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Expressions of natural cytotoxicity receptor, NKG2D and NKG2D ligands in endometriosis

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ABSTRACT

Pathogenesis of endometriosis is still unknown, and the relationship between NK cell activating receptors and endometriosis remains to be explored. We investigated the expression of NCRs and NKG2D in NK cells in peripheral blood (PB) and peritoneal fluid (PF) as well as expression of NKG2D ligands in endometrial cells, and illuminated their relationship with ovarian endometriosis. 20 patients with ovarian endometriosis and 13 subjects for control group were recruited. Flow cytometry was used for examining expressions of NCRs and NKG2D on NK cells. In PF with endometriosis, the expressions of NKp30 ($P = 0.006$) and NKG2D ($P = 0.010$) on $CD56^+$NK cells were decreased, whereas the expression of NKp46 ($P = 0.040$) on $CD16^+$NK cells was higher than that of control. Real time PCR and Western blotting were used for detecting expression of NKG2D ligands. mRNA level of NKG2D ligands on endometrial cells showed no noticeable difference. As for protein expression, the ULBP-2 expression on eutopic endometrial cells with pelvic endometriosis was lower than that on ectopic endometrial cells and eutopic endometrial cells without endometriosis ($P < 0.05$), and the ULBP-3 expression on ectopic endometrial cells was lower than that on eutopic endometrial cells with or without endometriosis ($P < 0.05$). These findings indicate that change of NKp30, NKp46 and NKG2D on NK cells in PF and ULBP-2, 3 on endometrial cells may relate to the pathogenesis of pelvic endometriosis. Especially, change of NK cell activating receptors in PF implies that pelvic endometriosis is probably due to local immune changes.

1. Introduction

Endometriosis is one of the most common gynecologic disorders, affecting about 5–10% of women during reproductive years (Bulun, 2009). It is defined as implantation of functional endometrial tissue outside the uterus, leading to diverse progressive symptoms such as dysmenorrhea, pelvic pain, irregular menstruation and infertility. This phenomenon was believed to be a result of retrograde menstruation (Zondervan et al., 2018). Although endometriosis has been described since the 1800s, the mechanisms underlying its pathogenesis and progression remain poorly understood. As a result, therapeutic options are rather limited with unsatisfactory efficacy.

Since the twentieth century, multiple hypotheses have been proposed to explain the presence of ectopic endometrial cells. Although Sampson's implantation theory is widely accepted (Sampson, 1927), there is no monophyletic theory that can conclusively explain the etiology. Based on the observation that 90% women have retrograde menstruation but only 10–15% suffer from endometriosis, we suspect that immune system plays an important role in the onset and development of endometriosis. The current consensus is that the function of immune-related cells is impaired, including natural killer (NK) cells. Consequently, the cytotoxicity of NK cells with endometriosis is decreased significantly (Oosterlynck et al., 1991; Xu et al., 2001).

NK cells, the same as B and T lymphocytes, are potent effector cells crucial for immunity to tumors and infections. In human, NK cells represent the third largest lymphocyte population, accounting for 5–10% of peripheral blood lymphocytes. They are characterized by expression of CD56 and CD16 surface markers and absence of CD3 complex ($CD3^-CD56^+CD16^+$ cells). Initially identified in 1975, NK cells did not receive much attention. However, during the last decades, multiple functions of NK cells, such as effectors, cytokine producers and potential regulators in adaptive immunity, have been detected. Recent research focused on the identification of NK cell receptors and effector molecules which are involved in target cell recognition.

Functionally, NK cell receptors are classified as activating and inhibitory. NK cells identify their targets through a set of activating or inhibitory receptors (Lanier, 2005). For many years, NK cells were thought to be solely controlled by inhibitory mechanisms. As for

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endometriosis and KIRs (killerimmunoglobulin-like receptors), Maeda and his colleagues (Maeda et al., 2002) found that the proportion of KIR2DL1$^+$NK cells was increased in peritoneal fluid and peripheral blood in women with endometriosis, and it may represent a risk factor in the pathogenesis of endometriosis. However, in the last decades, activation of NK cells was found to be more complicated, because NK cells receive specific activating signals (Karre, 2002). This realization was based on the discovery of a set of NK-triggering receptors that are able to directly induce cytotoxicity and cytokine production. In humans, NKG2D and natural cytotoxic receptors (NCRs), which are comprised of NKp46 (NCR1, CD335), NKp44(NCR2, CD336) and NKp30 (NCR3, CD337), are two main activating receptors of NK cells. NCRs are expressed exclusively in NK cells (Moretta et al., 2002; Moretta and Moretta, 2004). NKG2D, a surface receptor of the NKG2 family, can also be expressed in cytolytic T lymphocytes, γδT cells, macrophage, and dendritic cells (DC) in addition to NK cells. It functions predominantly under pathological conditions, and is involved in the process of innate immunity and acquired immunity simultaneously.

NK cells identify their targets through ligation of receptors and ligands. NKG2D is specific for stress-induced MICA and MICB, MHC class- I related chain A and B (Groh et al., 1999) or ULBPs (UL16-binding proteins). To date, there are six ULBP molecules that have been found, named ULBP 1–6 (Cosman et al., 2001; Sutherland et al., 2002; Chalupny et al., 2003). NKG2D ligands are absent or have low expression in normal tissue cells, but are expressed or highly expressed in a stressed state, such as heat shock, chemical stimulus, cytometaplasia and viral infection (Groh et al., 1999; Cerwenka and Lanier, 2003).

