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Original Article

IL-22 secreted by decidual stromal cells and NK cells promotes the survival of human trophoblasts

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Abstract: Interleukin-22 (IL-22) has been implicated as an important immune regulator in many physiologic and pathological processes, but little is known about the IL-22 in the fetal-maternal interface. In this study, we demonstrated that co-culture of decidual stromal cells (DSCs) and decidual natural killer (dNK) cells resulted in increased secretion of IL-22, compared to culture of DSCs or dNK cells alone. The trophoblast cell line, HTR8/SVneo, expresses IL-22 receptor α1 (IL-22R1). Combinant human (rh) IL-22 significantly promoted the proliferation and viability, and inhibited the apoptosis of HTR8/SVneo cells. By Western blotting and immunohistochemistry, we confirmed that villi expressed IL-22R1, and the villi from unexplained spontaneous miscarriage patients expressed reduced levels of IL-22R1 than those from normal early pregnancy. These findings indicate that the IL-22 secreted by DSCs and dNK might promote the survival of trophoblasts and participate in the maintenance of pregnancy by binding to the IL-22R1. The reduced level of IL-22/IL-22R1 in villi might be involved in the occurrence of spontaneous miscarriage.

Keywords: IL-22, decidual stromal cells, NK cells, HTR8/SVneo cells, miscarriage

Introduction

Trophoblast cells of the human placenta proliferate, differentiate and invade the endometrium leading to successful implantation of the embryo. It involves extensive cross-talk between the embryo-derived trophoblast cells and mother-derived cells at the site of implantation. A maternal component at the maternal-fetal interface is composed mainly of decidual stromal cells (DSCs), and a large and specific population of leukocytes (decidual leukocytes cells, DLCs). Among the various types of DLCs, decidual natural killer (dNK) cells are major cellular component in human decidua. However, dysfunction between the interaction of trophoblasts and decidual cells has been demonstrated in association with not only fetal intrauterine growth restriction and pre-eclampsia, but also first-trimester and late-term miscarriage [1-4].

IL-22 is one of the IL-10-family cytokines, which also include IL-10, IL-19, IL-20, IL-24, and IL-26, as well as more distantly related IL-28 and IL-29. IL-22 was first identified as an IL-10-related T cell-derived inducible factor (IL-TIF) from a lymphoma cell line and mainly secreted by active T helper cells and NK cells [5]. The functional IL-22 receptor complex consists of two chains, IL-22R1 and IL-10R2, which are ubiquitously expressed in various organs and cell types [6]. IL-22 activates a signal transduction cascade that results in the rapid activation of several transcription factors including STAT proteins via binding the receptor complex [7]. In addition, there is another receptor of IL-22, IL-22BP, a soluble molecule which inhibits cellular IL-22 effects [8].

Data from in vitro studies with various primary cells and cell lines implicate the potential roles of IL-22 in host defense, inflammation, and tissue repair [9-13]. Comprehensive analyses have demonstrated that IL-22 played an important role in the autoimmune diseases such as psoriasis [9, 12, 14, 15], inflammatory bowel disease [16, 17], rheumatoid arthritis [18], systemic lupus erythematous [19], infection and tumor [20]. However, the study of IL-22 in the reproductive system has not been widely per-

formed yet. Only in a very recently published article [21], expression of IL-22R1 mRNA was shown in equine pregnant endometrium. The aim of this study is to elucidate, the role of IL-22 in the fetal-maternal interface and its relevance to human pregnancy.

In this study, we showed co-culture of dNK and DSCs could secrete increased level of IL-22. This cytokine could promote the proliferation, enhance the cell viability and inhibit the apoptosis of trophoblast cell line HTR8/SVneo. Furthermore, expression of IL-22R1 was detected in the trophoblasts from both normal pregnant women and patients with spontaneous miscarriage and the level of IL-22R1 from the latter group was significantly lower than that from the former one.

Material and methods

Primary tissues

The decidual and villi tissues were obtained from clinically normal pregnancies, which were terminated for social reasons and unexplained spontaneous early abortions (USA) at the Obstetrics and Gynecology Hospital of Fudan University. Each patient completed a signed, written consent form. The Fudan University Human Investigation Committee approved this study and the use of the placental tissue.

Cell line

The human trophoblast cell line HTR8/SVneo was cultured in DMEM/F12 medium (GIBCO BRL Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT, USA) and 1% penicillin and streptomycin in a humidified incubator at 37°C and 5% CO_2.

Isolation of DSC and decidual NK cells (dNK)

The detailed description of the procedures used to isolate these cells from first-trimester placentas (n=16) have been reported in the literatures [22, 23]. DSC was cultured in DMEM/F12 medium supplemented with 10% FBS (Gibco) and incubated in 5% CO_2 at 37°C. Total decidual lymphocyte was incubated with microbeads of conjugated anti-human CD3 and anti-human CD56 mAb (Miltenyi Biotec), and then $CD3^-CD56^+$dNK cells were purified. Separation was performed with the AutoMACS instrument (Miltenyi Biotec).

