Chemokine CXCL16, a scavenger receptor, induces proliferation and invasion of first-trimester human trophoblast cells in an autocrine manner

Yu Huang, Xiao-Yong Zhu, Mei-Rong Du, Xia Wu, Ming-Yan Wang and Da-Jin Li

Laboratory for Reproductive Immunology, Hospital and Institute of Obstetrics and Gynecology, Shanghai Medical College, Fudan University, Shanghai, China

BACKGROUND: The aim of this study was to investigate whether CXCL16/CXCR6, a newly identified chemokine pair, is expressed in first-trimester human placenta and whether they affect the trophoblast cell biology, since we have found CXCR6 highly transcribed in first-trimester human trophoblast cells previously. METHODS: We analysed the transcription and translation of CXCR6 and CXCL16 in purified first-trimester human trophoblast cells by real-time RT-PCR and immunochemical staining. We then examined the kinetic secretion of CXCL16 in the supernatant of primary-cultured trophoblasts by enzyme-linked immunosorbert assay. We further investigated effects of CXCL16 on the proliferation and invasion of trophoblast cells in vitro. RESULTS: We found the chemokine pair CXCL16/CXCR6 was transcribed and translated in first-trimester trophoblast cells and JAR line. In addition, the primary-cultured trophoblasts secreted CXCL16 spontaneously and continuously in 100-h culture. Treating trophoblasts with CXCL16 induced marked proliferation and invasion in vitro. CONCLUSION: The findings from this study have demonstrated for the first time that CXCR6 and CXCL16 are co-expressed in first-trimester human trophoblast cells and stimulate their proliferation and invasion in an autocrine/paracrine manner. It suggests that CXCL16 plays important roles in human extravillous cytotrophoblast invasion and placentation.

Key words: chemokine/chemokine receptor/invasion/proliferation/trophoblast cell

Introduction

As a key cell of human placenta, fetal cytotrophoblast plays an important role in successful pregnancy. Its proliferation, differentiation, as well as invasion during implantation are a series of tightly controlled processes of intercellular signalling that are mediated by growth factors, cytokines and hormones (Wegmann and Guilbert, 1992; McMaster et al., 1994; Mellor and Munn, 2000; Norwitz et al., 2001). These cytotrophoblast cells differentiate along either the villous or the extravillous trophoblast (EVCT) pathway (Tarrade et al., 2001). At the tip of the anchoring villi, they proliferate and differentiate into EVCTs with invasive properties. On one hand, EVCT invades into decidua to form giant cells with two or three nuclei or replaces the uterine spiral arterial endothelial cells to remodel the placenta blood vessels; on the other hand, the cytotrophoblasts on the border layer of the floating villi differentiate by cell–cell fusion into multinucleate syncytiotrophoblasts (ST), which cover floating villi, and mediate substance exchange between fetus and mother. Moreover, they exert endocrine functions of placenta specifically, such as the expression of hCG, leptin, hPL and INSL4 (Handwerger, 1991; Jameson and Hollenberg, 1993; Laurent et al., 1998; Guibourdenche et al., 2000).

