Estrogen promotes the growth of decidual stromal cells in human early pregnancy

Jun Shao†, Ming-Qing Li†, Yu-Han Meng, Kai-Kai Chang, Ying Wang, Li Zhang, and Da-Jin Li*

Laboratory for Reproductive Immunology, Hospital & Institute of Obstetrics and Gynecology, Fudan University Shanghai Medical College, No. 413, Zhaozhou Road, Shanghai 200011, China

*Correspondence address. Tel: +86-21-63457331; Fax: +86-21-63457331; E-mail: djli@shmu.edu.cn

Submitted on December 16, 2012; resubmitted on April 26, 2013; accepted on April 29, 2013

Abstract: Interleukin-24 (IL-24) is a novel tumor suppressor gene, which has suppressor activity in a broad spectrum of human cancer cells. The present study aimed to elucidate the biological function of IL-24 and its receptors (IL-20R1, IL-20R2 and IL-22R1) in decidual stromal cells (DSCs) at human maternal–fetal interface. The DSCs behaviors in vitro were verified by viability (MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and apoptosis assay, respectively. Additionally, the effects of pregnancy-associated hormones on IL-24 and the effect of IL-24 on the correspondent functional molecules were investigated by ELISA, in-cell western and flow cytometry, respectively. Here we found that DSCs expressed IL-24 and its receptors, and IL-24 obviously suppressed the viability and stimulated the apoptosis in DSCs. On the contrary, both anti-IL-24 and IL-22R1 neutralizing antibodies markedly promoted growth and reduced the apoptosis. Estrogen but not progesterone could significantly decrease IL-24 but not its receptors, and these effects could be abolished by the antagonist of estrogen receptor beta (ERβ).

IL-24 significantly restricted the stimulatory effect of estrogen on the viability, anti-apoptosis, anti-apoptosis gene Bcl-2 and proliferation relative gene Ki-67 in DSCs. Our study has demonstrated that IL-24/IL-20R2/IL-22R1 axis is involved in the regulation of estrogen/ERβ signaling on the growth of DSCs through down-regulating IL-24.

Key words: estrogen receptor beta / IL-24 / DSCs / viability / apoptosis / early pregnancy

Introduction

Successful pregnancy requires coordinate progression of decidualization, placenta formation and embryo development. Decidualization is an ovarian steroid-induced remodeling/differentiation process of uterus essential for embryo implantation and placentaion (Logan et al., 2012), which is characterized by decidual stromal cells (DSCs) proliferation and differentiation. Decidualization of endometrial stromal cells is a prerequisite for successful embryo implantation and placentaion, which will sustain the pregnancy to term. Impaired decidualization can lead to implantation failure, spontaneous abortion or even pathological pregnancy such as pre-eclampsia or intrauterine growth restriction (Bulmer, 1995).

Estrogen and progesterone are essential steroid hormones that prepare the human uterine endometrium for embryo implantation and pregnancy (Brar et al., 1997; Telgmann and Gellersen, 1998; Carson et al., 2000), and activate transcription of target genes through binding their cognate receptors, the estrogen receptor (ER) and the progesterone receptor (PR). Following embryo attachment, de novo synthesis of estrogen by the decidual cells is critical for stromal differentiation (Logan et al., 2012). Moreover, hCG is also essential to sustain early pregnancy and is involved in the regulation of progesterone production, decidualization and cytotrophoblast differentiation.

Melanoma differentiation-associated gene-7 (MDA-7)/interleukin-24 (IL-24) is classified as a member of the IL-10 gene family because of the similarities in molecular biological characteristics (Jiang et al., 1995, 1996; Pestka et al., 2004). IL-24 gene is located at the 1q32 and expressed in primary melanoma and normal human’s immune-related tissues. The membrane receptors of IL-24, IL-20R1/IL-20R2 and IL-22R1/IL-20R2 are distributed in different cells and tissues (Sauane et al., 2008). IL-24 has been found to display ubiquitous anti-tumor biological properties such as inducing tumor cell apoptosis, inhibiting angiogenesis and enhancing the sensitivity to radiotherapy, without damage to the normal cells (Fisher et al., 2003; Su et al., 2003; Sauane et al., 2008).

