Interaction of steroid receptor coactivators and estrogen receptors in the human placenta

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Abstract

Female sex steroid hormones such as estrogen and progesterone have a pivotal role in maintaining pregnancy in human and animals. Especially, estrogen exerts specific effects on the cardiovascular system and angiogenesis, and thus affects significantly on placentation. Although the functions of estrogen have been emphasized during pregnancy, their signaling pathways in the placenta have not been fully understood. In this study, estrogen signaling was evaluated according to gestational age. Human placenta samples were collected and divided into early preterm (n = 10), late preterm (n = 18), and term (n = 20) groups. First, serum estrogen concentration and corticotropin-releasing hormone (CRH) mRNA expression, which is known as gestation clock gene, were increased following gestation age in our experimental condition, as we expected. Next, the expression of estrogen receptors (ERs) and steroid receptor coactivators (SRCs) in the placenta was evaluated. ERα (ESR1) and ERβ (ESR2) were expressed highly at term period compared with early preterm. In addition, SRC family including SRC1, SRC2, and SRC3 was expressed in the human placenta, and the levels of SRC1, SRC2, and SRC3 were increased in the placenta at the late stage of gestation. The interaction of ERs with SRCs was also examined, which was significantly enhanced at term period. In the immunostaining results, it was indicated that ERs and SRCs were all dominantly expressed in syncytiotrophoblast cells. These results suggested that SRC1, SRC2, and SRC3 were expressed and interact with ERs highly at the late stage of gestation, and may amplify the signaling of estrogen in the placenta to maintain pregnancy.

Introduction

The placenta is a specialized organ during pregnancy, which in combination with fetal membranes and amniotic fluid supports normal growth and development of the fetus. The placenta has a hemochorial villus, whereby maternal blood comes into direct contact with placental trophoblast cells and allows an intimate relationship with the developing embryo (Moore et al. 2015). The placental membrane is made of four layers, a maternal facing syncytiotrophoblast layer, a cytotrophoblast cell layer, connective tissue of the villus, and maternal endothelium lining the fetal capillaries (Gude et al. 2004). However, the cytotrophoblast cell layer of the villus becomes attenuated and disappears after approximately
20 weeks. Subsequently, in most chorionic villi, the membrane consists of three layers and becomes extremely thin in some areas such that the syncytiotrophoblast directly contacts the fetal capillary endothelium. Thus, in these positions, the maternal and fetal blood come into very close proximity (as little as 2–4μm) (Gude et al. 2004).

The main functions of the placenta can be categorized under the headings of transport and metabolism, protection, and endocrine functions. For transport, the placenta provides oxygen, water, carbohydrates, amino acids, lipids, vitamins, minerals, and other nutrients to the fetus, whereas removes carbon dioxide and other waste products (Gude et al. 2004). The placenta also metabolizes numerous substances and releases metabolic products into maternal and/or fetal circulations. It also helps to protect the fetus against certain toxic molecules, infections, and maternal diseases. For endocrine function, the placenta releases hormones into both the maternal and fetal circulations to affect pregnancy, metabolism, fetal growth, and parturition. The endocrine factors produced by the placenta include estrogen, progesterone, chorionic gonadotropin, placental lactogen, placental growth hormone, and many others (Gude et al. 2004, Lehrer et al. 1990).

During human pregnancy, the placenta produces large amounts of estrogen. Estrogen exerts a wide variety of effects on cellular growth, development, and differentiation. The physiological effects of estrogen include important regulatory functions within the reproductive systems of both females and males, which are related to mammary gland development and differentiation as well as regulation of the hypothalamic–gonadal axis (Pepe & Albrecht 1995). During pregnancy, estrogen regulates a sequence of events leading to initiation of labor (Gibb et al. 2006), biosynthesis of progesterone, and placental transfer mechanisms that modulate maturation of the fetal reproductive system (Pepe & Albrecht 1995). Furthermore, estrogen stimulates angiogenesis, which is a critical process for placental function, by regulating vascular endothelial growth factor (VEGF) and growth of endothelial cells in the placenta. Therefore, abnormal estrogen levels and angiogenesis in the placenta are associated with complications such as preeclampsia and preterm delivery (Albrecht & Pepe 2010).