Chemokines are small molecular polypeptides. According to the motif displayed by the first two cysteines at the amino end, they have been classified into four families: CXC, CC, XC and CX3C. The hallmark of chemokines is their ability to induce chemotaxis (Luster, 1998; Rossi and Zlotnik, 2000). However, chemokines are also involved in cellular proliferation, differentiation, apoptosis, angiogenesis, hematopoiesis, pro-tumour, antitumour and inflammatory diseases (Taub et al., 1996; Arici et al., 1998; Koch, 1998; Garcia-Velasco et al., 1999). To date, trophoblasts have been found to produce several chemokines, such as CCL1(I309), CCL3(MIP-1α), CCL9, CXCL6 and CXCL12, which contribute to decidual leukocyte recruitment and trophoblast proliferation (Douglas et al., 2001; Drake et al., 2001; Moussa et al., 2001; Red-Horse et al., 2001; Tsuda et al., 2002). Trophoblasts also express some chemokine receptors, particularly CXCR4 and CXCR6, which are known to function as co-receptors of HIV-1 and HIV-2 (Mognetti et al., 2000; Wu et al., 2004). These suggest that chemokines are an integral part of placentation, playing important roles in both immune and non-immune functions of placenta.
CXCR6, initially described under the names of Bonzo, STRL 33 and TYMSTR, is a newly characterized chemokine receptor expressed by subsets of memory/effector T cells (Kim et al., 2001), NK cells (Unutmaz et al., 2000), NKT cells (Kim et al., 2002; Johnston et al., 2003) and by plasma cells (Nakayama et al., 2003). CXCL16, the ligand for CXCR6, is one of the only two known members of the plasma membrane chemokines, consisting of chemokine domain followed by a glycosylated mucin-like stalk and a single transmembrane helix followed by a short cytoplasmic tail. CXCL16 is selectively expressed on DCs and bone marrow plasma cells via its receptor CXCR6, but the transmembrane molecule functions as a scavenger receptor for OX-LDL, phosphatidylserine, dextran sulphate and bacteria and can uptake OX-LDL and phagocytose bacteria as well as adhere to CXCR6-expressing cells (Shimaoka et al., 2004).

Our previous research showed that the purified first-trimester human trophoblast cells transcribed most chemokine receptors, particularly CXCR4 and CXCR6, and we have discussed the functions of CXCL12 and CXCR4 in detail (Wu et al., 2004, 2005). The aim of this study was to elucidate the expression and function of chemokine receptor CXCR6 and its sole ligand CXCL16 in human placenta and trophoblast cells.

Materials and methods

Human placental tissue collection

Informed consent was obtained from all patients from whom tissue was collected. Placentas were from elective termination of pregnancy (gestational age, 7–9 weeks) at the Obstetrics and Gynecology Hospital of Fudan University. The tissues were immediately collected into Dulbecco’s modified Eagle’s medium (DMEM; Life Technologies, Merelbeke, Belgium) high glucose and washed in Hanks’ balanced salt solution (HBSS) for trophoblast cell isolation.

Isolation and primary culture of the first-trimester human trophoblast cells

The detailed description of the procedure used to isolate trophoblast from first-trimester placentas has been reported in the literature (Wu et al., 2004). Briefly, the obtained placenta tissue was digested in four cycles of 10 min by 0.25% trypsin-50 000 U/ml DNase type I (Life Technologies) at 37°C with gentle agitation. Cell suspension was carefully layered over a discontinuous Percoll gradient (65 to 20%, in 20% steps) and centrifuged. The middle layer (density of 1.042–1.068 g/ml) was removed and washed with DMEM–high glucose medium. Cells were diluted to 5 × 10⁶ cells/ml and maintained in DMEM–high glucose complete medium (2 mM glutamine, 25 mM HEPES, 100 U/ml penicillin and 100 μg/ml streptomycin), supplemented with 20% FBS (Gibco) and incubated in 5% CO₂ at 37°C.

Human choriocarcinoma cell line JAR was cultured in DMEM complete medium supplemented with 10% FBS in 5% CO₂ at 37°C.

Quantitative RT-PCR

Trophoblast cells were isolated from pools of first-trimester placentas. Total RNA from trophoblast cells or JAR line was extracted reversely transcribed as previously described (Wu et al., 2004). cDNA (5 μl) was amplified by real-time PCR in a final volume of 50 μl containing 25 μl of Hot-start PCR Master Mix (RuiCheng Bio., Shanghai, China) and 200 nM of each primer probe. The following primers and probes were used: human CXCL16, FW: 5-GGC CCA CCA GAA GCA TTT AC-3, RW: 5-CTG AAG ATG CCC CCT CTG AG-3 and TM: 5-CCT ACC AGC CCC CCA ATT TCT CAG G-3; human CXCR6, FW: 5-ATG CCA TGA CCA GCT TTC ACT-3, RW: 5-TTA AGG CAG GCC CTC AGG TA-3 and TM: 5-ACC ATC ATG GTG ACA GAG GCC ATC GT-3 (Abel et al., 2004); human cyclophilin A, FW: 5-GTC AAC ACC ACC GTG TTC TT-3, RW: 5-CTG CTG TCT TTG GGA CCT TGT-3 and TM: 5-AGC TCA AAG GAG GAC GGC GCC A-3 (DaAn Gene, Guangzhou, China). Each sample was analysed in duplicate (2 min at 50°C, 20 min at 95°C, followed by 40 cycles of 0.5 min at 95°C and 1 min at 60°C using ABI Prism 7000 Sequence Detector). The PCR amplification was correlated against a standard curve. The reactions were performed in MicroAmp Optical 96-well reaction plates.