† Jun Shao and Ming-Qing Li contributed equally to this work.
Chen and Zou have shown that IL-24 was expressed in villous column, trophoblasts, stroma and blood vessels at the maternal–fetal interface, and recombinant IL-24 could inhibit the invasion of trophoblasts cell line (TEV-1 cells) in vitro (Cheng and Zou, 2008). However, we still do not know whether IL-24 is involved in modulating biological behaviors of decidual cells. Therefore, the present study is undertaken to identify the expression of IL-24 and its receptors in DSCs at the maternal–fetal interface in human early pregnancy and further analyze the regulative actions of estrogen and IL-24 on DSCs.

**Materials and Methods**

**Decidual tissue collection, isolation and culture of DSC**

All procedures involving participants in this study were approved by the Human Research Ethics Committee of Obstetrics and Gynecology Hospital, Fudan University, and all subjects completed an informed consent to collect tissue samples.

Decidual tissues were from selective termination of the first-trimester pregnancy (age, 26.31 ± 3.72; gestational age at 7.89 ± 1.23 weeks; mean ± SD) for no medical reasons. The tissues from the first-trimester pregnancy were immediately put into ice-cold Dulbecco’s modified Eagle’s medium (DMEM high glucose; Gibco Grand Island, NY, USA), transported to the laboratory within 30 min after surgery and washed with Hank’s balanced salt solution for isolation of DSCs.

The DSCs were isolated according to our previous procedures (Li et al., 2010; Meng et al., 2012). These methods supplied >98% vimentin-positive and cytokeratin-negative DSCs.

**Immunostaining**

For immunohistochemistry, paraffin sections (5 µm) of early human decidua from normal pregnancy (n = 10) were dehydrated in grade ethanol, and incubated with 3% hydrogen peroxide and 1% bovine serum albumin/Tris-buffered saline (TBS) to block endogenous peroxidase. The samples were then incubated with mouse anti-human IL-24 monoclonal antibody (20 µg/ml; R&D Systems, Abingdon, UK), IL-20R1 antibody (20 µg/ml; R&D Systems), IL-20R2 antibody (SC-99085; 1:50; Santa Cruz Biotechnology, CA, USA), IL-22R1 antibody (20 µg/ml; R&D Systems) or mouse/rabbit IgG isotype (Sino-America Co., Ltd, Shanghai, China) overnight at 4°C in a humid chamber. After washing three times with TBS, the sections were overlaid with peroxidase-conjugated goat anti-mouse/rabbit IgG (Golden Bridge International, Inc., Beijing, China), and the reaction was developed with 3,3-diaminobenzidine and counterstained with hematoxylin. The experiments were repeated 3 times with 10 different samples.

**Treatment of DSC with the pregnancy-associated hormones**

The DSCs were seeded at 2 × 10^4 cells in 96-well plates and treated with various concentrations of 17β estradiol (E2) (10^-10 – 10^-7 M), progesterone (10^-10 – 10^-7 M) or HCG (1.25 – 10 kU/L) in phenol red-free DMEM (Gibco) containing 10% dextran-coated charcoal-treated FBS (HyClone, Logan, UT, USA). The controls were treated with 0.1% dimethyl sulfoxide (DMSO) (for E2 and progesterone) or media only (for HCG). In 48 h of culture, we analyzed the protein level of IL-24, IL-20R1, IL-20R2 and IL-22R1 in DSCs by in-cell western.

Subsequently, we treated the DSCs with E2 (10^-8 M), E2 plus ERα antagonist-MPP (10^-6 M, Tocris Bioscience, Bristol, UK), E2 plus ERβ antagonist-PHTPP (10^-6 M, Tocris Bioscience) or E2 plus ER antagonist-ICI182780 (10^-6 M, Tocris Bioscience) for 48 h, with 0.1% DMSO as the control, in-cell western was then performed to analyze the expression of IL-24 in DSCs.