Estrogen acts by binding to its receptor, estrogen receptor (ER). ER exists in two main forms, ERα (ESR1) and ERβ (ESR2), which have distinct tissue-specific expression patterns (Mueller & Korach 2001). ERα and ERβ are nuclear steroid hormone receptors with similarities in terms of ligand-binding and nuclear DNA-binding regions (Arnal et al. 2007, Luksha & Kublikiene 2009). ERα and ERβ are encoded by separate genes, ESR1 and ESR2, respectively, located at different chromosomal locations, and numerous mRNA splice variants exist for both receptors in both diseased and normal tissues (Herynk & Fuqua 2004). In the ‘classical’ pathway, estrogen binds to ER to regulate the transcription of target genes in the nucleus by binding to estrogen response element (ERE) in the promoters of target genes and by recruiting coregulatory proteins such as coactivators or corepressors (McKenna & O’Malley 2002).

Several classes of coactivators have been identified and shown to be essential in estrogen responsiveness (McKenna & O’Malley 2002). The major coregulators involved in estrogen signaling are the p160 steroid receptor coactivator (SRC) family, consisting of SRC1/NCoA1, GRIP1/TIF2/SRC2, and pCIP/RAC3/ACTR/AIB1/TRAM1/SRC3, which enhance transcriptional activation and recruit other cofactors possessing histone acetyl transferase activity (Leo & Chen 2000). SRCs interact with ER and enhance estrogen signals. Coactivators such as SRCs are fundamental to the proper function of reproductive events such as fertility (Mukherjee et al. 2007), uterine growth, blastocyst implantation (Mukherjee et al. 2006), and mammary gland development (Xu et al. 2000). The pleiotropic actions of SRCs make them attractive candidates as biomarkers for a multitude of pathologies. In relation to cancer, SRC3 overexpression results in aggressive breast and lung cancers (Osborne et al. 2003, Wang et al. 2010), SRC2 mutations are associated with prostate cancer metastases (Taylor et al. 2010), and SRC1 overexpression leads to early resistance to breast cancer therapy (Redmond et al. 2009). Furthermore, the significance of SRC activity in shaping the metabolic landscape suggests that they may be etiological predictors for numerous metabolic diseases.

Despite their fundamental actions in reproductive organs, no study has examined the expression of SRCs in the human placenta. The objective of this study was to define the expression, localization, and possible function of SRCs in the human placenta according to gestational age. Interactions between SRCs and ERs were also explored to elucidate the actions of estrogen in the placenta during pregnancy.

Materials and methods

Tissue and plasma collection and processing

This study was approved by the Institutional Review Board of the Pusan National University Hospital Clinical Trials Center (H-1302-005-015) and all participants gave written informed consent. Placental tissue and plasma samples were obtained from pregnant women who met...
the following inclusion criteria: 1) singleton pregnancy, 2) normal pregnancy at the time of sample collection, and 3) healthy women with no preexisting clinical conditions such as diabetes, hypertension, or autoimmune disease. The placental samples were divided into early preterm, late preterm, and term groups after onset of labor. The early preterm period was determined to be 22–29 weeks of gestation, which is clinically important because preterm delivery frequently happens during this period, whereas the late preterm period was determined to be 30–36 weeks of gestation. The term placenta group was collected from a gestational age of 37–40 weeks. The clinical characteristics of sample groups are given in Table 1.

**Measurement of blood estradiol concentration**

From each volunteer, blood was collected in plastic tubes under aseptic conditions with EDTA as an anticoagulant and then centrifuged in order to separate plasma. The plasma was stored at −70°C, and the sample was slowly thawed at room temperature. Plasma estradiol concentrations were measured using an estradiol-ELISA kit No. 582251 (Cayman, Ann Arbor, MI, USA) following the manufacturer’s protocol.

**Quantitative real-time PCR**

Total RNA was extracted using Trizol reagent (Invitrogen), according to the manufacturer’s protocol. The concentration of total RNA was measured using a spectrophotometer. First-strand complementary DNA (cDNA) was prepared from total RNA (3μg) by reverse transcription (RT) using M-MLV reverse transcriptase (Invitrogen) and random primers (9-mers; TaKaRa Bio). Quantitative real-time PCR (Q-PCR) was performed with a cDNA template (2μL) and 2× Power SYBR Green (6μL; TOYOBO Co., Osaka, Japan) containing specific primers. The primer sequences for β-actin, namely CRH, ERα, ERβ, SRC1, SRC2, and SRC3, are given in Table 2. Q-PCR was carried out for 40 cycles using the following parameters: denaturation at 95°C for 15s, followed by annealing and extension at 70°C for 60s. Fluorescence intensity was measured at the end of the extension phase of each cycle. The threshold value for the fluorescence intensity of all samples was set manually. The reaction cycle at which PCR products exceeded this fluorescence intensity threshold during the exponential phase of PCR amplification was considered to be the threshold cycle (CT). The expression of the target gene was quantified relative to that of β-actin, a ubiquitous housekeeping gene, based on comparison of C Ts at constant fluorescence intensity.