Immunocytochemistry

In 48 h of culture, VCT and JAR cells were fixed in 4% PFA for 20 min at room temperature, washed in PBS and permeabilized for 4 min in 0.3% Triton X-100-PBS. The cells were then incubated with 7% horse serum in PBS for 30 min to reduce non-specific binding. Primary antibodies diluted in PBS containing 1% BSA were added. Antihuman cytokerin-7 monoclonal antibody (Zymed Laboratories, USA) and antihuman vimentin monoclonal antibody (Sino-America, Shanghai, China) were used as markers for cells of trophoblast lineage. Antihuman CXCR6 monoclonal antibody and CXCL16 antibody (R & D Systems, Abingdon, UK) were administered to detect whether trophoblast cells express CXCR6 and CXCL16, respectively. The cells were incubated with primary antibody (or matched control immunoglobulin) overnight at 4°C and then incubated with a biotinylated secondary antibody for 30 min at room temperature. Streptavidin-HRP was applied for another 30 min at room temperature (avidin–biotin histostain kit; Sino-America). The slides were stained with 3,3'-diaminobenzidine (DAB) and counterstained with haematoxylin. The experiments were repeated five times.

Immunohistochemical staining

Cryosections of human placental villous tissues were fixed with cold acetone for 5 min. They were then blocked with methanol containing 3% H₂O₂, sequentially 7% horse serum normal serum, and incubated with anti-CXCR6 and anti-CXCL16 antibodies, respectively, overnight at 4°C. The sections were then treated with appropriate avidin–biotin histostain kit according to the manufacturer’s instructions (Sino-America). Slides were stained with DAB and counterstained with haematoxylin. Immunohistochemical results were evaluated by a pathologist. The experiments were repeated five times.

Enzyme-linked immunosorbent assay

Purified trophoblast cells were seeded in a 24-well plate (600 μl/well) at various densities of 1 × 10⁴, 5 × 10⁴, 1 × 10⁵, 5 × 10⁵ and 5 × 10⁶ cells/ml. Supernatants of trophoblast cell cultures were collected after 12, 24, 36, 48, 60, 72 and 100 h of culture. Each supernatant was centrifuged at 2000g and stored at −80°C. Human CXCL16 ELISA kit (R & D Systems) was used to measure chemokine production in each supernatant according to the manufacturer’s instructions. The CXCL16 assay demonstrated a sensitivity of 0.007 ng/ml and an intra-assay coefficient of variation of 3.5 ~ 4.9%.

Cell proliferation assay

[³H]-thymidine incorporation as well as MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma Chemicals, St Louis,
MO, USA] assay was applied to measure and compare the effects of CXCL12 and CXCL16 on trophoblast cell proliferation. The isolated trophoblast cells were resuspended in DMEM with 10% FBS and seeded at a density of 2 × 10^4 to 3 × 10^5 cells/100 μl/well in 96-well, flat-bottom microplates precoated by Matrigel. After 24 h of culture, the medium was replaced with DMEM containing 1% FBS and then the culture was carried on for 12 h before treatment. The medium was removed once again, and the cells were stimulated with recombinant human CXCL1, 10, 50, 100, 200, 300, 400 and 500 ng/ml or rhCXCL16 (100 ng/ml), rhSDF-1α (100 ng/ml) and neutralizing antibodies to CXCL12 (50 μg/ml), CXCL16 (30 μg/ml) and CXCR4 (20 μg/ml) (agents from R & D Systems), respectively, or a combination of any two at 37°C for 48 h. In addition, goat IgG (30 μg/ml) or mouse isotype (20 μg/ml) (Sino-America) was added to some wells as negative control.