Flow cytometric analysis

The evaluation of cell surface expression of CD3/CD56 was performed on dNK cells by direct cell surface labeling after pre-saturation of cells at 4°C for 20 min with goat normal serum diluted 1/20 in PBS. The cells were then washed twice and incubated at 4°C for 20 min with Percp5.5-conjugated mouse anti-human CD3, Allophycocyanin (APC)-conjugated mouse anti-human CD56, or the relevant isotype control (all from Biolegend, San Diego, USA). Then their purity was detected by flow cytometry (FCM). Samples were analyzed in a FACS Calibur flow cytometer (Becton Dickinson, USA) using Cellquest software (Becton Dickinson). Three independent experiments were performed, each in triplicate.

Enzyme-linked immunosorbent assay (ELISA)

Primary cultured decidual stromal cells are seeded or not in the 24-well plate ($1*10^5$ cells/well), after attachment, the fresh isolated dNK ($1*10^5$ cells/well) were seeded in and co-cultured for another 24 hs. At last, the supernatants were collected and detected by IL-22 ELISA kit (R&D Systems, Abingdon, UK) according to the instructions of the manufacturer. The minimal detectable concentration for IL-22 was 2.7 pg/ml. 6 individual placental samples were tested independently.

Immunostaining

For immunocytochemical staining, HTR8/SVneo cells were fixed in 4% paraformaldehyde for 20 min at room temperature after cultured for 48 hours, washed in PBS and permeabilized for 20 min in 0.1% Triton X-100-PBS. The cells were then incubated with 10% FBS in PBS for 30 min to reduce non-specific binding. Rabbit Anti-human IL-22R1 polyclonal antibody (10 ug/ml, 5 ug/ml, 2.5 ug/ml; Abcam, USA) and isotype were administrated overnight at 4°C to detect whether HTR8/SVneo cells express IL-22R1. The cells were then incubated with a biotinylated secondary antibody for 30 min at room temperature. Streptavidin-HRP was applied for another 30 min at room temperature. The cells were stained with DAB (Zhongshan Cambridge Company, Beijing,

China) and counterstained with haematoxylin. The experiments were repeated three times.

For immunohistochemistry (IHC), paraffin-embedded villi specimens (6 samples of normal pregnancies and unexplained miscarriages selectively) were sliced at a 5 um thickness. Tissue sections were de-waxed in xylene, rehydrated in alcohol, and antigen retrieval was performed by heating each section at 100°C for 30 min in 0.01 mol/L sodiumcitrate buffer (pH 6.0). Then the sections were immersed in 3% hydrogen peroxide for 15 min to suppress endogenous peroxidase activity. After blocking with 10% fetal bovine serum for 1 hour at room temperature, the sections were incubated with anti-IL22R1 polyclonal antibody (5 ug/ml, Abcam, USA) and isotype overnight at 4°C. They were then sequentially treated with a biotinylated anti-rabbit antibody and HRP-labeled streptoavidin. Development was performed by treating the sections with a Liquid DAB-Plus Substrate kit (Zhongshan Cambridge Company, Beijing, China). After counterstaining with hematoxylin, immuno-staining of IL22R1 on the tissue sections was detected by light microscopy.

Western blot

100 mg fresh villi tissues from normal pregnancies (n=6) or unexplained miscarriages (n=6) were added 1 ml RIPA lysis and 10 μl PMSF, centrifuged at 12,000 g at 4°C for 30 min, and cell lysates were assayed for protein concentration using the Bradford protein assay (Beyotime Company, Shanghai, China). Proteins (50 ug) were separated on 10% SDS-polyacrylamide gels, then transferred to a nitrocellulose membrane. After being blocked in blocking buffer, the membrane was incubated with anti-IL22R1 polyclonal antibody (1:400, Abcam, USA) overnight at 4°C, washed and incubated with HRP labeled secondary antibody (1:5000, Santa Cruz, CA, USA). Blots were developed using Amersham ECL detection kit (Amersham Pharmacia Biotech, Freiburg, Germany). The experiments were repeated three times.

Cell viability/proliferation/apoptosis assays

Two different techniques, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma Chemicals, St. Louis, MO) assay and BrdU (5-Bromo-2-deoxyUridine) (Millipore, USA) assay, were applied to measure the effect of rhIL-22 (Peprotech, USA) on viability/proliferation of HTR8 cells. HTR8 cells were re-suspended in DMEM/F12 with 10% FBS and seeded at a density of 3000 cells/well in 96-well flat-bottom microplates. After 24 h, we replaced the medium by DMEM/F12 containing 1% FBS and cultured the cells for another 12 h. Then the medium was removed again, and the cells were stimulated with rhIL-22 (0, 25, 50, 100, 200 ng/ml), at 37°C for 24 h.

For the MTT assay, 20 ul of the MTT reagent was added to each well of the 96-well microplates and incubated at 37°C for 4 h, and 150 ul of DMSO was added, absorbency was measured at a wavelength of 570 nm on an automatic microplate reader.

For BrdU assay, 20 ul of BrdU reagent was added to each well of the 96-well microplates and incubated at 37°C for 24 h. Then we decanted the medium and washed cells for 3 times with PBS, added 200 ul fixation dilution and incubated for 30 min, washed 3 times, added 100 ul 1×BrDU primary monoclonal antibody, incubated for 1 hour at room temperature, washed 3 times, added 100 ul goat anti-mouse HRP-IgG, incubated for 30 min at room temperature, washed 3 times, added 100 ul TMB substrate solution for 30 min at room temperature and added 100 ul stop solution, at last, absorbency was measured at a wavelength of 450 nm - 570 nm on an automatic microplate reader. The experiments were repeated three times.