**Immunoprecipitation and western blotting analysis**

Protein samples were extracted with Pro-prep solution (iNtRON Biotechnology, Korea) by following the manufacturer’s protocol. Immunoprecipitation was performed based on a previous study (An et al. 2006). Briefly, 200μg of protein was precleared with protein A/G agarose beads (Santa Cruz Biotechnology) for 1h at 4°C, followed by exposure to antibodies together with beads overnight. Immunoprecipitates were then washed three times, eluted in 3× sodium dodecyl sulfate (SDS) loading buffer, and processed for western blotting. Twenty microgram of protein was loaded for input. The proteins were separated by 8% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes (Daebil Service Co, Seoul, Korea). The membranes were then blocked for 2h with 5% skim milk (Difco, Sparks, MD, USA) in phosphate-buffered saline (PBS) with 0.05% Tween-20 (PBS-T). After blocking, the membranes were incubated with anti-ERα rabbit (Santa Cruz Biotechnology, diluted 1:500), anti-ERβ rabbit (Santa Cruz Biotechnology, diluted 1:500), antibody together with beads overnight. Immunoprecipitates were then washed three times, eluted in 3× sodium dodecyl sulfate (SDS) loading buffer, and processed for western blotting. Twenty microgram of protein was loaded for input. The proteins were separated by 8% SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellulose membranes (Daebil Service Co, Seoul, Korea). The membranes were then blocked for 2h with 5% skim milk (Difco, Sparks, MD, USA) in phosphate-buffered saline (PBS) with 0.05% Tween-20 (PBS-T). After blocking, the membranes were incubated with anti-ERα rabbit (Santa Cruz Biotechnology, diluted 1:500), anti-ERβ rabbit (Santa Cruz Biotechnology, diluted 1:500),

<table>
<thead>
<tr>
<th>Table 1 Clinical characteristics of pregnancies</th>
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<tbody>
<tr>
<td>Early preterm (n = 10)</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td><strong>Gestational age (weeks)</strong></td>
</tr>
<tr>
<td><strong>Birth weight (g)</strong></td>
</tr>
<tr>
<td><strong>Systolic BP (mmHg)</strong></td>
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<tr>
<td><strong>Diastolic BP (mmHg)</strong></td>
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<tr>
<td><strong>Parity</strong></td>
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<tr>
<td><strong>Gravidity</strong></td>
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<td><strong>BMI (kg/m²)</strong></td>
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</tbody>
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**Table 2 Primer sequences for real-time PCR analyses**

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Primer</th>
<th>Sequence (5’-3’)</th>
<th>Fragment (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>Forward</td>
<td>GGACTTCGAGCAAGAGATGG</td>
<td>234</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>AGACGTGTTGGCGTACAGG</td>
<td>178</td>
</tr>
<tr>
<td>CRH</td>
<td>Forward</td>
<td>GCCAGGGCCTATGATTAT</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>TCCCCCTCAGTCTCTCAATG</td>
<td>173</td>
</tr>
<tr>
<td>ERα</td>
<td>Forward</td>
<td>AGACAGCTGGAAGGAGTAGGA</td>
<td>195</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>AAGAGATTCGCCGTTGTTT</td>
<td>225</td>
</tr>
<tr>
<td>ERβ</td>
<td>Forward</td>
<td>GATCAGTGCCGATTTGCTGTG</td>
<td>206</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>ATAGCGCGTGAATGCTGTG</td>
<td>206</td>
</tr>
</tbody>
</table>

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anti-SRC1 mouse (Thermo Fisher Scientific diluted 1:500),
anti-SRC2 mouse (Thermo Fisher Scientific, diluted
1:1000), and anti-SRC3 mouse antibodies (Santa Cruz
Biotechnology, diluted 1:2000) for overnight, followed
by HRP-conjugated anti-rabbit and anti-mouse secondary
antibodies (diluted 1:2000) in 5% skim milk with PBS-T
for 1 h. Luminol reagent (Bio-Rad) was used to visualize
the antibody binding. Each blot was then stripped by
incubation with 2% SDS and 100 mM mercaptoethanol in
62.5 mM Tris–HCl (pH 6.8) for 30 min at 50–60°C. The
membranes were subsequently probed with antibody
against actin (Santa Cruz Biotechnology, diluted 1:2000)
as an internal control. The blots were scanned using Gel
Doc 1000, version 1.5 (Bio-Rad), and band intensities
were normalized to β-actin levels.