**In-cell western**

According to the description by Egorina et al. (2006) and our previous procedures (Li et al., 2010, 2011, 2012), we used a newly set-up assay called in-cell western to determine the in-cell protein levels of IL-24, IL-20R1, IL-20R2 and IL-22R1. The procedure was as follows: hormones-treated DSCs in 96-well plate were immediately fixed with 4% paraformaldehyde for 20 min at room temperature. After washing with 0.1% Triton, these cells were blocked by 150 µl of LI-COR Odyssey Blocking Buffer (LI-COR Biosciences, Lincoln, Nebraska, USA) for 90 min at room temperature, and then incubated with mouse anti-human IL-24 (25 µg/ml), IL-20R1 (25 µg/ml), IL-22R1 (25 µg/ml) or with rabbit anti-human IL-20R2 (25 µg/ml) antibody, the primary rabbit or mouse (for IL-20R2 group) antibody with actin (Santa Cruz Biotechnology Shanghai Co., Ltd) as control. After overnight treatment at 4°C, the wells were incubated with corresponding second IRDyeTM700DX-conjugated affinity purified (red fluorescence) anti-mouse antibody and IRDyeTM800DX conjugated affinity purified (green fluorescence) anti-rabbit antibody, fluorescence antibodies recommended by the manufacturer (Rockland, Inc., Gilberts, PA, USA). This procedure was carried out in the dark. Images of the target gene were obtained by using the Odyssey Infrared Imaging System (LI-COR Biosciences German version of Ltd). The expression level of the correspondent molecules was calculated as the ratio of the intensity of target protein to that of actin. The experiments were carried out in triplicate, and repeated three times.

**IL-24 silence in DSCs**

According to the description by our previous method (Li et al., 2010), IL-24 in DSC was effectively silenced by siRNA transfection. These siRNAs and related non-silencing controls were synthesized by Invitrogen (Carlsbad, CA, USA). The successful gene knockdown was confirmed by ELISA.

**Enzyme-linked immunosorbent assay for determination of IL-24**

To evaluate the secretion level of IL-24, DSCs (1 × 10^5, 5 × 10^5 and 1 × 10^6 cells/well) were seeded in 6-well plates for 24, 48 and 72 h, and then the culture supernatant was harvested, centrifuged to remove cellular debris and stored at −80°C until being assayed by ELISA for IL-24 determination (R&D Systems).

DSCs (1 × 10^5 cells/well) in 24-well plates were treated with E2 (10^-10 – 10^-7 M) for 48 h, and then the IL-24 level in the supernatant of DSCs were detected by ELISA. Moreover, after transfection of siRNA, we collected the supernatant of transfected DSCs and analyzed the IL-24 level in the supernatant by ELISA.

**Cell viability assay, cell apoptosis assay and flow cytometry**

The isolated DSCs were re-suspended in phenol red-free DMEM with 10% dextran-charcoal-treated FBS, and seeded at a density of 5 × 10^5 cells/well in 96-well flat-bottom microwells for MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay or 2 × 10^5 cells/well in 12-well flat-bottom microwells (cell apoptosis assay and flow cytometry), and were stimulated with recombinant human (rh) IL-24 (0, 1, 10 or 100 ng/ml), anti-IL-24, IL-20R1 or IL-22R1 (0, 0.625, 1.25, 2.5 or 5 µg/ml) neutralizing antibody for 48 h. In addition, mouse isotype (Sino-America Co., Ltd) or media was added to some wells as a negative control. Then we treated DSCs with E2 (10^-8 M) or E2 plus ERβ antagonist-PHTPP (10^-6 M, Tocris Bioscience) for 48 h, with 0.1% DMSO as the control, cell viability was then performed to analyze the metabolic activity of DSCs.
as the control. The MTT assay, annexin V-FITC assay and flow cytometry were applied to evaluate the effects of estrogen and IL-24 on cell viability, apoptosis and relative molecules in DSCs, respectively.