For apoptosis assay, the cells were digested by 0.25% trypsin without EDTA, and then centrifuged 1000 g for 5 min, re-suspended with PBS, labeled the cells by Annexin V and PI according to the instruction manual, and detected the percentage of early apoptosis cells by flow cytometry. The apoptosis detection kit was obtained from Merk Company (Merk, Germany).

Statistical analysis

All values are shown as mean±SEM. Comparison between controls and various treatments was performed by one-way ANOVA, with application of the Dunnett test. Differences were accepted as significant at $P<0.05$.

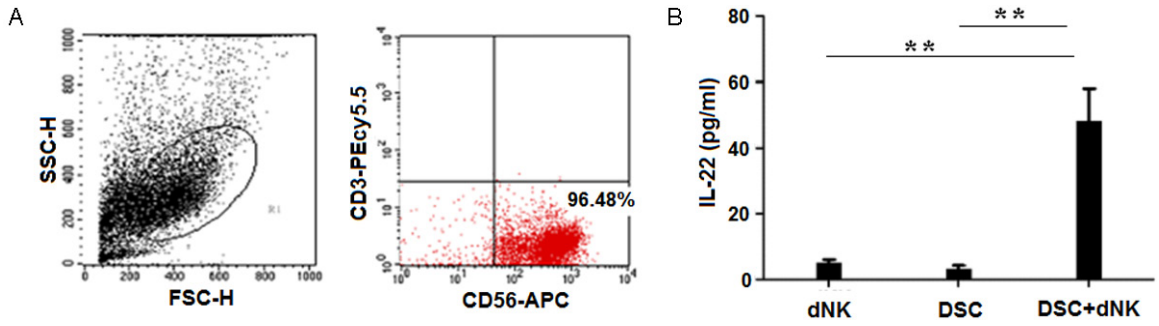

Figure 1. *Co-culture of DSCs and dNK cells produces increased level of IL-22.* A: dNK cells were isolated and purified by CD56 and CD3 MACS beads from human first-trimester decidual tissues. The cells were then stained with CD56-APC and CD3-Percp5.5 and analyzed by FCM. Three independent experiments were performed, each in triplicate. Representative figures are shown. B: Primary dNK cells or DSCs were cultured alone or co-cultured for 48 h, and the supernatants were harvested and the level of IL-22 was measured by ELISA. $^{**}P<0.01$ was compared to dNK cells alone or DSCs alone.

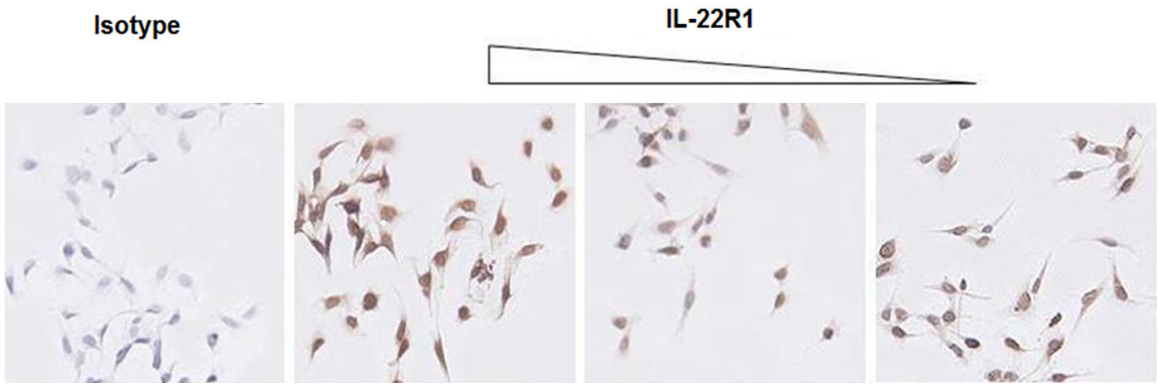

Figure 2. *Human extravillous trophoblast cell line HTR8/SVneo expresses IL-22R1.* HTR-8/SVneo cells were stained with anti-IL-22RA1 polyclonal antibody in different concentration (1:50, 1:100, and 1:200) and the immunostain signal weakened followed by the dilution of antibody. Original magnification: ×100. These pictures are representatives of three individual experiments.

Results

Co-culture of DSCs and dNK cells produces increased level of IL-22

DSC and decidual NK cells (dNK) were isolated from human first-trimester placentas (n=16) as described before [22, 23]. Using immunofluorescent labeling for the CD56, CD3 and subsequent flow cytometric analysis, it was confirmed that the purity of the $CD3^{-}CD56^{+}$dNK cells isolated in our study was more than 95 percent (**Figure 1A**). And then, we mimicked the microenvironment of fetal-maternal interface by co-culturing dNK with DSCs, and detected the level of IL-22 in the cell culture supernatant. The results showed that both dNK cells and DSCs could secrete low level of IL-22. The co-culture system significantly elevated the secretion of IL-22 in the supernatant ($P<0.01$) (**Figure 1B**). These results indicated that the interaction between dNK and DSCs might play some roles at the fetal-maternal interface by secreting IL-22.