**Histological analysis**

The placental tissues were fixed with 10% formalin,
embedded in paraffin wax, routinely processed, and
then sectioned into 4 µm thick slices. The tissue sections
were then stained with hematoxylin & eosin (Sigma-Aldrich).
For immunohistochemistry, the sections were
deparaffinized and rehydrated through a graded series of
alcohol using standard procedures. Endogenous peroxidase
activity was quenched using a 10 min incubation step
with 3% H2O2 in MeOH. Nonspecific binding reactions
were blocked by incubation with 10% bovine serum
albumin and incubated with primary antibody solution
overnight at 4°C. The tissue sections were then rinsed
three times for 2 min, each in PBS while slowly warming
to room temperature, followed by incubation with second
antibody for 10 min. The primary antibody was detected
using a Polink-2 Plus HRP DAB kit (GBI Labs, Mukilteo,
WA, USA). The images of the tissue were captured at 40 ×
using a model BX50F–3 optical microscope (Olympus).

**Statistical analysis**

The results are presented as the mean ± standard deviation
(s.d.). Data were analyzed using Sigma Plot 10.0 (Systat
Software Inc, San Jose, CA, USA). The P-values <0.05 were
considered to be statistically significant.

**Results**

**Plasma estradiol concentration and CRH mRNA
expression level during gestational age**

For the initial experiment, plasma estradiol concentration
during gestational period was measured. The plasma
samples were obtained from 23 normal pregnant females,
and estradiol concentration ranged between 2990 and
12,650 pg/mL with a median value of 6092 pg/mL.
Estradiol plasma concentrations gradually increased with higher
gestational age (Fig. 1A). The mean values of estradiol were
4279 pg/mL (early preterm), 6898 pg/mL (late preterm), and
7738 pg/mL (term). Next, we examined the expression of
corticotrophin-releasing hormone (CRH), which is a well-
known biomarker of gestational age in the human placenta.
As expected, transcriptional levels of CRH increased
according to the gestational age. The expression levels
increased to 2.6- and 7.9-fold in the late preterm and term
groups, respectively, compared with early preterm (Fig. 1B).

**Expression of ERα and ERβ in placenta during gestation**

For the next experiment, we evaluated expression of both
ERα and ERβ in placental tissues by Q-PCR (Fig. 2). As shown
in Fig. 2A and B, mRNA levels of both ERα and ERβ were
significantly elevated in the term placenta compared with
early preterm placenta by 2.3- and 1.8-fold, respectively.
The protein levels of ERs were also analyzed by western
blot assay, and their photographic figures are represented
in Fig. 2C. The results indicate that ERα and ERβ protein
levels in the term gestational placenta were dramatically
greater than those in early preterm (Fig. 2D and E), which
was consistent with the results obtained for mRNA levels.
The uniformity of the β-actin (internal standard) bands in
these western blots further confirms that equal amounts
of protein were loaded onto the gels.

**Expression of SRCs in placenta during gestation**

Expression levels of SRC mRNA in the placenta were
examined by Q-PCR. SRC1 and SRC2 mRNA levels were
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Correlation between ERs and SRCs in placenta according to gestational age

To confirm the interaction between ER and SRCs proteins, we performed co-immunoprecipitation experiments. We immunoprecipitated total proteins from early preterm and term placenta using antibodies for SRC1, SRC2, and SRC3, and performed western blots for ERα and ERβ. First, both ERα and ERβ interacted with SRC1 more strongly at term period compared with early preterm period (Fig. 4A). The basal interactions of SRC1 with ERα and ERβ were not significantly different in early preterm. However, interaction of SRC1 with ERα was drastically stronger than that with ERβ at term period, suggesting that SRC1 was more closely associated with ERα than ERβ during this period. Next, interaction of ERs with SRC2 and SRC3 was evaluated (Fig. 4B and C). SRC2 and SRC3 strongly interacted with both ERα and ERβ at term compared with early preterm. In contrast to SRC1, interactions of SRC2 and SRC3 with ERα and ERβ at term period were not significantly altered.