For MTT cell viability assay, MTT reagent (20 μl; Sigma-Aldrich Shanghai Trading Co., Ltd, Shanghai, China) was added to each well of 96-well microplates and incubated at 37°C for 4 h. The medium was discarded and 150 μl DMSO was added to solubilize the reactive crystals. Absorbency was measured at a wavelength of 570 nm on an automatic microplate reader. The samples were run in triplicate and experiments were repeated four times.

As a marker of apoptosis, phosphatidylserine externalization was quantified by flow cytometry by using a commercially available annexin V-FITC apoptosis detection kit (Invitrogen) according to the manufacturer’s guideline. DSCs were trypsinized and collected. The cells were resuspended in PBS, and washed twice and resuspended in the kit-binding buffer (100 ml/pellet) containing annexin V solution (5 ml/pellet) and propidium iodide (2.5 mg/ml). Samples were incubated in the dark for 15 min, and the percent of annexin V-positive cells in DSCs was determined by FACS Calibur flow cytometry. The experiments were carried out in triplicate, and repeated three times.

Finally, flow cytometry was performed to analyze the expression of Bcl-2 (BD, NJ, USA), Ki67 (Biolegend, San Diego, USA) and proliferating cell nuclear antigen (PCNA; BD) in DSCs. Samples were analyzed in a FACS Calibur flow cytometer (Becton Dickinson, NJ, USA) by using Cellquest software (Becton Dickinson). Statistical analysis was conducted by using isotype matched controls.

**Statistics**

All values are shown as the mean ± SD. One-way ANOVA analysis of variance was used to detect the difference of IL-24, Bcl-2, Ki67 expression, viability and apoptosis in DSCs. Differences were considered as statistically significant at P < 0.05.

**Results**

**IL-24 and its receptors are co-expressed in DSCs**

Immunohistochemistry was used to localize IL-24 and its receptors (IL-20R1, IL-20R2 and IL-22R1) proteins in the paraffin section at first. As depicted in Fig. 1, our results showed moderate-to-strong staining for IL-24 in the cytoplasm and on the cytomembrane of DSCs in early pregnancy, and the cytoplasm and cytomembrane of the primary DSCs was also strongly positive for IL-20R2, and moderate staining for IL-20R1 and IL-22R1. Moreover, both decidual epithelial cells (DECs) and vascular endothelial cells from the decidua tissues in the first-trimester also expressed a certain level of IL-24, IL-20R1 and IL-20R2, not IL-22R1, respectively.

Subsequently, we found that DSCs secrete IL-24 in a time-dependent manner (P < 0.01) (Fig. 1b), after 72 h of culture, the concentration of IL-24 in the supernatant of DSCs was > 15 ng/ml (Fig. 1b).

![Figure 1](image_url) **Figure 1** IL-24 and its receptors are co-expressed in DSCs. (a) The expression of IL-24 and its receptors in decidua (n = 10) was analyzed by immunohistochemistry. IL-24, IL-20R1, IL-20R2 and IL-22R1 expression was localized to the cytoplasm and cytomembrane in the decidua. Original magnification: ×200 or ×400. (b) DSCs (n = 6) (1 × 10⁷, 5 × 10⁷ and l × 10⁸ cells/well) was seeded in 6-well plates for 24, 48 and 72 h, and then ELISA was performed to analyze the secretion level of IL-24 in the supernatant. Results were highly reproducible in three independent experiments. **P < 0.01 compared with 24 h control."
Collectively, these results suggest that IL-24 and its receptors are abundantly expressed in DSCs, which may participate in regulating the biological behaviors of DSCs at the maternal–fetal interface in an autocrine manner.

