Mifepristone Inhibited the Expression of B7-H2, B7-H3, B7-H4 and PD-L2 in Adenomyosis

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Research

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Abstract

Background

The immune mechanism was shown to be involved in the development of adenomyosis. The current study aims to evaluate the expression of immune checkpoint B7-H2, B7-H3, B7-H4 and PD-L2 in adenomyosis and to explore the effect of mifepristone on the expression of these immune checkpoints.

Methods

The expression of B7-H2, B7-H3, B7-H4 and PD-L2 in normal endometria and adenomyosis treated with or without mifepristone was determined by immunohistochemistry analysis.

Results

In adenomyosis, the expression of B7-H2, B7-H3 and B7-H4 was increased in the eutopic and ectopic endometria compared with normal endometria, both in the proliferative and secretory phase. Moreover, the expression of B7-H2 and B7-H3 was higher in adenomyotic lesions than in the corresponding eutopic endometria, both in the proliferative and secretory phase. The expression of PD-L2 was higher in adenomyotic lesions than in normal endometria, both in the proliferative and secretory phase. In secretory phase but not the proliferative phase, the expression of B7-H4 and PD-L2 in adenomyotic lesion showed a significantly higher level than that in the corresponding eutopic endometria. In normal endometria and eutopic endometria, the expression of B7-H4 showed elevated expression in proliferative phase compared with that in the secretory phase, while this change altered in ectopic endometria with decreased B7-H4 expression in proliferative phase than the secretory phase. In addition, the expression of B7-H2, B7-H3, B7-H4 and PD-L2 was significantly decreased in adenomyosis after treated with mifepristone.

Conclusions

Expression of immune checkpoint proteins B7-H2, B7-H3, B7-H4 and PD-L2 is up-regulated in adenomyosis and down-regulated with mifepristone treatment. The data suggests that the B7 immunomodulatory molecules are involved in the pathophysiology of adenomyosis.

Background

Adenomyosis is a chronic inflammatory disease characterised by the invasion and growth of functional endometrial glands and stroma in the myometrium. Adenomyosis always causes dysmenorrhea menorrhea and subfertility, which seriously affect physical and psychological health of women. However, its pathogenesis remains poorly understood[1]. Growing evidence from diverse studies showed that aberrant immune responses play a vital role in the pathogenesis of adenomyosis[2]. Both systemic and local immune alterations exist in women affected by adenomyosis, with the coexistence of changes in inflammatory as well as anti-inflammatory signals[2]. This underscored the immune contributions to the disease.

The B7-CD28 family are immunomodulatory molecules and indispensable members for full activation of T cells. Recently, B7-H2, B7-H3, B7-H4 and programmed death ligand 2 (PD-L2, CD273, B7-DC), as new B7 proteins, attracts increasing interests. These co-signaling molecules not only provide critical positive signals that stimulate T cell growth, up-regulate cytokine production and promote T cell differentiation, but also contribute key negative signals to limit, terminate and/or attenuate T cell responses[3, 4] Aberrant expression of these B7 family members are associated with the emergence of T cell exhaustion in many disorders, including cancers, pregnancy, autoimmune diseases, etc[5]. Therefore, we wondered whether altered expression of these B7 family members could be observed in adenomyosis.

To date, hysterectomy is still the most common and effective treatment for adenomyosis as it nearly ensures cure. However, it is challenge for many patients with desiration for uterus reservation[6]. For those patients contradictive to hysterectomy, drug therapy including oral contraceptives, progestogen, mifepristone, danazol and gonadotropin-releasing hormone analog is therapeutically important. Evidences have shown that mifepristone can inhibit endometrial proliferation or suppress adenomyotic lesions, resulting in inhibition of prostaglandin production and endometrial atrophy in animal models[7]. It has been demonstrated that treatment with 50-mg of mifepristone daily lead to improved pain and regression of adenomyosis[8].

Therefore, in the current study, we estimated the expression and localization of four B7 molecules including B7-H2, B7-H3, B7-H4 and PD-L2 in adenomyosis and control group. In addition, we also investigate the effect of mifepristone on the expression of these four B7 molecules in adenomyosis.

Methods

Collection of tissues

The study participants were enrolled in the Department of Obstetrics and Gynaecology, Provincial Hospital affiliated to Shandong First Medical University from January 2016 to March 2018. Adenomyotic lesion as well as the corresponding eutopic endometria were obtained from patients with adenomyosis undergoing hysterectomy. Drug-untreated adenomyotic group comprised 58 patients with adenomyosis (proliferative phase: n= 35; secretory phase: n= 23) with no use of any hormone therapy within at least 6 months before surgery. The mifepristone-treated adenomyotic group consists of 11 patients with adenomyosis treated with mifepristone at a dose of 12.5-mg daily for 3 months. 74 samples of normal endometrium collected from hysterectomy specimens checked for leiomyoma pathology (proliferative phase: n= 47; secretory phase: n= 27) were included as controls. Participants in control group had no evidence of adenomyosis in the histopathological examination of their hysterectomy specimens and had no visible pelvic inflammation or endometriosis at the time of hysterectomy. The characteristics of patients in each group were shown in Table 1. Ectopic and eutopic endometria of adenomyosis were collected during the surgery. Adenomyosis was confirmed by histological examination. Written informed consent was obtained from all participants prior to the biopsy procedure. This study was approved by the Institutional Review Board of Shandong First Medical University.