Immunohistochemical detection of ERs and SRCs in the placenta

To further explore the expression patterns of ERs and SRCs in placental tissues, formalin-fixed tissues were subjected to immunohistochemical analysis using antibodies specific for ERs and SRCs as described in the Materials and methods section. The morphology of placental tissue was first confirmed by H&E staining (Fig. 5A). The cytotrophoblast section was surrounded with layer of syncytiotrophoblasts, which is a general morphological feature of the placenta. As shown in Fig. 5B, tissue was stained only with secondary antibody in order to test the specificity of the immunoreaction as a negative control. ERα and ERβ were localized to both cyto- and syncytiotrophoblast cells. However, signals were more dominant in the nuclei of syncytiotrophoblast cells (Fig. 5C and D). The expression patterns of SRC-2 and −3 proteins were similar with those of ERs by localizing more at syncytiotrophoblasts (Fig. 5E and G). The signal of SRC1 was strongly shown in the cytotrophoblast cell than any other antibody (Fig. 5E).

Discussion

During pregnancy, estrogen synthesized by the uteroplacental unit using steroid precursors from maternal and fetal adrenal glands plays a crucial role in maintaining pregnancy (Albrecht & Pepe 1990, Magness 1998). Therefore, the plasma levels of estrogen are potentially useful for understanding the physiological cause of clinical complications. In this study, we measured the concentration of plasma estrogen during an experimental gestational period (22–40 weeks of gestation). Many studies have reported that serum estrogen levels increase...
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progressively from 22 week of gestation to regulate placental growth (Mesiano 2001, You et al. 2006).

In this study, the experimental groups were separated by early preterm, late preterm, and term period. Depending on the period of gestation, estrogen may exert different functions. For example, during preterm, it is closely involved in the angiogenesis and growth of placenta, which is necessary for the growth of fetus. However, in term period, estrogen is associated with the preparation of delivery by regulating uterine contraction (Mesiano et al. 2002). To confirm this, our placenta samples were arranged according to gestational age, we examined the expression of CRH, which is proposed to regulate the placental clock and controls a cascade of physiological events leading to parturition (Smith et al. 2002). CRH is synthesized primarily in the paraventricular nucleus of the hypothalamus, whereas it is produced by the placenta during pregnancy. Placental CRH production increases dramatically over the course of normal human gestation (McLean et al. 1995), reaching levels at term observed only in the hypothalamic portal system (median eminence) during physiological stress (Lowry 1993). Abnormally accelerated rates or excessive levels of placental CRH are the significant risk factors for earlier onset of spontaneous birth (Holzman et al. 2001, Ng et al. 2002, Wadhwa et al. 2004). In our results, CRH expression progressively increased depending on gestation age, as expected, suggesting that our placental samples reflected proper gestational age.

Estrogen exerts its function via binding to its specific receptors, ERα and ERβ, which play important roles in maintaining uteroplacental and systemic circulations during pregnancy (Byers et al. 2005). In a previous report, reduced ERα expression caused by a single-nucleotide polymorphism was implicated in the pathogenesis of spontaneous abortion (Lehrer et al. 1990). Furthermore, a woman with prior preeclampsia also showed reduced risk of breast cancer, which is consistent with reduced ER

Figure 3
The mRNA and protein levels of SRcs in human placenta according to gestational age. Total mRNA and proteins were harvested from the human placenta after onset of labor. Transcriptional levels of SRC1 (A), SRC2 (B), and SRC3 (C) were analyzed by Q-PCR depending on gestational age. Proteins from at least six different samples were examined and quantified for graphs, and the representative samples were shown in (D). The values for SRC1 (E), SRC2 (F), and SRC3 (G) were represented as schematic graphs. Total mRNA and protein expression levels were normalized to that of β-actin. Data are expressed as the mean ± s.d. *P<0.05 compared with the early preterm group.
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Figure 4
Interactions between ER and SRC proteins in human placenta according to gestational age. Proteins in the placenta were immunoprecipitated using anti-SRC1 (A), anti-SRC2, and anti-SRC3 (B) antibodies. The immunoprecipitates were then probed with anti-ERα and anti-ERβ antibodies, and values are represented as graphs. *P < 0.05 compared with the control group; #significant compared ERα.