For the [³H] thymidine assay, [³H] thymidine (0.5 μCi/well) was added to each well and incubated for an additional 6 h. The cells were harvested onto a glass-fibre paper using a semiautomatic cell harvester, and thymidine incorporation was measured in a liquid scintillation counter.

For the MTT assay, 20 μl of the MTT reagent was added to each well and incubated at 37°C for 4 h. The medium was decanted, and 100 μl of ethanol was added to solubilize the reactive crystals. Absorbency was measured at a wavelength of 570 nm on an automatic microplate reader.

The result is expressed as the ratio of the cpm value (or optical density) of cells with treatment to that without treatment. The experiments were repeated four times.

**Matrigel invasion assay**

The Matrigel invasion assay was carried out as previously described with slight modification (Sato et al., 2002). We used Transwell plates (6.5 mm diameter) (Corning, Corning, NY, USA) containing polycarbonate filters with 8.0 μm pore size. The upper surface of the filter was coated with 5 μl pure Matrigel and air-dried the filter aseptically.

Before use, the Matrigel was rehydrated with 100 μl warm DMEM for 2 h. The purified trophoblast cells (1 × 10^5 in 200 μl DMEM with 1% FBS) were plated in upper chamber. CXCL16 at various final concentrations of 0, 1, 10, 50, 100 and 200 ng/ml and rhCXCL16 at a concentration of 100 ng/ml with its neutralizing antibody (30 μg/ml) were added, respectively. The lower chamber was filled with 800 μl DMEM with 1% FBS. The cells were allowed to invade for 12 h in 5% CO₂ at 37°C. Then, we removed the cells attached to the upper surface of the filter by scrubbing with a cotton swab. The cells remaining on the lower surface were fixed in methanol for 10 min at room temperature and stained with haematoxylin. For quantification, the cells that had migrated to the lower surface were counted under a light microscope in five predetermined fields at a magnification of ×200. The assay was repeated three times, and the results are expressed as a percentage to the mean number of three controls.

**Statistics**

Comparisons between controls and various treatments were performed to measure cell proliferation and cell invasion by analysis of variance with post hoc Dunnett’s t-tests. All the error bars in the figures indicate SE. The differences were accepted as significant at P < 0.05.

**Result**

**Quantitative analysis of mRNA for CXCL16 and CXCR6 in first-trimester human trophoblasts**

To validate the gene levels of CXCL16 and CXCR6 in the trophoblast, we analysed the gene expression intensities for both isolated trophoblast and JAR line by the quantitative real-time RT-PCR. Figure 1 shows the mRNA copy for CXCL16 (3.45 × 10^7), CXCR6 (1.15 × 10^7) and Cyclophilin A (3.78 × 10^6) in first-trimester trophoblast. Their mRNA were also detected in JAR cells. Three independent experiments were performed (including 15 placental samples), and the results were similar. The quantitative RT-PCR showed that first-trimester human trophoblasts expressed a 102-fold and threefold increase in CXCL16 and CXCR6 mRNA, respectively, than JAR cells.

**Expression of CXCR6 and CXCL16 in human first-trimester trophoblast**

In 48 h of culture, we verified the expression of cytokeratin-7 and vimentin in isolated trophoblast cells (Figure 2). These cells isolated were almost all stained for cytokeratin-7, whereas no cells were found to be stained with antivimentin antibody. We observed that the purity of isolated trophoblast cells was above 95%. Figure 3 shows that trophoblast and JAR cells stained positively for CXCR6 and CXCL16. The cells incubated with the isotypic control immunoglobulin showed no staining. In culture, we found that some cytotrophoblasts fused.
to form multinucleated ST, and JAR cell clustered because of their active proliferative character. Cytotrophoblast and ST both stained positively for CXCL16 and CXCR6.