The pathogenesis of pelvic endometriosis is still not clear, and the relationship between activating receptors in NK cells and the pathogenesis of endometriosis remains to be explored. In this study, we investigated the expression of NCRs and NKG2D in NK cells in peripheral blood (PB) and peritoneal fluid (PF) as well as the expression of NKG2D ligands in endometrial cells, and explored its relationship with pelvic endometriosis.

2. Materials and methods

2.1. Subjects and ethical approval

We recruited 20 patients with ovarian endometriosis (range of age: 22–49 years, mean: 34.9 years) who underwent a laparoscopic or a laparotomy surgery at Renji Hospital, Shanghai Jiaotong University School of Medicine. All subjects were diagnosed with endometriosis by pathological examination. They were classified according to the Revised American Fertility Society (r-AFS) classification: 2 patients were at stage II, 6 at stage III and 12 at sage IV. All the 18 patients of stage III and stage IV suffered from ovarian endometrioma. Fourteen patients suffered menorrhalgia and 6 had a history of infertility. All patients had not received any endometriosis medication and/or any hormonal therapy within three months before surgery. The 13 subjects in the control group (range of age: 20–50 years, mean: 34.6 years) were randomly selected from patients confirmed to only have simple ovarian cysts, and underwent laparoscopy or laparotomy during the same period at the same hospital. All the participants have regular menstrual cycles. In the case group, 17 cases were proliferative phase and 3 cases were secretory phase, and in the control group, 11 cases were proliferative phase and 2 cases were secretory phase. None of the participants had any other immunological disorders.

The study was approved by the ethics committee/Institutional Review Board (IRB) of Renji Hospital. Both verbal and written informed consent were obtained from each participant prior to inclusion in the study.

2.2. Flow cytometry

The following fluorescently-labeled antibodies were used to analyze cell surface antigens: fluorescein isothiocyanate (FITC)-conjugated mouse, anti-human CD16 monoclonal antibody (Clone 3G8) and phycoerythrin-anthocyanin (PE-Cy5)-conjugated mouse, anti-human CD56 monoclonal antibody (Clone B159) (BD Pharmingen, San Diego, CA, USA); phycoerythrin (PE)-mouse, anti-human NKp30 monoclonal antibody (Clone Z25) and PE-mouse, anti-human NKp44 monoclonal antibody (Clone Z231) (Beckman Coulter, Marseille, France); PE-mouse, anti-human NKp46 monoclonal antibody (Clone 195314) and PE-mouse, and anti-human NKG2D monoclonal antibody (Clone 149810) (R&D Systems, Wiesbaden, Germany). Samples were evaluated with a FACS Calibur system (Becton Dickinson, San Jose, CA, USA).

Heparinized blood samples and peritoneal fluid/peritoneal lavage fluid samples were collected from patients with all participants during surgery. Direct tricolor staining with fluorescent antibodies, CD16-FITC/CD56-PE-Cy5/NCR (NKp30, NKp44, NKp46) and NKG2D-PE was completed within 2–4 h. Standard techniques were used to label peripheral blood leukocytes with these panel of monoclonal antibodies (mAbs). Briefly, 100 μL of whole blood was incubated with mAbs (10 μL each) for 30 min, away from light at room temperature. Red cells were then lysed with 3 mL erythrolysin for 5–10 min. Cells were then washed in PBS and fixed with 1% paraformaldehyde. Cell mixtures from peritoneal fluid were centrifuged and hemolyzed before staining, then labeled in the same way as peripheral blood leukocytes.

FACS Calibur flow cytometer was used for immunofluorescence and three-color flow cytometric analysis. CellQuest software (BD Biosciences, San Jose, CA, USA) was used for data collection and storage. For each sample, at least 1×10^4 events were recorded. FlowJo software(FlowJo LLC, Ashland, Oregon, USA) was used for data analysis. Gates were set with lymphocyte/SSC region as R1 and the subsets of CD56$^+$ cell region as R2. The percentage of NCRs and NKG2D on CD56^{+NK} cells and CD16$^+$NK cells was determined.

2.3. RNA extraction and quantitative real-time RT-PCR

Total RNA was extracted from homogenized eutopic and ectopic endometrial tissues by using TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA). 400 ng RNA of each sample was reverse-transcribed with a PrimeScript RT Reagent kit (Takara Bio Inc., Kusatsu, Shiga, Japan). cDNA products were diluted to 1: 5 and then used to quantify mRNA expression level with a SYBR Premix Ex Taq RT-PCR kit (Takara Bio Inc., Kusatsu, Shiga, Japan). All the reactions were performed on an ABI Prism 7900 Real-Time PCR System (Applied Biosystems Inc., Foster City, CA, USA). The relative expression levels were normalized to endogenous expression of GAPDH (glyceraldehyde-3-phosphate dehydrogenase) and ACTB (Actin, cytoplasmic 1) and calculated by the $2^{-\Delta\Delta Ct}$ method. The primer sequences used in real-time RT-PCR were listed in Table 1.