Figure 5
Immunostaining of ERs and SRCs in human placental tissue. Placental tissue was stained with H&E to confirm placental morphology (A, 40x magnification). The section was stained only with secondary antibody as a negative control (B). To examine localization of each protein, tissues were stained with specific antibodies for ERα (C), ERβ (D), SRC1 (E), SRC2 (F), and SRC3 (G). A full colour version of this figure is available at http://dx.doi.org/10.1530/JME-15-0248.

expression (Forman et al. 2005). ERα and ERβ are necessary for estrogen-mediated protection against vascular injury (Karas et al. 2001, Pare et al. 2002). In this study, transcriptional and translational levels of ERα and ERβ in the placenta increased at the late stage of gestation. Although many studies have focused on the concentration of estrogen, only one study examined ER expression in the human placenta. Specifically, only mRNA levels of ERα and ERβ in the placenta were tested from the first trimester to term (Fujimoto et al. 2005). The mRNA levels of both ERα and ERβ increased from the first to second trimester and then suddenly decreased until normal term delivery, which differs from our results. They also observed that the mRNA expression of estrogen-related receptors (ERRs), ERRα, ERRβ, and ERRγ, all gradually increased until term delivery. On the other hand, placental ER expression in rats has been shown to gradually decrease during gestation (Al-Bader 2006).

To understand estrogen-related signaling, interactions of ERs with their coregulators should be elucidated, because coregulators such as SRCs critically modulate initiation of ER/ERE transcription. Although the expression
and regulation of SRC family proteins have been well established in other tissues such as the uterus and mammalian glands, they have not been explored in the placenta. We thus measured the mRNA levels of SRCs in the placenta. First, SRC1, SRC2, and SRC3 were all present in the human placenta. Specifically, mRNA expression levels of SRC1, SRC2, and SRC3 were enhanced in the term placenta compared with early preterm placenta, although the result of SRC3 was not significant, but the protein levels of SRC1, SRC2, and SRC3 were upregulated in term placenta compared with early preterm placenta. These results indicate that SRC1, SRC2, and SRC3 may regulate estrogen signaling in the placenta and play pivotal roles during pregnancy.

We also evaluated the interactions between ERs and SRCs in the human placenta by immunoprecipitation assays. In the results, the signal of input was different among IP samples. The intensity of input in IP sample for SRC1 was stronger than SRC2 and SRC3. It may be due to the basal interaction of SRC1 with ERs, which was weaker than those of SRC2 and SRC3. The interactions of ERs with SRC1, SRC2, and SRC3 were significantly augmented in the term placenta. Interestingly, SRC1 interacted with ERα more than ERβ at term, whereas SRC2 and SRC3 did not show any change depending on the isoforms of ER. Numerous studies have shown that SRC and ER interactions are closely involved in the estrogen-mediated pathway. In breast cancer cells, knockdown of SRC1 abolished estrogen-related signaling (Klinge 2000). Furthermore, another previous study showed that the fertility of both sexes is impaired in Sr2-knockout mice. Specifically, male hypofertility was found to be due to defects in spermatogenesis and testicular degeneration, while that of female was because of placental hypoplasia, suggesting the possible critical function of SRC2 in the placenta (Gehin et al. 2002).

A recent report has shown that ERα and SRC1 proteins are expressed in distinct subsets of cells within the epithelium of the estrogen-responsive rat mammary gland (Shim et al. 1999). Results from Sre1-null mice revealed decreased mammary responsiveness to estrogen (Xu et al. 1998), suggesting that ERα and SRC1 have distinct patterns of expression and function within the mammary gland. In contrast, ERα and SRC1 were colocalized to the rat uterine epithelium (Shim et al. 1999). Taken together, these results indicate that SRC1 plays a cell type-specific role in ER actions. To determine whether or not ERs are colocalized with SRCs, we performed immunohistochemistry analysis. ERs and SRCs were detected mainly in syncytiotrophoblasts but weakly in cytotrophoblasts, suggesting that ERs and SRCs may be associated.

In summary, our results demonstrate that estrogen signaling may be amplified by several pathways in the placenta during gestation. High levels of plasma estrogen that increased the expression of ERs and SRCs and stimulated the interactions between ERs and SRCs in the placenta are involved in this signaling. The amplified signaling of estrogen may stimulate the function and growth of placenta in the late stage of gestation to maintain pregnancy.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Author contribution
B.S. An, and S.C. Kim initiated and designed the study and played a role in recruitment of patients and sample collection. M.N. Park contributed towards acquisition of data. B.S. An, M.N. Park, S.C. Kim, Y.J. Lee, and J.K. Joo were involved in analysis and interpretation of the data. M.N. Park drafted the manuscript. All authors contributed to the revision of the manuscript and approval of the final draft.

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