Expression of CXCR6 and CXCL16 in first-trimester placental villous tissue
The identification of trophoblast cells was confirmed by immunohistochemistry with anticytokeratin-7 and antivimentin antibody. We analysed the expression of CXCR6 and CXCL16 in placental tissues by immunohistochemistry. The results showed specific brown-coloured staining in the cytoplasm and the cytomembrane of villous cytotrophoblasts, STs and EVCT (Figure 4).

Secretion of CXCL16 by first-trimester trophoblast cells in vitro
Trophoblast cells had a weak proliferative ability and a short survival period in vitro. When seeded on Matrigel-coated plate, they can survive for more than 1 week. We cultured the isolated trophoblast in Matrigel-precoated 24-well plate for 12–100 h at different densities and examined the release of soluble CXCL16 in culture medium of trophoblasts by ELISA every 12 h. Primary-cultured trophoblast cells secreted CXCL16 constitutively at nearly a constant rate, though 70% of the trophoblasts were confluent after 72 h of culture. The production of CXCL16 correlated positively to the plating density. When the trophoblast cells were seeded in $1 \times 10^6$ cells/ml, the accumulated concentration of CXCL16 was $2.690 \pm 0.180$ ng/ml after culture for 100 h (Figure 5). The $100 \text{ h}$-accumulated levels of CXCL16 were $0.190 \pm 0.014$ ng/ml and $0.170 \pm 0.04$ ng/ml, respectively, when seeded in $5 \times 10^4$ cells/ml and $1 \times 10^4$ cells/ml. When seeded in $5 \times 10^3$ cells/ml, the level of CXCL16 was undetectable since few cells survived.

CXCL16 induces cellular proliferation of first-trimester human trophoblast
We then investigated effects of CXCL16 on cell proliferation of human first-trimester trophoblast. The results showed that CXCL16 induced human trophoblast cell proliferation in a dose-dependent manner (Figure 6A). When the concentrations of CXCL16 ranged from 100 to 500 ng/ml, the number of viable trophoblasts increased significantly compared to the negative control. Meanwhile, addition of U0126 or neutralizing antibody to CXCL16 inhibited completely the stimulatory effect of exogenously administered CXCL16. The unavailability of the neutralizing antibody to CXCR6 refrained us from confirming our result through blocking CXCR6 of trophoblast cells. When CXCL16 was absent and trophoblast cells were
were collected and measured after 12, 24, 36, 48, 60, 72, 100 h of culture. The results showed CXCL16 accumulation in the medium during the course of culture. Each point represents the mean ± SD obtained from duplicate dishes, and the data are representative of two independent experiments.

treated with CXCL16 antibody alone, the viability of these cells was significantly lower than that of the cells without such treatment (Figure 6B).

In a previous study, we found that trophoblast cells secreted CXCL12 and induced proliferation in an autocrine manner. In this research, we confirmed the results (Figure 6C) and compared the proliferative effects induced by chemokine CXCL16 and CXCL12. Either CXCL16 or CXCL12 alone stimulated the trophoblast proliferation; however, treatment with a combination of CXCL16 and CXCL12 appeared to have a stimulatory effect on proliferation similar to treatment with CXCL16 or CXCL12 alone (Figure 6D). Furthermore, addition of neutralizing antibodies of CXCL12 and CXCR4 was unable to reduce the stimulatory effect of CXCL16, which suggested that CXCL16 and CXCL12 stimulated the proliferation of trophoblast in an independent extracellular way.

CXCL16 induced cellular invasion of first-trimester human trophoblast

To evaluate the role of CXCL16 in trophoblast invasion, Matrigel invasion assay was performed. As shown in Figure 7A, CXCL16 caused a specific dose-responsive increase in trophoblast invasion. This stimulatory effect on cell invasion was first seen at 10 ng/ml CXCL16 (P < 0.05) and was approximately 2.8-fold higher at 200 ng/ml of CXCL16. The stimulation of trophoblast invasion was readily apparent upon microscopic visual examination of the lower surface of the filter (Figure 7B–D). The specificity of CXCL16 action on trophoblast invasion was further demonstrated by a complete inhibition of CXCL16-stimulated trophoblast invasion by CXCL16 neutralizing antibody (Figure 7E).