2.4. Western blotting

Eutopic or ectopic endometrial tissues were collected and washed with cold 1 × PBS. Thereafter, the tissues were lysed in 1 mL RIPA lysis buffer containing 10 μL protease inhibitor, 10 μL phenylmethylsulfonylfluoride (PMSF) and 10 μL phosphotase inhibitor on ice. The lysates were subject to sodium dodecyl sulfate–polyacrylamide (SDS-PAGE) gel electrophoresis and transferred onto PVDF membrane which was subsequently incubated in 5% BSA blocking buffer for 1 h. The membrane was probed with the primary antibodies overnight at 4°C, washed with 1 × TBST buffer three times, probed with HRP-labeled secondary antibody for 1 h, washed again with 1 × TBST buffer three times, and finally visualized with ECL substrate (Thermo Fisher Scientific Inc., United States). We employed the following antibodies: goat anti-MICA, MICB and ULBP-1, 2, 3 (diluted 1:300; R&D Systems, Minneapolis, MN, USA). ImageJ(Schindelin et al., 2015) was used to quantitatively analyze western blots results. The ratio of the gray-scale value of target protein band to beta-ACTIN band represented relative expression of the target protein.

Table 1
Primer sequences used in real-time RT-PCR.

Gene Symbol		Primer Sequence (5′ - 3′)
MICA	Forward	CCC TCT TGG TGC CTC AGT T
	Reverse	CTG CAA ACG CCT CAT ATC TAC
MICB	Forward	TTA GCC ACC ATG CCT GTC
	Reverse	GGC TGG TCA GGA GGA GTC
ULBP1	Forward	TGG AGC CAA GAA GAT GAC AG
	Reverse	CAT CTT ACA ATC CCC CAG TG
ULBP2	Forward	TCT TCC TCC TCT TTG ACT CAG
	Reverse	GCC ACA ACC TTG TCA TTC TC
ULBP3	Forward	GTG AGT GTG AAG CCG ATG G
	Reverse	AAG GTG GTC AGT CCG CTA TC
ULBP4	Forward	CCT CCT CTT TGA CGC AAT G
	Reverse	TGA GCC AGT GAT CGC AGT C
GAPDH	Forward	GAA GGT GAA GGT CGG AGT C
	Reverse	GAA GAT GGT GAT GGG ATT TC
ACTB	Forward	TCG ACA ACG GCT CCG GCA T
	Reverse	AAG GTG TGG TGC CAG ATT TTC

2.5. Statistical analysis

SPSS version 17.0 were used for statistical analysis (SPSS Inc., Chicago, IL, USA). For data from flow cytometry assay, nonparametric Mann Whitney U test was used to compare the endometriosis group and the control groups. For data from quantitative PCR and protein expression of NKG2D ligands, one-way ANOVA was used. P-values < 0.05 were considered statistically significant.

3. Results

3.1. Expression of NKp30 and NKp46

Flow cytometry was used to analyze the expression of NCRs in NK cells. There was no significant difference in the expression of NCRs in $CD3^-CD56^+$NK cells and $CD3^-CD16^+$NK cells from PB between the pelvic endometriosis group (n = 20) and the control group (n = 13) (Fig. 1). But in NK cells from PF, the expression of NKp30 in $CD56^+$NK cells in patients with endometriosis (n = 20) was much lower than that in the control group (n = 13) (P = 0. 006; Fig. 2), whereas the expression of NKp46 in $CD16^+$NK cells in patients with endometriosis (n = 20) was higher than that in the control group (n = 13) (P = 0.040) (Fig. 2). NKp44 was not expressed in NK cells from both PB and PF in all subjects.

3.2. Expression of NKG2D

Flow cytometry was also used to analyze expression of NKG2D in NK cells. There was no significant deference in NKG2D expression in $CD3^-CD56^+$NK cells and $CD3^-CD16^+$NK cells from PB between the pelvic endometriosis group (n = 20) and the control group (n = 13). However, there was significant difference in NKG2D expression between the two groups: the expression of NKG2D in $CD56^+$NK cells from PF with endometriosis (n = 20) was lower than that of the control group (n = 13) (P = 0.01; Fig. 3B).