Human extravillous trophoblast cell line HTR8/SVneo expresses IL-22R1

To further study the functions of IL-22 at the fetal-maternal interface, we used the cell line HTR8/SVneo. The source of HTR8/SVneo is primary cultured trophoblast, so it has the most similar characterization to that of the primary cells. Firstly we identified the expression of IL-22 receptor IL-22R1 on this cell line by immunocytochemistry. We stained the cells with anti-

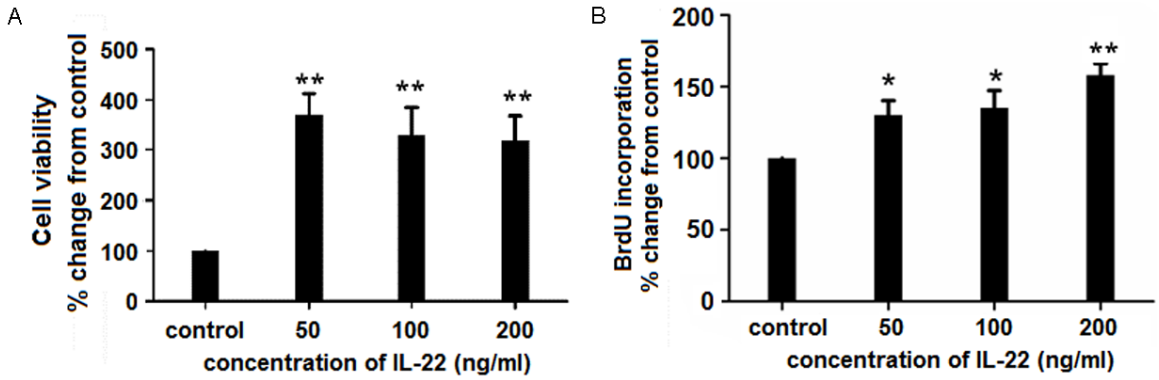

Figure 3. *Recombinant human IL-22 promotes viability and proliferation of HTR-8/SVneo cells.* A: HTR-8/SVneo cell line was treated with different concentrations of rhIL-22 (0, 50, 100 and 200 ng/ml) for 48 h and then the cell viability and proliferation were detected by MTT and BrdU proliferation assays, respectively. The OD value of the control group which was not treated by the rhIL-22 was standardized as 1. The values of the treated cells were compared with the values generated from the untreated control cells and reported as the percentage viability and proliferation. $^{*}P<0.05$ or $^{**}P<0.01$ was compared to the vehicle control. Error bars represent the standard error of the mean.

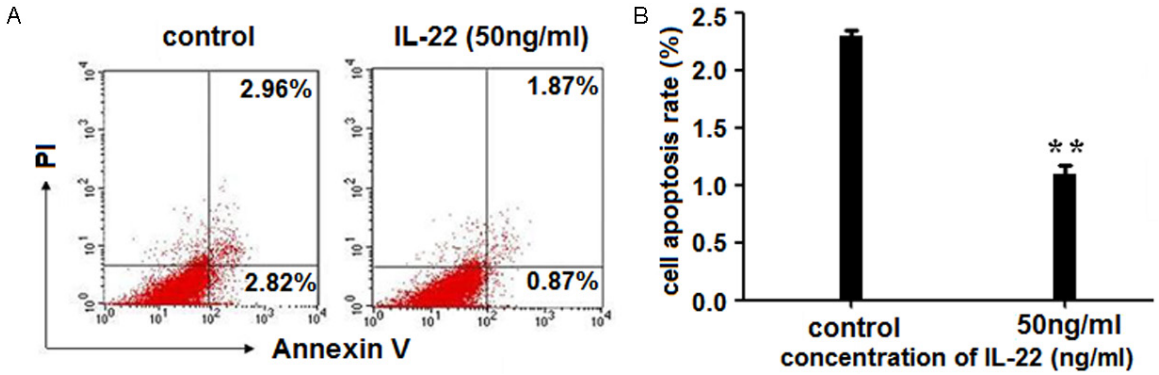

Figure 4. *rhIL-22 restricts the apoptosis of HTR-8/SVneo cells.* A, B: HTR-8/SVneo was treated with or without 50 ng/ml rhIL-22 for 48 h and then the apoptosis rate was analyzed by apoptosis assay. $^{**}P<0.01$ was compared to the vehicle control. Error bars represent the standard error of the mean. Three independent experiments were performed.

human IL-22R1 antibody at different concentrations (1:50, 1:100, and 1:200) and demonstrated IL-22R1 specific expression. We repeated the experiment for 3 times and the results were accordant (**Figure 2**).

Recombinant human IL-22 promotes viability and proliferation of HTR-8/SVneo cells

The effect of recombinant human IL-22 (rhIL-22) on the viability and proliferation of HTR-8/SVneo cells was studied. The results of MTT assay showed that rhIL-22 could enhance the viability of HTR8 ($P<0.01$) (**Figure 3A**). Moreover, rhIL-22 promoted the proliferation of HTR8/SVneo in a dosage dependent manner ($P<0.05$ or $P<0.01$) (**Figure 3B**).

rhIL-22 restricts the apoptosis of HTR-8/SVneo cells

Result in **Figure 4** showed that the apoptosis percentage of HTR-8/SVneo cells pretreated with 50 ng/ml IL-22 decreased from 2.1%± 0.13% to 1.17%±0.13% ($P<0.01$) (**Figure 4A** and **4B**). These data suggest that rhIL-22 significantly stimulated the viability and proliferation, inhibited the apoptosis of HTR-8/SVneo cells, which might promote the growth and survival of trophoblasts in early pregnancy.