**IL-24 suppresses the viability and promotes the apoptosis of DSCs through binding IL-20R2/IL-22R1**

To evaluate whether IL-24 regulates viability and apoptosis of DSCs, primary DSCs were incubated with different concentrations of recombinant human IL-24 (rhIL-24), anti-IL-24, IL-20R1 or IL-22R1 neutralizing antibody for 48 h. Data presented in Fig. 2 showed that rhIL-24 inhibits viability ($P < 0.05$) (Fig. 2a), while it promotes apoptosis of DSCs ($P < 0.05$) (Fig. 2c and d). Conversely, anti-IL-24 or IL-22R1 antibodies could notably promote the viability ($P < 0.05$ or $P < 0.01$) (Fig. 2b) and reduce apoptosis ($P < 0.05$) (Fig. 2c and d) of DSCs in vitro. These results indicate that IL-24 derived from DSCs can modulate the viability and apoptosis of DSCs in an autocrine manner. Moreover, as shown, IL-20R1 treatment failed to influence the viability and apoptosis of DSCs ($P > 0.05$) (Fig. 2b–d), which suggests that the regulatory effect of IL-24 on the viability and apoptosis of DSCs is dependent on IL-20R2/IL-22R1 rather than IL-20R1/IL-20R2.

**Estrogen down-regulates IL-24 in DSCs**

Taking into account the important role of pregnancy-related hormones in the regulation of DSC differentiation and function, we further investigated the influence of estrogen, progesterone or HCG on IL-24 and its receptor of DSCs. At first, we treated DSCs with different concentrations of estrogen, progesterone or HCG for 48 h, and then performed MTT assay and apoptosis assay to detect the viability and apoptosis of DSCs. These results indicate that estrogen, progesterone or HCG can down-regulate IL-24 expression in DSCs in a dose-dependent manner.

---

**Figure 2** IL-24 suppresses the viability and promotes the apoptosis of DSCs through binding IL-20R2/IL-22R1. Primary DSCs ($n = 6$) were incubated with different concentrations of rhIL-24 [0, 1, 10 and 100 ng/ml for (a); 100 ng/ml for (c and d)], anti-IL-24, IL-20R1 or IL-22R1 neutralizing antibody [0, 0.625, 1.25, 2.5 and 5 μg/ml for (b); 2.5 μg/ml for (c and d)] for 48 h, mouse isotype or media was added to some wells as a negative control, and then we performed MTT assay (a and b) and apoptosis assay (c and d) to detect the viability and apoptosis of DSCs. These pictures are representatives of three individual experiments. Error bars depict the standard deviation of the mean. *$P < 0.05$, **$P < 0.01$ compared with the control. ***$P < 0.01$ compared with the control [for anti-IL-22R1 neutralizing antibody treatment in (b)].
concentrations of 17-β estradiol (E2) (10^{-10}–10^{-7} M), progesterone (10^{-10}–10^{-7} M) or HCG (0, 1.25, 2.5, 5 and 10 kU/L) for 48 h. Then we found that E2 decreases the expression and secretion of IL-24 in DSCs in a dosage-dependent manner (P < 0.05 or <0.01) (Fig. 3a, left and b). The optimal concentration of E2 was 10^{-8} M. However, E2 did not change the levels of IL-20R1, IL-20R2 or IL-22R1 (P > 0.05) (Fig. 3a, left). In addition, both progesterone and HCG had no effect on IL-24 expression (P > 0.05) (Fig. 3a, middle and right). Our results indicate that estrogen can down-regulate the expression of IL-24, and therein participate in regulating the biological behavior of DSCs at the maternal–fetal interface.

**Estrogen-ERβ axis mediates down-regulation of IL-24 in DSCs**

To analyze the possible mechanism of estrogen on the IL-24 in DSCs, we used in-cell western to measure the expression of IL-24 in DSCs treated with E2 (10^{-8} M), E2 plus ERα antagonist-MPP (10^{-6} M), E2 plus ERβ