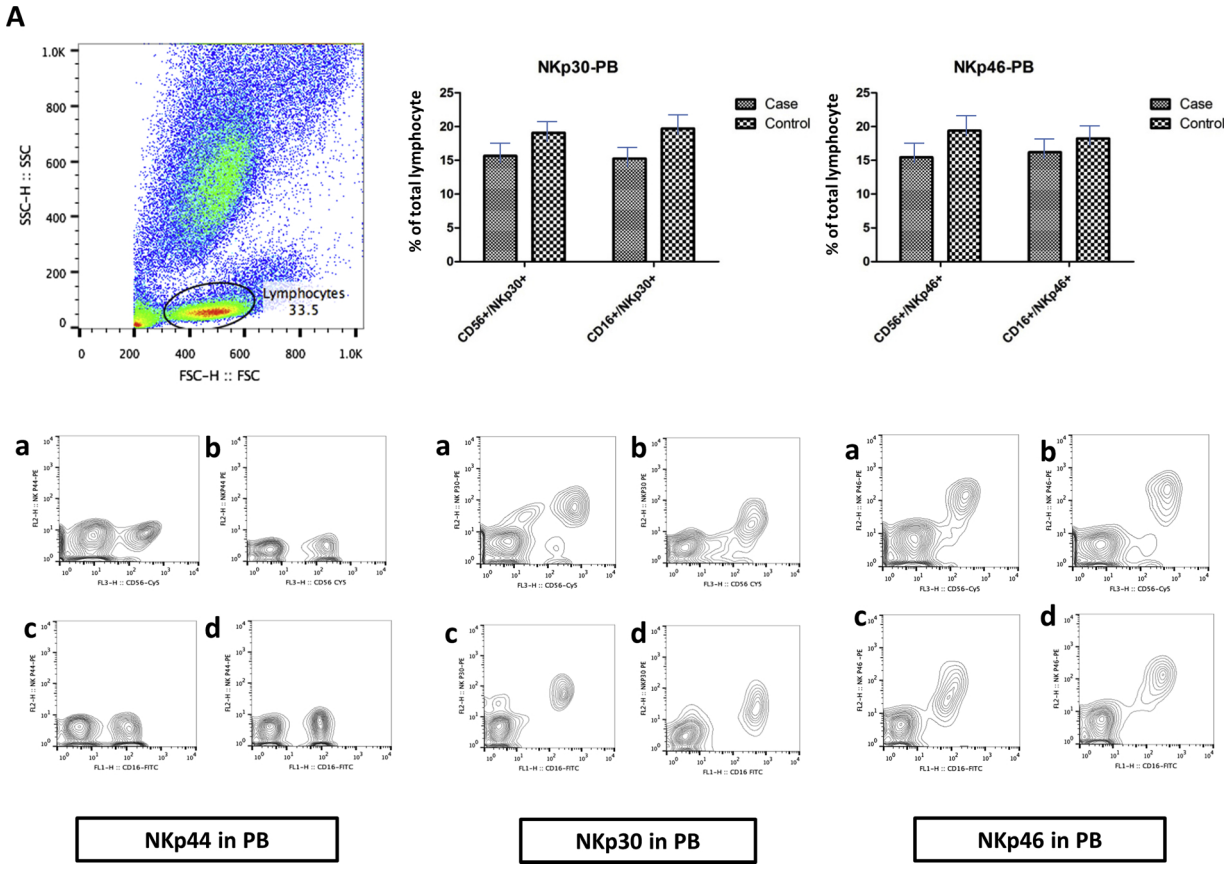

Fig. 1. Expression of NCRs in $CD56^+$NK cells and $CD16^+$NK cells from PB.
a) expression in $CD56^+$NK cells of the pelvic endometriosis group; b) expression in $CD56^+$NK cells of the control group; c) expression in $CD16^+$NK cells of the pelvic endometriosis group; and d) expression in $CD16^+$NK cells of the control group.
There was no significant difference in the expression of NCRs in NK cells from PB between the pelvic endometriosis group (n = 20) and the control group (n = 13). NKp44 was not expressed in NK cells from PB in all subjects.
NCR, natural cytotoxicity receptor; PB, peripheral blood