The expression of IL-22R1 is decreased in the villi from patients with miscarriage

Finally, we analyzed the expression level of IL-22R1, a subunit of IL-22 receptor complex in

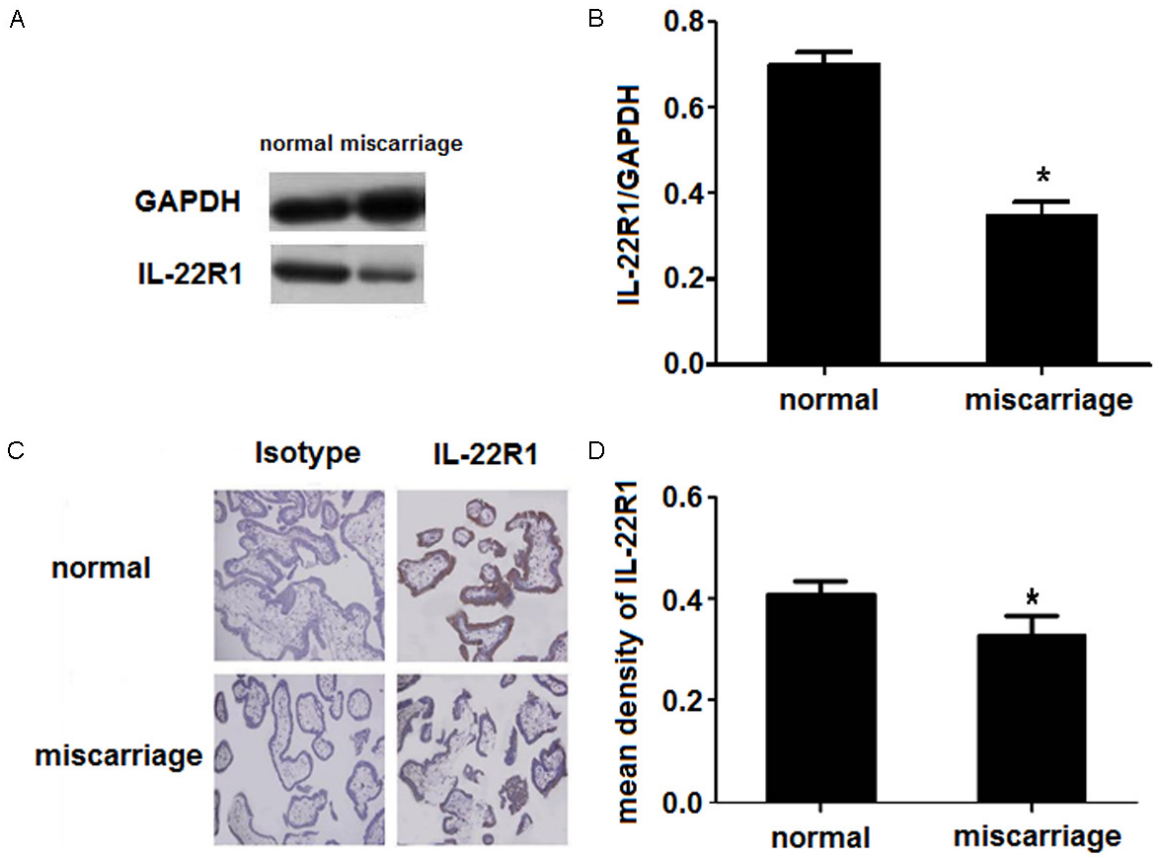

Figure 5. *The expression of IL-22R1 is decreased in the villi from patients with miscarriage.* The expression of IL-22R1 on human first-trimester villi of normal early pregnancy women (n=6) and unexplained miscarriage patients (n=6) were detected by Western blotting (A, B) and immunohistochemistry (C, D), respectively. Original magnification: ×100. *P<0.05 was compared to normal early pregnancy group. Error bars represent the standard error of the mean.

human villi tissues from 6 normal pregnant women and 6 unexplained miscarriage patients by IHC and Western blot. The results showed that the villi from both groups could express IL-22R1, while the villi from the normal group had significantly higher expression (P<0.05) (**Figure 5A-D**), indicating that villi tissue might be one of the target tissues of IL-22, and IL-22/IIL-22R1 signaling might participate in the maintenance of normal pregnancy.

Discussion

During the first trimester of pregnancy, DSCs play an important role in regulating the growth and invasion of trophoblasts at the maternal-fetal interface [22]. The dNK cells account for more than 70 percent of total lymphoid population in the maternal deciduas [24]. This group of NK cells displays a unique functional profile and also plays important roles including promoting trophoblast cell invasion, vascular growth [25-27] and the induction of regulatory T cells that modulate the maternal physiologic immune response to prevent fetal rejection [28]. It is known that dNK cells may participate in the maintenance of successful pregnancy through secreting some Th2 type cytokines such as IL-4 and IL-10 [29]. In recent years, the Th-17 subset of T helper cells was confirmed in the fetal-maternal interface and was confirmed to induce the proliferation and invasion of trophoblast by secreting IL-17 (unpublished data). The concept of Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy is being established.