---

**Figure 3** Estrogen down-regulates IL-24 in DSCs. (a) DSCs (n = 6) were incubated with 17beta-estradiol (10^{-10}–10^{-7} M) (left), progesterone (10^{-10}–10^{-7} M) or HCG (0, 1.25, 2.5, 5 and 10 kU/L) for 48 h, and then in-cell western was performed to evaluate the expression of IL-24, IL-20R1, IL-20R2 and IL-22R1 in DSCs. (b) The supernatant of 17beta-estradiol (10^{-10}–10^{-7} M)-treated DSCs (n = 6) was collected, and ELISA was used to analyze IL-24 secretion level: IL-24, IL-20R1, IL-20R2 and actin (for IL-20R2 group) (red); IL-20R2 and actin (green). The y-axis represents the ratio of the intensity of target proteins to that of actin. These pictures are representatives of three individual experiments. Error bars depict the standard deviation of the mean. *P < 0.05, **P < 0.01 compared with the control.
antagonist-PHTPP (10^{-6} M) or E2 plus ER antagonist-ICI182780 (10^{-6} M) for 48 h. As shown in Fig. 4, estrogen significantly inhibited IL-24 levels (P < 0.05) (Fig. 4a), and these effects could be abrogated by PHTPP or ICI182780 but not MPP. Collectively, our data indicate that estrogen–ERβ axis is involved in decreasing the IL-24 expression, and may further modulate the biological behaviors of DSCs.

**Figure 4** Estrogen-ERβ axis mediates down-regulation of IL-24 in DSCs. In-cell western was used to analyze the expression of IL-24 in DSCs (n = 6) treated with E2 (10^{-8} M), E2 plus ERα antagonist-MPP (10^{-6} M), E2 plus ERβ antagonist-PHTPP (10^{-6} M) or E2 plus ER antagonist-ICI182780 (10^{-6} M) for 48 h. Here IL-24 (red); actin (green). The y-axis represents the ratio of the intensity of IL-24 to that of actin. These pictures are representatives of three individual experiments. Error bars depict the standard deviation of the mean. **P < 0.01 compared with the vehicle control. *P < 0.05 compared with MPP treatment.**

**Estrogen promotes viability, anti-apoptosis and the expression of Bcl-2 and Ki67 in DSCs through down-regulating IL-24 expression**

To test whether estrogen modulates the biological function by decreasing IL-24 secretion, we analyzed the viability and apoptosis of DSCs after treatment with E2 (10^{-8} M) or rhIL-24 or E2 plus rhIL-24 for 48 h. As shown in Fig. 5, estrogen could markedly stimulate the viability and anti-apoptosis of DSCs (P < 0.05) (Fig. 5a and b). In contrast, IL-24 not only decreased the DSCs viability and promoted apoptosis (P < 0.05) (Fig. 5a and b), but also reversed the effect of estrogen on the viability and apoptosis of DSCs.

We next evaluated the expression of anti-apoptosis gene Bcl-2 and proliferation relative gene Ki67 and PCNA by flow cytometry, and found that IL-24 could also abolish the increase of Bcl-2 and Ki67 induced by estrogen (P < 0.05) (Fig. 6a and b). However, both estrogen and IL-24 failed to change PCNA expression level (P > 0.05) (Fig. 6a and b).

Subsequently, we have successfully silenced IL-24 of DSCs after transfection with siRNA 3 (P < 0.01) (Fig. 6c). Both estrogen and IL-24 silence obviously promoted the expression of Bcl-2 and Ki67 in DSCs, although combination of estrogen and IL-24 silence had no synergistic effect (Fig. 6d). However, rhIL-24 could inhibit these effects induced by estrogen, which suggested that the effect of estrogen on Bcl-2 and Ki-67 is achieved partly through down-regulating IL-24 production of DSCs.

**Figure 5** Estrogen promotes viability and anti-apoptosis of DSCs through down-regulating IL-24 expression. We evaluated the viability and apoptosis of DSCs (n = 6) after treatment with E2 (10^{-8} M), rhIL-24 (100 ng/ml) or E2 plus rhIL-24 for 48 h by MTT (a) or apoptosis assay (b). Error bars depict the standard deviation of the mean. *P < 0.05 compared with the vehicle control. **P < 0.05 compared with estrogen treatment alone.