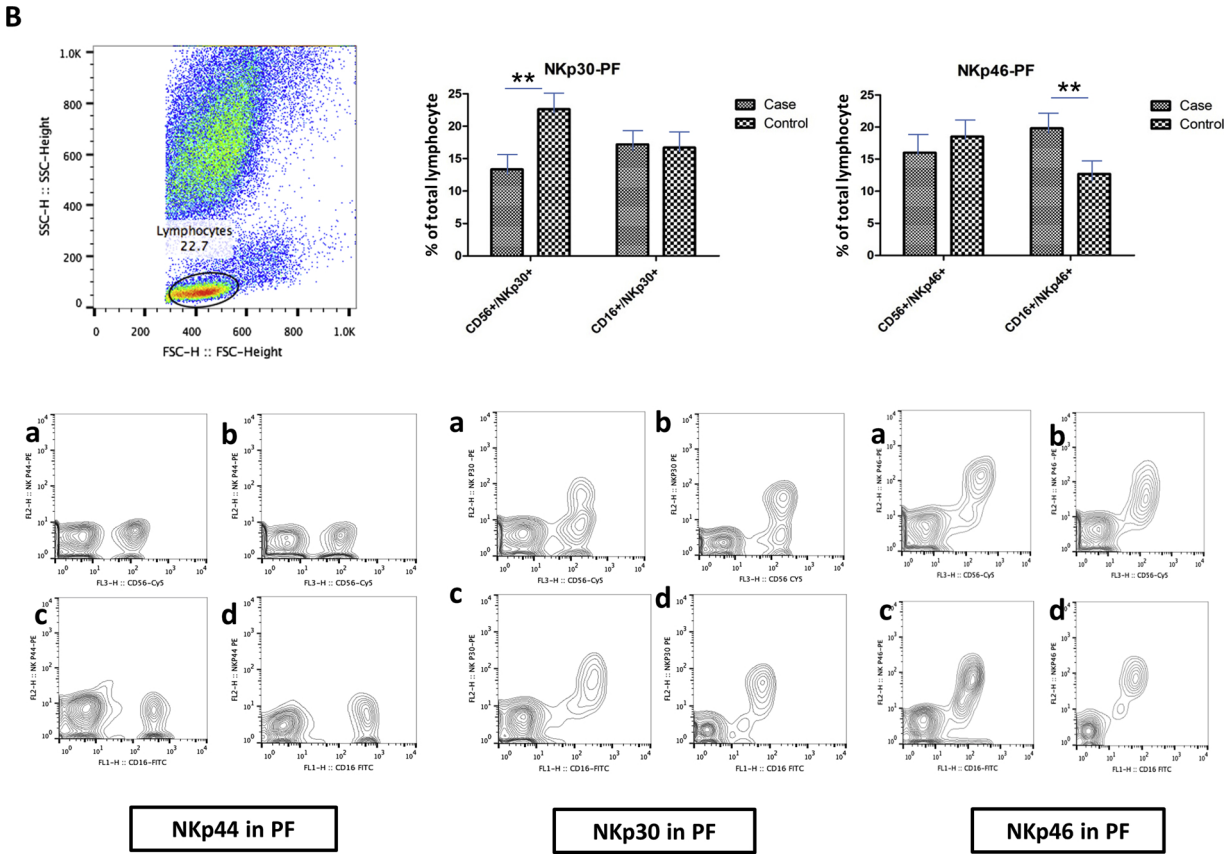

Fig. 2. Expressions of NCRs in CD56$^+$NK cells and CD16$^+$NK cells from PF.
Figure a, b, c and d represent similar expression as in Fig. 1. From the figure for NKp30 in PF, the expression in CD56$^+$NK cells of the pelvic endometriosis group was lower than that of the control group (P = 0.006). From the figure for NKp46 in PF, the expression in CD16$^+$NK cells of the pelvic endometriosis group (n = 20) was higher than that of the control group (n = 13) (P = 0.04). "**" means there had difference between the two groups.
NKp44 was not expressed in NK cells from both PF in all subjects.
NCR, natural cytotoxicity receptor; PF, peritoneal fluid

3.3. Expression of NKG2D ligands in eutopic and ectopic endometrial cells

Real time PCR and Western Blotting were usedto determine the expression of NKG2D ligands in endometrial cells (Figs. 4 and 5). We divided the subjects into three groups:normal eutopic endometrium group, eutopic endometrium with endometriosis and ectopic endometrium. The mRNA expression had no noticeable difference, but there was significant difference at translational level. ULBP-2 expression in eutopic endometrial cells with pelvic endometriosis was lower than that in ectopic endometrial cells and eutopic endometrial cells without endometriosis (P < 0.05). ULBP-3 expression in ectopic endometrial cells was lower than that in eutopic endometrial cells with or without endometriosis (P < 0.05). We found no difference in MICA/MICB and ULBP-1 expressions in endometrium among the three groups.

4. Discussion

Endometriosis is a perplexing medical condition and its exact pathogenesis remains elusive. The pathogenesis of endometriosis is likely multi-factorial. Several speculations have been proposed for the pathogenesis of this disease, such as genetics, environmental factors and alterations in immune and/or endocrine functions (Vercellini et al., 2014), with local and systemic aberrations in immune responses likely being important pathogenic factors. Significantly diminished NK cytotoxicity is well known in pelvic endometriosis patients.

NCRs and NKG2D are two principal receptors involved in NK cells activation. NKp46 (Sivori et al., 1997; Pessino et al., 1998) and NKp30 (Pende et al., 1999) are found in both resting and activated NK cells, whereas NKp44 (Vitale et al., 1998; Cantoni et al., 1999) is selectively expressed upon NK cell activation. In this study, we examined the expressions of NCRs and NKG2D in NK cells from PB and PF with pelvic endometriosis. We found no significant difference in NCRs and NKG2D expression in NK cells from PB between the endometriosis group and the control group. In contrast, the expression of NKp30 and NKG2D in CD56$^+$NK cells decreased in NK cells from PF in patients with endometriosis, and the expression of NKp46 in CD16$^+$NK cells with endometriosis was higher than that in the control (P < 0.05). We also found that NKp44 was not expressed in NK cells in pelvic endometriosis, implying that NKp44 was in an inactivated state.There has difference of NK cells from peripheral blood and peritoneal fluid (Belisle et al., 2007). The NK cells from peripheral blood is activated phenotype, and most are CD56^{dim} CD16^{bright}. CD16$^+$NK cells are nearly equal to CD56^{dim} NK cells. These cells are more cytotoxic via proferin and granzyme secretion. However, NK cells from peritoneal fluid with endometriosis is CD56^{bright} CD16$^-$/low, and is regulated phenotype, especially in late stage.These later cells produce more cytokines, such as GM-CSF, IFN-γ, IL-10, TNF-α, and etc (Wagner et al., 2017). According to this and based on our research results, we speculate that with late stage of pelvic endometriosis, the decreased NK cell cytotoxicity within pelvic microenviroment was due to the decreased expression of NKp46 in CD56^{dim} CD16^{bright} NK cells which maybe very small amount in the pelvic cavity. As for the expression changes of NKp30 and NKG2D in CD56^{bright}CD16$^-$/lowNK cells in the pelvic cavity, it should be related to many cytokine expresssions, such as IL-10, TNF-α, and etc. There have been many reports about cytokine changes with endometriosis (Funamizu et al., 2014; Monsanto et al., 2016). Since expression