As a typical Th17 type cytokine, IL-22 is one of the IL-10-like family cytokines and first identified as an IL-10-related T cell-derived inducible factor (IL-TIF) from a lymphoma cell line [5]. Th17 cells are clearly the dominant IL-22 pro-

ducer both at the mRNA and protein levels [30]. IL-22 could also be produced by innate immune cells, such as NK [14], gamma/delta T [31] and NKT cells. No expression was found in monocyte-derived macrophages or dendritic cells [32]. Victoria Male *et al* [33] found that uterine stage 3 NK cells, the $CD34^{-}CD117+CD94^{-}$ NK cells, constitutively produced IL-22 by FCM. Similarly, we also found that dNK cells produce IL-22, and the co-culture with DSCs could increase the level of IL-22 in the co-culture unit.

In view of the biological effects, IL-22 increases the innate immunity of tissue cells, protects tissues from damage, and enhances their regeneration. Until recently, researchers have focused the function of this cytokine in a variety of immune diseases such as psoriasis. In fact, some unsuccessful pregnancies are the result of maternal immune rejection of fetus. Thus, we speculated whether there was relationship between IL-22 and unexplained miscarriage. IL-22 mediates its effects via a heterodimeric trans-membrane receptor complex consisting of IL-22R1 and IL-10R2 and subsequent Janus kinase–signal transducers and activators of transcription (JAK–STAT) signaling pathways including Jak1, Tyk2, and STAT3 [6, 7]. In addition to this cell surface-associated IL-22 receptor complex, there is a secreted ("soluble"), single-chain, high affinity IL-22 binding receptor named IL-22 binding protein (IL-22BP) which could negatively regulate the activity of IL-22 [8]. Early studies [34] demonstrated that in contrast to other T and NK cell cytokines, no expression of IL-22R1 was detected in bone marrow, blood mononuclear cells, thymus, or spleen or in a variety of isolated resting or activated primary immune cells including monocytes, B cells, T cells, NK cells, macrophages, and immature and mature Dendritic cells. Some tissues and organs forming the body barriers and containing epithelial cells such as skin, kidney and those from the digestive (pancreas, small intestine, liver, colon) and the respiratory (lung, trachea) systems express the IL-22R1 and IL-10R2 complex. Recently, researchers [21] published the expression of IL-22R1 on the endometrium of D32 and D34 pregnancy and believed IL-22 facilitated re-epithelialization of the endometrium after trophoblast migration. In our previous studies [23], we have shown that trophoblasts derived from epithelial and were CK7 positive. Moreover, this study showed that the trophoblast cell line HTR-8/SVneo expressed the receptor IL-22R1. Therefore, trophoblast cells might be the target of IL-22.

To further study the detailed functions of IL-22 on trophoblasts, we used IL-22 to stimulate the HTR-8/SVneo cells and found that IL-22 markedly promoted the proliferation, enhanced the cell viability and reduced the apoptosis of HTR-8/SVneo cells. That is to say, IL-22 played an important role in modulating the functions of trophoblasts. The formulation of placenta represents an important biologic behavior in early pregnancy, in which process the proliferation, differentiation and invasion of trophoblasts are the critical events. The proliferation ability of trophoblasts was strong in early pregnancy and weakening alongside the gestation proceeding. The trophoblasts with high proliferation ability help to promote the embryo development and pregnancy maintenance while those with disturbance in the proliferation ability may lead to the occurrences of pregnancy-associated diseases such as recurrent spontaneous abortion (RSA), Fetal growth restriction (FGR) and pre-eclampsia. Other researchers [35] also found IL-22 could promote liver cell regeneration by increasing hepatic cell proliferation and hepatocyte migration through the activation of Akt and STAT signaling. Zhang W *et al* reported that IL-22 protected human lung cancer cells from starvation and chemotherapy drug-induced apoptosis via activation of STAT3 and its downstream anti-apoptotic proteins (Bcl-2 and Bcl-xL) and inactivation of extracellular signal regulated kinase (ERK1/2) [20]. To some extent, trophoblast cells have similar characterizations to tumor cells. Furthermore, both ERK1/2 [36] and STAT3 [37] signaling are involved in regulating the biological behavior of trophoblasts. So, we believed that IL-22 from dNK cells and DSCs might stimulate the growth and survival of trophoblasts at the fetal-maternal interface in a paracrine manner by binding the IL-22 receptor on the trophoblasts through regulating ERK1/2 and or STAT3 signaling.

Subsequently, we compared the expression of IL-22R1 in villi from normal early pregnant and unexplained miscarriage patients. The results showed that trophoblast from both normal early pregnant and miscarriage patients expressed IL-22R1. Our results also showed that trophoblast from miscarriage patients expressed significantly lower levels of IL-22R1,

which might involve in the occurrence of disease by limiting the growth and proliferation of trophoblast.

Most recently, there was report [21] that IL-22 was expressed by a non-immune cell type, the invasive trophoblast cells of the equine chorionic girdle. This was the first study of non-lymphocytes derived IL-22 and expanded the resources of this cytokine and indicated the more widespread functions of it. Based on these results and our findings, IL-22 might be a beneficial cytokine for a successful pregnancy. The IL-22 secreted by the dNK cells, DSCs and trophoblasts at the maternal-fetal interface might promote the proliferation, enhance the cell variability and repress the apoptosis of trophoblasts in paracrine and autocrine manners through the cross-talking between dNK cells, DSCs and Trophoblasts. While the abnormal lower level of IL-22R1 in trophoblasts may be the reason for the dysfunction of trophoblasts growth and survival, which is not conducive to the formation and development of placenta and maintenance of normal pregnancy and may finally be involved in the occurrence of miscarriage.