**Discussion**

Embryo implantation into the maternal uterus is a crucial step for the successful establishment of mammalian pregnancy. Following the attachment of embryo to the uterine luminal epithelium, uterine stromal cells undergo steroid hormone-dependent decidualization, which is characterized by stromal cell proliferation and differentiation. The mechanisms underlying steroid hormone-induced stromal cell proliferation and differentiation during decidualization are still poorly understood.
A lot of research focused on progesterone (Kim et al., 2005; Kodama et al., 2010; Menkhorst et al., 2010) in regulating biological function of DSCs. However, as shown in Fig. 7, the key finding of the present study is that down-regulation of IL-24 induced by estrogen but not progesterone can promote the proliferation and reduce the apoptosis of DSCs by interacting with ERβ but not ERα. In addition, we have found that these effects are dependent on the elevated expression of downstream molecules, such as Bcl-2 and Ki67, in an IL-20R2/IL-22R1-dependent manner. These findings suggest that estrogen has an important role in regulating IL-24 expression, and subsequently stimulating the proliferation and growth of DSCs. Moreover, we have found that other cells in decidua, such as DECs and vascular endothelial cells may regulate the growth of DSCs through secreting IL-24 in a paracrine manner, and jointly maintain the appropriate growth state of DSCs at the maternal–fetal interface, and further participate in the establishment and maintenance of normal pregnancy.

Estrogen action is mediated by two receptors, ERα and ERβ, the two transcription factors of a large family of nuclear receptors. About 40–60% of ovarian cancers express ERα (Greenlee et al., 2000), but it is intriguing to observe that only a small proportion of them will benefit from anti-estrogen therapy (Hatch et al., 1991). Smuc and Rizner established that ERβ in 16 endometrial cancer samples was lower than that of the adjacent normal endometrium (Smuc and Rizner, 2009). However, the role of ERβ remains poorly understood, although recent research suggests it to be different from that of ERα, and plays an important role in ovarian carcinogenesis (Bossard et al., 2012; Häring et al., 2012) and regulation of insulin-like growth factor-1 (IGF-1) signal of lung cancer (Tang et al., 2012). As we all know, IGF signaling pathway plays a crucial role in...
the functions of decidual endometrial cells (Ganeff et al., 2009). Therefore, we propose that estrogen may down-regulate IL-24 and further influence the growth, differentiation and remodeling through ERβ/IGF signaling, but the specific mechanism needs further elucidation.

Based on its structure, chromosomal location, and biochemical properties, mda-7 has been classified as a member of the IL-10 family of cytokines that includes IL-10, IL-19, IL-20, IL-22 and IL-26 and has been designated IL-24 (Pestka et al., 2004; Chaiken and Williams, 1996). IL-24 stimulates growth and suppresses apoptosis in a broad spectrum of human cancer cells, including those from melanoma, malignant glioma, fibrosarcoma and carcinomas of the breast, cervix, colon, rectum, liver, lung, ovary and prostate, without exerting any deleterious effects on their normal counterparts (Lebedeva et al., 2003; Yacoub et al., 2003; Su et al., 2005; Lebedeva et al., 2007).

IL-24 binds to currently recognized MDA-7/IL-24 receptor complexes consisting of two sets of heterodimeric chains, IL-20R1/IL-20R2 or IL-22R1/IL-20R2 (Dumoutier et al., 2001; Wang et al., 2002; Parrish-Novak et al., 2002). Most human tissues express the IL-20R1/IL-20R2 complex. However, IL-22R1 is found in a few tissues lacking IL-20R2, such as adult and fetal liver, small intestine, colon and pancreas. Our present work has found that human DSCs co-express IL-24, IL-20R1, IL-20R2 and IL-22R1. Moreover, the inhibitory effect of IL-24 on the viability and anti-apoptosis can be reversed by blocking IL-22R1 not IL-20R1, although DSCs predominantly express IL-20R1. Further research is needed to evaluate the different function of IL-20R1/IL-20R2 and IL-22R1/IL-20R2 in DSCs.

