In human, ATX activity in serum was...
increased during pregnancy (Tokumura et al., 2002). In agreement with previous studies, we show that HESCs expressed ATX at very low level. Interestingly, we found that ATX was significantly up-regulated during in vitro decidualization. It has been shown previously that ATX was up-regulated in sheep and pig endometrium during pregnancy (Seo et al., 2008; Liszewska et al., 2012). These data indicate that ATX up-regulation in HESCs may be required for decidualization process and pregnancy maintenance.

Extracellular LPA and S1P are mainly dephosphorylated by the ecto-activities of the LPPs (Brindley, 2004). The expression of LPPs in HESCs has not been studied yet. A previous study shows that the mRNA of PPAP2a (LPP1) and PPAP2b (LPP3) but not PPAP2c (LPP2) were increased in human decidua during late pregnancy (Yamamoto et al., 2010). Here, we show the first time that LPP1 has a higher expression than LPP2 and LPP3 in HESCs. Interestingly, LPP3 (∼4-fold) and LPP1 (∼1.5-fold) genes were highly up-regulated. These data

**Figure 6** Effect of progesterone and estradiol on LPA and S1P metabolic pathway proteins and their receptors expression in HESCs during decidualization at mid-secretory phase. SGPP1, S1P phosphatase 1; SGPL1, S1P lyase 1; SPHK1, Sphingosine kinase 1; LPAR, lysosphatidic acid receptor; LPP, lipid phosphate phosphatase; S1PR3, sphingosine-1-phosphate receptor 3.
suggest a role of LPPs in decidualization of human endometrium. The up-regulation of LPP3 suggests that it may specifically regulate the decidualization either by controlling the turnover of LPA concentration and/or other related lipids such as S1P. The functions of LPPs other than phosphatases cannot be excluded, and should be explored during early pregnancy.

SIP binds and activates five specific G protein-coupled receptors (SIPR1–5) of the EDG family (Maceyka et al., 2012). We recently showed the expression of SIP receptors in trophoblast cells (Goyal et al., 2013). We show first time that SIPR2 was the only receptor that is highly expressed in HESCs. SIPR2 and SIPR3 were much less expressed in comparison to SIPR3 in these cells. It has been shown before that activation of SIPR1 and SIPR3 induces migration and invasion in various cell types including endothelial cells and breast epithelial cells (Takua et al., 2008; Kim et al., 2011). Migration of HESCs is required for proper embryo implantation and for remodeling of the endometrium during the menstrual cycle (Salamonsen, 2003; Grewal et al., 2008). Higher expression of SIPR3 in HESCs suggests a role in cell proliferation and migration of cells that might play an important role in implantation and endometrium remodeling. Previously, it was shown that the expression of SIPR1, and SIPR3 was reduced in decidualizing cells throughout the decidua in a mouse model (Skaznik-Wikiel et al., 2006). SIPR3 protein expression increased with gestational age and in term compared with preterm in women (Yamamoto et al., 2010). We found that SIP receptors were down-regulated during decidualization of HESCs. These data suggest that precise regulation of SIPR3 expression is essential for proper preparation of endometrium to implantation and misregulation may lead to pregnancy disorder.

SIP is synthesized via the phosphorylation of Sphingosine by SPHK1 and SPHK2 (Maceyka et al., 2012). Impaired decidualization was shown in in SPHK-deficient female mice (SPHK1−/− SPHK2), and it was also found that prostaglandin signaling did not seem to be critical for the decidualization (Mizugishi et al., 2007). We report here that SPHK1 is highly expressed in HESCs and further up-regulated after hormonal treatment in vitro. Other studies also reported the up-regulation of SPHK1 in the mouse uterus (Jeng et al., 2007; Mizugishi et al., 2007). These date indicate that expression of SPHK1 regulates decidualization processes possibly via increasing the growth and differentiation of HESCs.

Production of S1P is tightly controlled by reversible dephosphorylation by two phosphatases, SGPP1 and SGPP2, and degradation by one lyase, SGPL1 (Van Veldhoven et al., 2000; Mandala, 2001). SGPP2 and SGPL1 mRNA were the more abundant in endometrium of healthy women (Santulli et al., 2012). In another human study, Yamamoto et al. showed that the expression of SGPL1 at mRNA and protein level was increased in term compared with preterm suggesting increased S1P turnover in decidual at term (Yamamoto et al., 2010). Higher expression of SGPL1 and SGPP1, and up-regulation of SGPP1 during decidualization of HESCs suggest that higher turnover of extracellular S1P and/or intracellular S1P is required for normal decidualization process in human.

In summary, this study shows the first time that LPA and SIP receptors and their metabolizing enzymes are highly regulated during decidualization of human endometrium (Fig. 6). Based on the previous studies (Ye et al., 2005) together with our data we suggest that LPAR1 receptor-mediated signaling in HESCs is crucial for decidualization and LPAR2 is crucial for embryo implantation. SPHK1 activity and high turnover of SIP and LPA might be essential for precise regulation of their signaling during decidualization of human endometrium.

Knowledge about cellular and molecular mechanisms governing the physiology of decidua will allow in further understanding the mystery of implantation, increase the success of assisted reproductive technology, and develop the treatments for some causes of infertility and gestational diseases.

Supplementary data

Supplementary data are available at http://molehr.oxfordjournals.org/.

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Authors’ roles

D.B., P.G. and M.S.: conceived and designed the experiments; P.G., D.B., M.S., F.B. and N.T.: performed the experiments; P.G., D.B., M.S., F.B. and M.Z.: analyzed the data; M.Z. and S.W.: contributed reagents/materials/analysis tools; P.G., D.B., M.Z. and S.W.: wrote the paper.

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Conflict of interest

None declared.

References