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Disclosure of conflict of interest

None.

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References

[1] Kaufmann P, Black S, Huppertz B. Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod 2003; 69: 1-7.

[2] Bose P, Kadyrov M, Goldin R, Hahn S, Backos M, Regan L, Huppertz B. Aberrations of early trophoblast differentiation predispose to pregnancy failure: lessons from the antiphospholipid syndrome. Placenta 2006; 27: 869-875.

[3] Torry DS, Labarrere CA, McIntyre JA. Uteroplacental vascular involvement in recurrent spontaneous abortion. Curr Opin Obstet Gynecol 1998; 10: 379-382.

[4] Kimatrai M, Oliver C, Abadia-Molina AC, García-Pacheco JM, Olivares EG. Contractile activity of human decidual stromal cells. J Clin Endocrinol Metab 2003; 88: 844-849.

[5] Dumoutier L, Louahed J, Renauld JC. Cloning and characterization of IL-10-related T cell-derived inducible factor (IL-TIF), a novel cytokine structurally related to IL-10 and inducible by IL-9. J Immunol 2000; 164: 1814-1819.

[6] Kotenko SV, Izotova LS, Mirochnitchenko OV, Esterova E, Dickensheets H, Donnelly RP, Pestka S. Identification of the functional interleukin-22 (IL-22) receptor complex - The IL-10R2 chain (IL-10R beta) is a common chain of both the IL-10 and IL-22 (IL-10-related T cell-derived inducible factor, IL-TIF) receptor complexes. J Biol Chem 2001; 276: 2725-2732.

[7] Lejeune D, Dumoutier L, Constantinescu S, Kruijer W, Schuringa JJ, Renauld JC. Interleukin-22 (IL-22) activates the JAK/STAT, ERK, JNK, and p38 MAP kinase pathways in a rat hepatoma cell line - pathways that are shared with and distinct from IL-10. J Biol Chem 2002; 277: 33676-33682.

[8] Gruenberg BH, Schoenemeyer A, Weiss B, Toschi L, Kunz S, Wolk K, Asadullah K, Sabat R. A novel, soluble homologue of the human IL-10 receptor with preferential expression in placenta. Genes Immun 2001; 2: 329-334.

[9] Wolk K, Witte E, Wallace E, Döcke WD, Kunz S, Asadullah K, Volk HD, Sterry W, Sabat R. IL-22 regulates the expression of genes responsible for antimicrobial defense, cellular differentiation, and mobility in keratinocytes: a potential role in psoriasis. Eur J Immunol 2006; 36: 1309-1323.

[10] Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, Reinhart TA, McAllister F, Edeal J, Gaus K, Husain S, Kreindler JL, Dubin PJ, Pilewski JM, Myerburg MM, Mason CA, Iwakura Y, Kolls JK. IL-22 mediates mucosal host defense against Gram-negative bacterial pneumonia. Nat Med 2008; 14: 275-281.

[11] Sonnenberg GF, Fouser LA, Artis D. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. Nat Immunol 2011; 12: 383-390.

[12] Ma HL, Liang S, Li J, Napierata L, Brown T, Benoit S, Senices M, Gill D, Dunussi-Joannopoulos K, Collins M, Nickerson-Nutter C, Fou-