Previous study has revealed that IL-24 over-expression induces autophagic death in glioma as well as an initial cytoprotective autophagy in specific target cells, which switches to apoptosis in prostate cancer (Park et al., 2008a, b; Bhutia et al., 2010; Bhutia et al., 2011). Recently, it has been reported that IL-24 induces cancer cells selective anti-tumor activity through endoplasmic reticulum stress by inhibiting the translation of the anti-apoptotic protein Mcl-1 in prostate cancer (Dash et al., 2010). A nuclear form of clusterin (CLU) protein (nCLU) generated by IL-24 not only causes G2/M phase arrest followed by apoptosis, but also alters cytoskeleton and results in decreased cell migration (Bhutia et al., 2012). IL-24 also decreases cell proliferation and colony formation with apoptosis induction CLU over-expressing prostate cancer cells (Bhutia et al., 2012). Moreover, IL-24 can decrease the expression of anti-apoptosis gene Bcl-2 and proliferation relative gene Ki-67, and further modulate the growth and apoptosis of DSCs, which partly echoed the results from Tian et al. (2012).

It is well documented that IL-24 can induce the secretion of high levels of IFN-γ, IL-6 and tumor necrosis factor-a and low levels of IL-1, IL-12 and granulocyte macrophage colony stimulating factors from human PBMCs (Caudell et al., 2002; Sauane et al., 2008). It is suggested that human IL-24 acts as an immune regulatory molecule to mediate the induction of Th1 type cytokines (Ma et al., 2011). Exogenous IL-24 had a protective effect against Salmonella typhimurium infection in mice through the stimulation of early production of the Th1 cytokines IFN-γ and IL-12 by neutrophils, which in turn activate CD8+ T cells (Ma et al., 2009). The maternal–fetal interface exhibits a Th2 bias characterized by IL-4, IL-5 and IL-10 secretion during pregnancy (Guo et al., 2010). Therefore, based on these report, we propose that DSCs-derived IL-24 can stimulate the secretion of Th1 type cytokine and further induce Th1 bias in a paracrine manner at the maternal–fetal interface. However, estrogen may promote Th2 polarization and maintain the Th2-dominant milieu possibly through inhibiting IL-24 production.

Therefore, our finding indicates that the IL-24 produced by first-trimester human DSCs decreases the expression of Bcl-2 and Ki67, and inhibits growth and promotes apoptosis of DSCs through binding IL-22R1/IL-20R2. In contrast to progesterone and HCG, estrogen not only enhances the survival and anti-apoptosis of DSCs in an autocrine manner, but also participates in the complicated maternal–fetal
免疫调节通过调节ERβ/IL-24信号通路。这些整合性效应将促进DSCs的生长和正常妊娠的建立。进一步的研究将阐明IL-24及其受体在DSCs和其他细胞中的作用，这将有助于更深入地理解母胎界面的细胞间交流，并最终导致对母胎界面交叉调节机制的理解。

**Authors’ roles**

J.S. conducted all experiments and prepared the figures and the manuscript. Y.-H.M., K.-K.C., Y.W. and L.Z. assisted with immunohistochemistry, flow cytometry assay and in-cell western analysis, respectively. M.-Q.L. and D.-J.L. initiated and supervised the project and edited the manuscript. All the authors were involved in writing the manuscript.

**Funding**

This study was supported by Major International Joint Research Project of NSF of China 30910103909, NSF of China 31270969 to D.-J.L.; National and Shanghai Leading Academic Discipline Project (2111XK22) to D.-J.L.; Program for Outstanding Medical Academic Leader of Shanghai to D.-J.L.; NSF of China 31101064 to M.-Q.L.; Research Program of Shanghai Health Bureau to M.-Q.L. (2011Y080), Ministry of Education Research Fund for Doctoral Program to M.-Q.L. (20110071120092) and Program for ZhouXue of Shanghai Health Bureau to M.-Q.L. (2011Y080), Ministry of Education Research Fund for Doctoral Program to M.-Q.L. (20110071120092) and Program for ZhouXue of Fudan University to M.-Q.L.

**Conflict of interest**

None declared.

**References**


Lebedeva IV, Su ZZ, Emmad L, Kolomeyer A, Sarkar D, Kitada S, Waxman S, Reed JC, Fisher PB. Targeting inhibition of K-ras enhances...