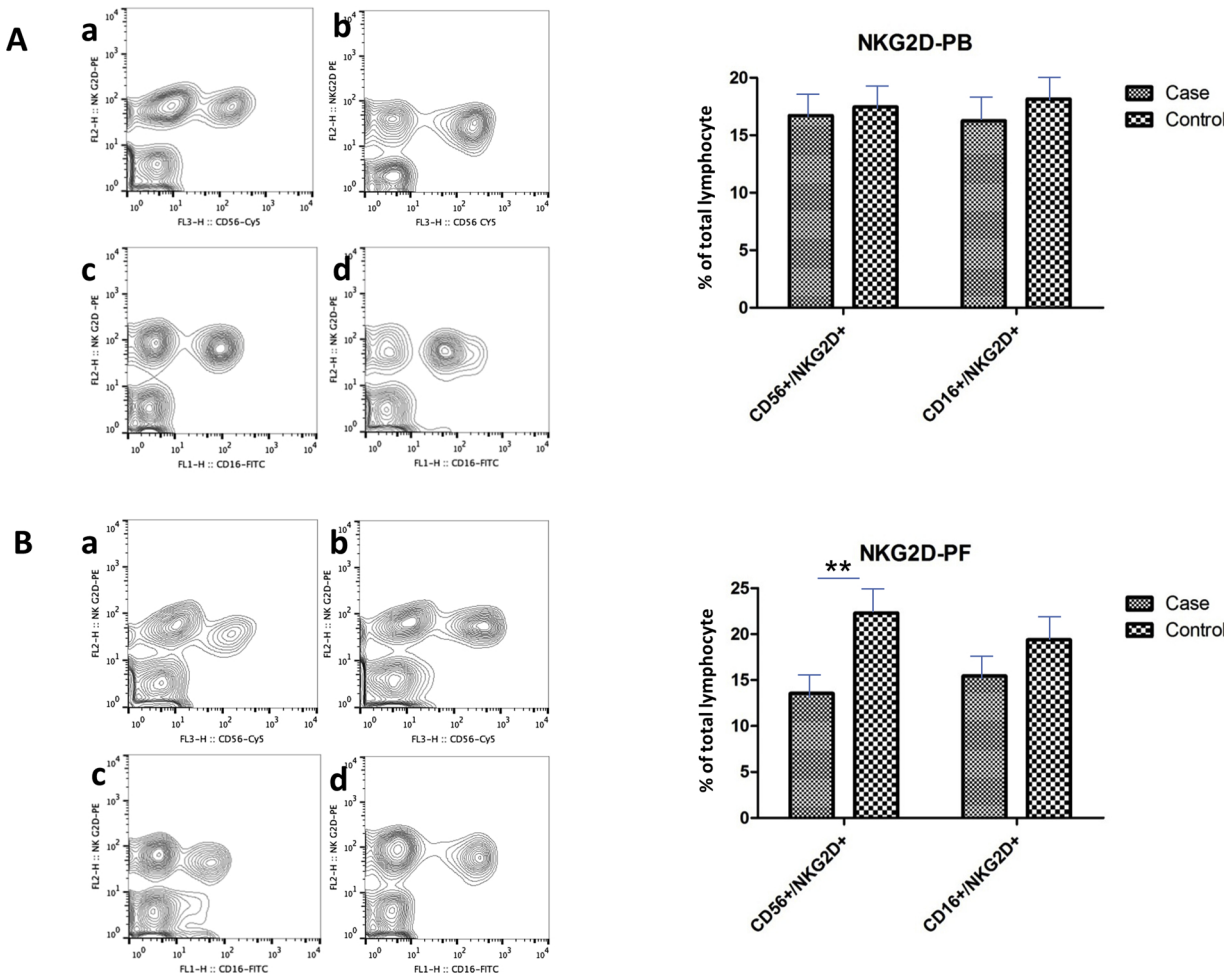

Fig. 3. Expressions of NKG2D on CD56$^+$NK cells and CD16$^+$NK cells from PB and PF.
Figure a, b, c and d represent similar expression as in Fig. 1.
A. NKG2D expression in NK cells from PB. There was no significant difference in NKG2D expression in NK cells from PB between the pelvic endometriosis group (n = 20) and the control group (n = 13).
B. NKG2D expression in NK cells from PF. The expression of NKG2D in CD56$^+$NK cells of the pelvic endometriosis group (n = 20) was significantly lower than that of the control group (n = 13) (P = 0.01). "**" means there had difference between the two groups.
NKG2D can be detected in CD56$^-$CD16$^-$NK cells from both PB and PF. There was no significant difference in expression between the pelvic endometriosis group and the control group.
PB, peripheral blood; PF, peritoneal fluid

occurred primarily in PF, the occurrence of pelvic endometriosis is probably due to functional changes in the local immune system.

Previous research found that the percentage of NKp46 expressed both on CD56$^+$NK cells and CD56^{dim} NK cells were significantly lower in the severe endometriosis group in PF (Funamizu et al., 2014). Their findings were partly consistent with our results. The cases included ovarian type, peritoneal type and deep infiltrating endometriosis(DIE), but unclassified. Parts of the cases were received hormonal therapy, and the control group included patients with uterine fibroids. Hormone levels may have an impact on outcomes. A recent study also found that the percentage of NKG2D$^+$NK cells in CD56$^+$NK cells was significantly lower in the endometriosis group than that in the control group, and the percentage of CD56$^+$ NKG2D NK cells was correlated negatively with TGF-β1 concentration in PF (Guo et al., 2016). Similarly, the percentage of NKG2D$^+$NK cells in CD56^{dim} NK cells was also correlated negatively with platelet activation rate and TGF-β1 concentration in PF. These findings implied that platelet-derived TGF-β1 may be responsible for reduced NKG2D expression in PF of women with endometriosis, and for reduced cytotoxicity of NK cells in women with endometriosis (Du et al., 2017). Guo and his colleges' papers were about the effect of platelet-derived TGF-β1 on NK cell function and on inhibition of NKG2D expression. Our research described the expressions of activating receptors, including both NCRs and NKG2D, on NK cells and the ligands of NKG2D with ovarian endometriosis systematically. Although the lesions sometimes coexisted, we only took samples from ovarian type. We collected peripheral blood, peritoneal fluid, eutopic and ectopic endometrium simultaneously, which means from the same individual. Humans were used as samples. There were no interference with hormonal therapy in all cases. The control group was limited to women with a simple ovarian cyst or even a completely normal control.