ser LA, Young DA. IL-22 is required for Th17 cell-mediated pathology in a mouse model of psoriasis-like skin inflammation. J Clin Invest 2008; 118: 597-607.
[13] Dudakov JA, Hanash AM, Jenq RR, Young LF, Ghosh A, Singer NV, West ML, Smith OM, Holland AM, Tsai JJ, Boyd RL, van den Brink MR. Interleukin-22 drives endogenous thymic regeneration in mice. Science 2012; 336: 91-95.
[14] Wolk K, Kunz S, Asadullah K, Sabat R. Cutting edge: Immune cells as sources and targets of the IL-10 family members? J Immunol 2002; 168: 5397-5402.
[15] Wolk K, Haugen HS, Xu W, Witte E, Waggie K, Anderson M, Vom Baur E, Witte K, Warszawska K, Philipp S, Johnson-Leger C, Volk HD, Sterry W, Sabat R. IL-22 and IL-20 are key mediators of the epidermal alterations in psoriasis while IL-17 and IFN-gamma are not. J Mol Med 2009; 87: 523-536.
[16] Ouyang WJ. Distinct roles of IL-22 in human psoriasis and inflammatory bowel disease. Cytokine Growth Factor Rev 2010; 21: 435-41.
[17] Monteleone I, Sarra M, Pallone F, Monteleone G. Th17-related cytokines in inflammatory bowel diseases: friends or foes? Curr Mol Med 2012; 12: 592-597.
[18] Ikeuchi H, Kuroiwa T, Hiramatsu N, Kaneko Y, Hiromura K, Ueki K, Nojima Y. Expression of interleukin-22 in rheumatoid arthritis - Potential role as a proinflammatory cytokine. Arthritis Rheum 2005; 52: 1037-1046.
[19] Pan HF, Zhao XF, Yuan H, Zhang WH, Li XP, Wang GH, Wu GC, Tang XW, Li WX, Li LH, Feng JB, Hu CS, Ye DQ. Decreased serum IL-22 levels in patients with systemic lupus erythematosus. Clin Chem Acta 2009; 401: 179-180.
[20] Zhang W, Chen Y, Wei H, Zheng C, Sun R, Zhang J, Tian Z. Antiapoptotic activity of autocrine interleukin-22 and therapeutic effects of interleukin-22-small interfering RNA on human lung cancer xenografts. Clin Cancer Res 2008; 14: 6432-6439.
[21] Brosnahan MM, Miller DC, Adams M, Antczak DF. IL-22 is expressed by the invasive trophoblast of the equine (Equus caballus) chorionic girdle. J Immunol 2012; 188: 4181-4187.
[22] Li MQ, Tang CL, Du MR, Fan DX, Zhao HB, Xu B, Li DJ. CXCL12 controls over-invasion of trophoblasts via up-regulating CD82 expression in DSCs at maternal-fetal interface of human early pregnancy in a paracrine manner. Int J Clin Exp Pathol 2011; 4: 276-286.
[23] Wu X, Jin LP, Yuan MM, Zhu Y, Wang MY, Li DJ. Human first-trimester trophoblast cells recruit CD56(bright)CD16(-) NK cells into decidua by way of expressing and secreting of CXCL12/stromal cell-derived factor 1. J Immunol 2005; 175: 61-68.
[24] Bulmer JN, Morrison L, Longfellow M, Ritson A, Pace D. Granulated Lymphocytes in human endometrium: histochemical and immuohistochemical studies. Hum Reprod 1991; 6: 791-798.
[25] Hanna J, Goldman-Wohl D, Hamani Y, Avraham I, Greenfield C, Natanson-Yaron S, Prus D, Cohen-Daniel L, Arnon TI, Manaster I, Gazit R, Yutkin V, Benharroch D, Porgador A, Keshet E, Yagel S, Mandelboim O. Decidual NK cells regulate key developmental processes at the human fetal-maternal interface. Nat Med 2006; 12: 1065-1074.
[26] Le Bouteiller P, Tabiasco J. Killers become builders during pregnancy. Nat Med 2006; 12: 991-992.
[27] Vacca P, Cantoni C, Prato C, Fulcheri E, Moretta A, Moretta L, Mingari MC. Regulatory role of NKp44, NKp46, DNAM-1 and NKG2D receptors in the interaction between NK cells and trophoblast cells. Evidence for divergent functional profiles of decidual versus peripheral NK cells. Int Immunol 2008; 20: 1395-1405.
[28] Vacca P, Cantoni C, Vitale M, Prato C, Canegallo F, Fenoglio D, Ragni N, Moretta L, Mingari MC. Crosstalk between decidual NK and CD14(+) myelomonocytic cells results in induction of Tregs and immunosuppression. Proc Natl Acad Sci U S A 2010; 107: 11918-11923.
[29] Lidström C, Matthiesen L, Berg G, Sharma S, Ernerudh J, Ekerfelt C. Cytokine secretion patterns of NK cells and macrophages in early human pregnancy decidua and blood: Implications for suppressor macrophages in decidua. Am J Reprod Immunol 2003; 50: 444-452.
[30] Chung YS, Yang XX, Chang SH, Ma L, Tian Q, Dong C. Expression and regulation of IL-22 in the IL-17-producing CD4+T lymphocytes. Cell Res 2006; 16: 902-907.
[31] Ma SD, Lancto CA, Enomoto S, Abrahamsen MS, Rutherford MS. Expression and regulation of IL-22 by bovine peripheral blood gamma/delta T cells. Gene 2010; 451: 6-14.
[32] Wolk K, Witte K, Witte E, Proesch S, Schulze-Tanzil G, Nasilowska K, Thilo J, Asadullah K, Sterry W, Volk HD, Sabat R. Maturing dendritic cells are an important source of IL-29 and IL-20 that may cooperatively increase the innate immunity of keratinocytes. J Leukocyte Biol 2008; 83: 1181-1193.
[33] Male V, Hughes T, McClory S, Colucci F, Caligiuri MA, Moffett A. Immature NK Cells, Capable of Producing IL-22, Are Present in Human Uterine Mucosa. J Immunol 2010; 185: 3913-3918.
[34] Wolk K, Kunz S, Witte E, Friedrich M, Asadullah K, Sabat R. IL-22 increases the innate immunity of tissues. Immunity 2004; 21: 241-254.

[35] Brand S, Dambacher J, Beigel F, Zitzmann K, Heeg MH, Weiss TS, Prüfer T, Olszak T, Steib CJ, Storr M, Göke B, Diepolder H, Bilzer M, Thasler WE, Auernhammer CJ. IL-22-mediated liver cell regeneration is abrogated by SOCS-1/3 overexpression in vitro. Am J Physiol Gastrointest Liver Physiol 2007; 292: G1019-G1028.

[36] Du MR, Zhou WH, Yan FT, Zhu XY, He YY, Yang JY, Li DJ. Cyclosporine A induces titin expression via MAPK/ERK signalling and improves proliferative and invasive potential of human trophoblast cells. Hum Reprod 2007; 22: 2528-2537.

[37] Pu HH, Duan J, Wang Y, Fan DX, Li DJ, Jin LP. Thymic stromal lymphopoietin promotes the proliferation of human trophoblasts via phosphorylated STAT3-mediated c-Myc upregulation. Placenta 2012; 33: 387-391.