Figure 5. The accumulated concentration of CXCL16 in culture medium of first-trimester trophoblast cells was examined by ELISA. Purified trophoblast cells were seeded at 1 × 10⁴, 5 × 10⁴, 1 × 10⁵, 5 × 10⁵, 5 × 10⁶, 5 × 10⁷ cells/ml. Supernatants of trophoblast cell cultures were collected and measured after 12, 24, 36, 48, 60, 72, 100 h of culture. The results showed CXCL16 accumulation in the medium during the course of culture. Each point represents the mean ± SD obtained from duplicate dishes, and the data are representative of two independent experiments.

Discussion

In the present study, we showed that CXCR6 and CXCL16 are expressed in first-trimester human trophoblast cells using immunostaining and quantitative RT-PCR analysis. Both cytotrophoblast and ST, especially extravillous cytotrophoblast, expressed CXCL16 and CXCR6 protein. Moreover, we analysed JAR cells in the same way and found similar results. In addition, primary-cultured trophoblast cells continually secreted soluble CXCL16 into the culture medium when cultured for 100 h. It seems that the confluence of trophoblasts has no impact on the yield of the chemokine, which suggests that both cytotrophoblasts and terminal trophoblasts could produce CXCL16. As the expression of CXCR6 may render trophoblasts a target for autocrine and paracrine regulation by locally produced CXCL16, we are interested in exploring the possible roles of CXCR6/CXCL16 in cellular proliferation and cell invasion of the trophoblasts.

Coinciding with our prediction, CXCL16 is in fact an effective inducer to proliferation of human first-trimester trophoblasts. When the extrinsic concentration of CXCL16 was up to 500 ng/ml, the stimulatory effect was more than fourfold of the control. Although the neutralizing antibody to CXCR6 is unavailable for us to verify directly that the proliferation requires chemokine receptor CXCR6, the present data clearly demonstrates that CXCR6 is expressed in trophoblast of the first-trimester placenta, and we propose that CXCL16, combined with CXCR6, induced proliferation of HAMSC in vitro via heterotrimERIC G protein, PI3K, PDK-1, Akt and IKK pathway. Thus, we predict that CXCL16 takes its stimulatory effect in trophoblast proliferation via CXCR6 in an autocrine or paracrine manner. Meanwhile, this study confirmed our result in a previous research that CXCR4/CXCL12 induces proliferation of trophoblasts in an autocrine manner. At last, we tried to compare the stimulatory effects of CXCL16 and CXCL12. The data suggests that both induce trophoblast proliferation via their respective receptors, independently of each other. Whether there are similar intracellular signal transduction pathways or junctions remains unknown and is under further investigation. The pro-proliferation effect of CXCL16 appears stronger than that of CXCL12, which invites more research in the future.

The human placenta is characterized by extensive invasion of trophoblast into the maternal uterus, allowing direct contact of trophoblasts with the maternal blood. A defect of this trophoblast invasion is directly involved in serious and specific complications of human pregnancy, such as pre-eclampsia and placenta increta. The EVCT invasion is precisely regulated and controlled in contrast to tumour cell invasiveness. EVCT never fuses, but migrates and invades the Matrigel, so we examined the invasive capacity of the isolated first-trimester trophoblasts with transwell plates containing Matrigel-coated filters in upper compartments. CXCL16 increases the invasive capacity of trophoblast distinctly with the minimal therapeutic concentration of 10 ng/ml. The mechanism by which CXCL16 increases the invasion of trophoblasts is currently unknown. It may be due to either the promotion of invasive capacity of EVCT or induction of cytotrophoblast cell differentiation toward EVCT after treatment with CXCL16, or a combination of both of them.
The expression of CXCL16 and CXCR6 in first-trimester trophoblasts may play other important roles in both immune and non-immune functions at the materno–fetal interface. First, transmembrane CXCL16 is thought to act as an adhesion molecule for CXCR6-expressing leukocytes to form firm adhesion, as suggested by Shimaoka et al. (2004), and both chemokine...
Expression of CXCL16 in first-trimester human trophoblast cells