The role of receptors depends on their interaction with ligands in target cells. To date, however, little information is available regarding NCRs ligands. In this study, we focused on the expression of NKG2D ligands, MICA/MICB and ULBPs. Using real-time PCR, we found no difference in mRNA expression of MICA/MICB and ULBP1–3 among normal eutopic endometrium, case eutopic endometrium and ectopic endometrium (P > 0.05). Whereas using Western blotting, we found that protein expression of ULBP-2 on eutopic endometrium of patients with endometriosis was much lower than that on normal eutopic endometrium and ectopic endometrium. Protein expression of ULBP-3 on ectopic endometrium of patients with endometriosis also decreased significantly, but protein expression of MICA/MICB and ULBP-1 showed

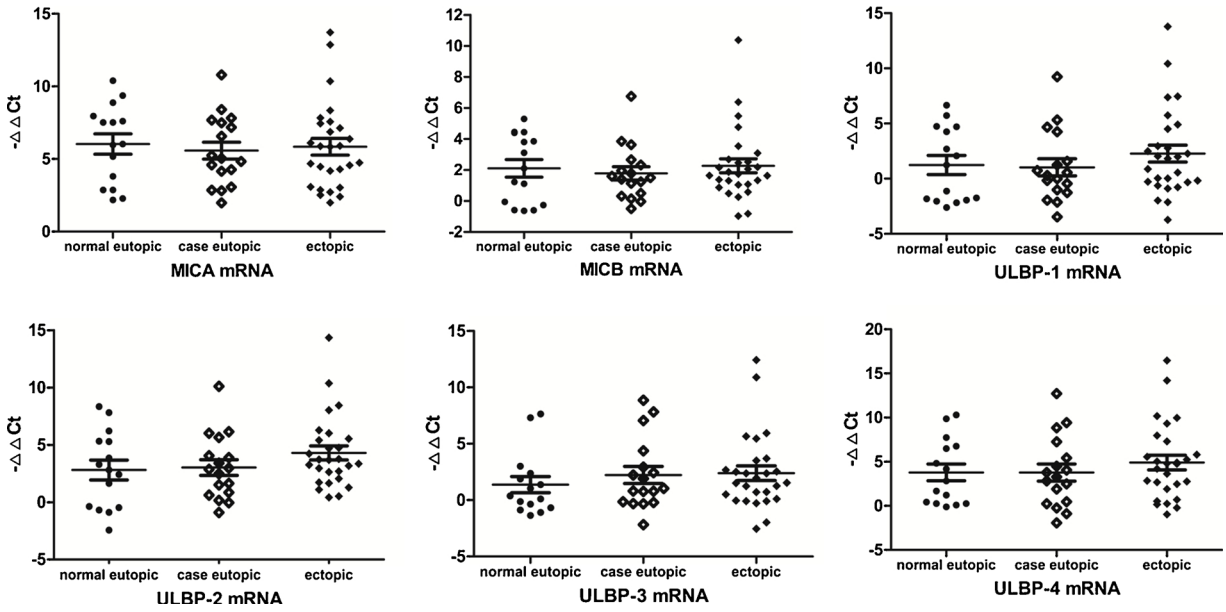

Fig. 4. mRNA expression of human NKG2D ligands, MICA/MICB and ULBP 1–4 ligands in eutopic and ectopic endometrial cells.
Real-time PCR indicated that the mRNA expression of MICA/MICB and ULBP 1–4 was similar between the pelvic endometriosis group and the control group in eutopic endometrium as well as in ectopic endometrium. The middle lines meant median, and the whiskers indicated the distribution of data.
PCR, Polymerase Chain Reaction

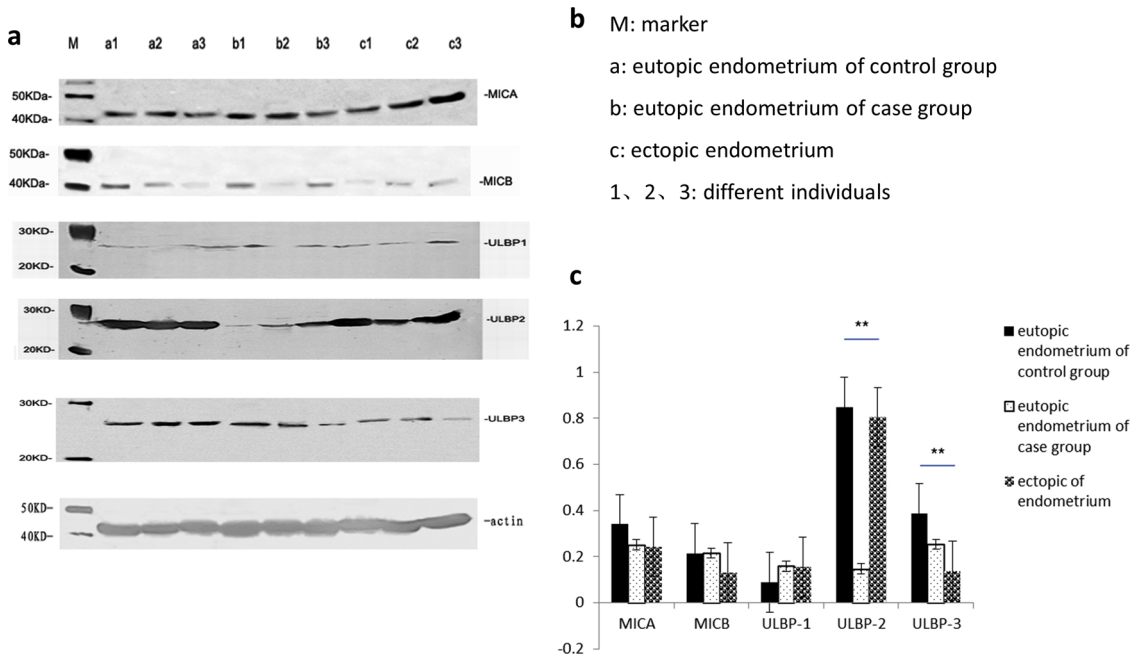

Fig. 5. Protein expression of human NKG2D ligands, MICA/MICB (38 kD) and ULBP 1–3 (27 kD) in endometrial cells.
Western blotting was used to detect the expression. The expression of ULBP-2 in eutopic endometrium and in ectopic endometrium of the pelvic endometriosis group was much lower than that of the control group. In both the pelvic endometriosis group and the control group, the expression of ULBP-3 in ectopic endometrium was lower than that in eutopic endometrium. There was no difference in the expression of MICA/MICB and ULBP-1 in endometrium among the three groups.
a. The expression of protein levels of NKG2D ligands.
b. M: marker; a: eutopic endometrium of the control group; b: eutopic endometrium of the pelvic endometriosis group; c: ectopic endometrium; and 1, 2 and 3 represent different individuals.
c. The bar indicates mean, and the error bar indicates SEM.
d. "**" means there had difference among the three groups.

no differences, compared with normal eutopic endometrium. The discrepancy between RNA expression and protein expression occurred in the translation process. That means the protein molecules were shedding from the cell membrane, and can not be detected on the endometrium. But, the soluble ULBP-2 of the peritoneal fluid may be increased. It was consistent with Gonzalez-Foruria's finding (Gonzalez-Foruria et al., 2015).

The decreased expression in ULBP-2 and ULBP-3 may help a patient's eutopic/ectopic endometrial cells escape immune surveillance, and play a role in NKG2D-mediated decline in NK cell cytotoxicity.

NKG2D receptor recognizes a broad and structurally diverse range of ligands. NKG2D ligands are generally absent on the surface of normal cells, but often are induced by tumorigenic cells and are further upregulated by chemotherapy or radiation. NKG2D ligands are expressed in almost all tumor cells from all tissues, but different expressions occur within different organs. It is related to the sensitivity of tumor cells to NK cells. Carlsten and his colleges found that MICA, ULBP1 and ULBP3 was lower expressed on ovarian cancer cells, and ULBP2 was higher level (Moroni et al., 2007). Similarly, the expressions of NKG2D ligands were also different with endometriosis. ULBP-2 plays a major role in the pathogenesis of endometriosis.

In general, the expression of NKG2D ligands is an indicator which means the cell was under stress, such as viral infection and malignant transformation. It is rarely expressed on healthy cells. This is related to the sensitivity of stressed cells to NK cells. ULBP transcripts are widely expressed in the kidney, uterus, prostate, tonsil and lymph nodes of healthy adults. Although mRNA of NKG2D ligands exists in normal embryonic tissues and adult mature tissues, there has no expression on these normal cells, because the regulation of post-transcription prevents translation and expression of these ligands in healthy individuals, then the self-recognition and autoimmune responses will be prevented (Zhang et al., 2015). In our research, we find that ULBP-2 was lower expression with endometriosis, that means the endometrium back to the pelvic cavity with the menstruation can not be recognized and cleared by NK cells, and escape from immune surveillance. On the other hand, tumor cells frequently escape the immune surveillance of NKG2D pathways by proteolytic-mediated shedding of NKG2D ligands from tumor cell surface. The shedding form of NKG2D ligands, sNKG2D-L, has been demonstrated to be highly immune suppressive.

NKG2D ligands were overexpressed in autoimmune tissues near tumor cells (Ogasawara et al., 2004; Hudspeth et al., 2013). The dysregulation of NKG2D ligands expression and the abnormal activation of NKG2D-mediated immune responses are known as a feature of autoimmune diseases (Jabri and Meresse, 2006). The significant increase of ULBP-2 protein expression in ectopic endometrial cells as observed in this study may be associated with autoimmune response. Therefore, we speculate that endometriosis may be an autoimmune disease, which needs further confirmation in future studies.

Endometriosis remains to be a disease with no specific treatment. Based on our results, we propose that regulation of NKp30, NKp46, NKG2D and NKG2D ligands expression can lead to increased cytotoxicity of NK cells with pelvic endometriosis. It reveals a promising approach of immunotherapy by using immunomodulatory methods to enhance NK cell function to treat this enigmatic disease.

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