and mucin domains of CXCL16 are necessary for specific and efficient adhesion between CXCR6 and CXCL16. Likewise, co-expression of CXCL16 and CXCR6 in trophoblast cells may lead to cell–cell adhesion, which is important for trophoblasts to differentiate towards VCT and transfer cell–cell signal. Second, EVCT invade into maternal uterus and encounter decidual leukocyte population that is uniquely composed compared to peripheral blood. However, little is known about how they migrate into decidua and settle down to exert their activity. Since NKT cells express CXCR6 (Kim et al., 2002; Johnston et al., 2003), we think that CXCL16 produced by trophoblast cells may be responsible for recruitment of NKT cells and maintaining them adjacent to the trophoblast cells. Our previous study analysed the transcription of 18 chemokine receptors in the decidual CD56brightCD16−NK cells and found CXCR6 mRNA moderately expressed (Wu et al., 2005). If the result is confirmed, CXCL16 from trophoblasts will contribute to the recruitment and localization of the decidual CD56brightCD16−NK cells. These possibilities are currently under investigation.

Moreover, the expression of chemokine receptor CXCR6 by trophoblast cells could have other important roles. As aforementioned, CXCR6 functions as a principal co-receptor for some HIV-1 and HIV-2 strains, and trans-placental HIV passage might occur through successive infection of placental cells as little as 8 weeks after conception (Lewis et al., 1990). In fact, successful early trophoblast infection in vitro conflicts with the natural materno–fetal transmission rate of only 22%. The reasons for the paradoxically low frequency of HIV transmission in uterus during pregnancy and selective trans-placental

Figure 7. Effect of CXCL16 on the invasive capacity of the primary-cultured first-trimester trophoblast. The invasion index (II) of cells under different conditions was normalized to isotype-treated control cells. Invasion of the trophoblast was stimulated by the addition of CXCL16 in a dose-dependent fashion (A). Microscopic morphologies of the trophoblast after invasion through the Matrigel-coated membranes were taken at ×200 magnification. Trophoblast incubated in the presence of goat isotype (B), in the presence of 100 ng/ml CXCL16 (C), in the presence of 100 ng/ml CXCL16 with its neutralizing antibody (D). The results expressed by invasion index suggested that addition of a neutralizing antibody to CXCL16 completely inhibited stimulation of trophoblast invasion (E). Data represents the II ± SE of triplicates of one experiment representative of three separate experiments. The viability of trophoblast cells in this experiment was above 70% by Trypan blue staining. Con: control, anti: antibody, *P < 0.05, **P < 0.01, ***P < 0.001. Error bars depict the standard error of the mean.
transmission are not fully understood. This might depend on TC exposure to many different concentrations of cytokines and growth factors (Mognetti et al., 2000). Since CXCL16 is constitutively expressed and spontaneously secreted by the first-trimester trophoblast cells, we postulate that the combination of CXCL16 and CXCR6 should protect HIV from infecting STs by occupying the co-receptor of HIV.

In summary, we analysed the expression and function of chemokine pair CXCL16/CXCR6 in first-trimester human trophoblast for the first time. Our research demonstrates that CXCL16 promotes the primary-cultured trophoblast proliferation and invasiveness in an autocrine manner, which indicates that chemokine pair CXCL16/CXCR6 plays an important role in the development and progression of human placenta. Meanwhile, since CXCR6 can serve as a co-receptor of HIV, chemokine CXCL16 or its analogue may provide a new strategy for AIDS. Furthermore, ADAM10 (disintegrin-like metalloproteinase 10) acts as a CXCL16 sheddase, it is important to explore its modulation in the expression and function of CXCL16 in first-trimester trophoblasts (Abel et al., 2004). The biological and signal transduction pathways elicited by CXCL16 in trophoblast are required to prove the hypothesis.

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References


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