Immunohistochemistry analysis

The tissue processing and staining procedure have been described in detail as our previous study[9]. Immunohistochemical staining was performed using polyclonal antibody rabbit anti-human ICOSL (B7-H2) (1:100, ab233151, Abcam, Cambridge, UK), monoclonal antibody mouse anti-human CD276 (B7-H3) (1:200, ab105922, Abcam), monoclonal antibody rabbit anti-human VTCN1 (B7-H4) (1:300, ab209242, Abcam) and polyclonal antibody rabbit anti-human PD-L2 (1:100, ab244332, Abcam,). Goat anti-rabbit and goat anti-mouse HRP-conjugated secondary antibodies and Diaminobenzidine(DAB) staining kits were obtained from ZSGB-BIO (Beijing, China).

The sections were viewed under a Leica DM4000B microscope (Leica, Wetzlar, Germany), and photographs were taken using the IM50 image analysis system (Leica). Immunohistochemical staining was evaluated by semi-quantitative immunoscore which was a product of a quantity score and a staining intensity. Quantity score(Pi) also as the percentages of positively stained glandular epithelial cells was estimated from four randomly chosen views and 100 counting cells. The staining intensity (I) of the glandular epithelial cells was estimated as follows: 0: negative; 1: weak staining; 2: moderate staining; and 3: strong staining. H-score equals Pi* (I + 1) . Two sections of each sample were assessed by two investigators blinded to any pathological or clinical data about the tissues. The average score of the two investigators was used.

Statistical analysis

Statistical analysis of data was performed by analysis of variation using SPSS 19.0 (SPSS Inc., Chicago, IL). Data are presented as means ± SD. Differences between two groups were determined by the two-tailed student's t-test. $P<0.05$ was considered to be statistically significant.

Results

1. Overexpression of B7-H2 in adenomyotic eutopic and ectopic endometria

B7-H2 protein was expressed both in glandular and stromal cells in endometrial tissues, and mainly in glandular epithelial cells. Positive expression was mainly located in the cell membrane and cytoplasm, but not the nucleus. The immune expression of B7-H2 protein in the endometrial tissues of the control group is extremely faint and almost no expression, regardless of glandular epithelial or stromal cells (Fig 1 E&F). In contrast, B7-H2 protein was moderately expressed in partial epithelial and stromal cells in the eutopic endometrium of adenomyosis (ADE-EU) (Fig.1 C&D) and intensely expressed in the ectopic endometrium of adenomyosis (ADE-EC) (Fig.1 A&B). Compared with the control group, ADE-EU and ADE-EC showed significantly increased immune expression of B7-H2, both in the proliferative and secretory phase. [Fig.1 J ADE-EU vs control P(proliferative stage)<0.001; P(secretory stage =0.015; ADE-EC vs control (proliferative and secretion stage): both $P<0.001$]. Moreover, the expression of B7-H2 in ADE-EC was significantly higher than that of ADE-EU in the same menstrual stage. [Fig.1 J ADE-EC vs ADE-EU (proliferative and secretion stage): both $P<0.001$]

During the menstrual cycles, the expression of B7-H2 in the ADE-EU was statistically significant stronger in the proliferative stage than that in the secretory stage (P=0.03), while no significant difference was noted in ADE-EC (P=0.78) or in the control group (P=0.82).

2. Overexpression of B7-H3 in adenomyotic eutopic and ectopic endometria

B7-H3 protein was mainly expressed in glandular epithelium instead of stromal cells in endometrial tissues. The positive expression was mainly located in the cell membrane and cytoplasm. The immune expression of B7-H3 protein in the endometrial tissues of the control group is weak and almost no expression, regardless of glandular or stromal cells (Fig.2 E&F). However, B7-H3 was moderately expressed in glandular epithelium of ADE-EU (Fig.2 C&D), and intensely expressed in that of ADE-EC (Fig.2 A&B). Compared with the control group, ADE-EU showed significantly increased B7-H3 expression, both in the proliferative and secretory phases [Fig.2 J ADE-EU vs control: P(proliferative phase)<0.001， P(secretory phase)=0.002]. Moreover, ADE-EC showed statistically higher expression of B7-H3 compared with the corresponding ADE-EU in the same menstrual phase [Fig.2 J ADE-EC vs ADE-EU (proliferative and secretion stage): both P < 0.001].

3. Overexpression of B7-H4 in adenomyotic eutopic and ectopic endometria

In endometrium of patients with or without adenomyosis, the immunostaining of B7-H4 showed similar characteristics. Positive staining was mainly observed in the glandular epithelial cells, with almost no staining in the stroma. The positive immunostaining of B7-H4 was located mainly in the cell membrane and cytoplasm.

In the control group, the endometria showed extremely low, almost no immunostaining of B7-H4 protein (Fig.3 E&F). While in the adeomyotic group, the ADE-EU showed moderately immunostaining of B7-H4 in glandular epithelium cells(Fig.3 C&D). ADE-EC showed strong immunostaining intensity of B7-H4 in almost all glandular epithelium(Fig.3 A&B). Compared with the control group, ADE-EU and ADE-EC showed statistically significant higher expression of B7-H4 both in the proliferative phase and in secretory phase (Fig.3 J P<0.001). ADE-EC in secretory phase showed statistically significant higher level of B7-H4 expression than the corresponding ADE-EU in the same menstrual phase (Fig.3 J P<0.001). However, no significant difference of B7-H4 expression was shown between ADE-EU and ADE-EC in the proliferative phase (Fig.3 J P=0.06).

Both in ADE-EU and the control groups, the expression of B7-H4 was higher in proliferative phase than in secretory phase [Fig.3 J control group， P=0.01, ADE-EU， P=0.03]. In contrast, that expression in ADE-EC was lower in the proliferative phase than in the secretory phase (Fig.3 J P< 0.001).

4. Overexpression of PD-L2 in ectopic endometria of patients with adenomyosis

The expression of PD-L2 protein in control and adenomyotic endometrium was detected using immunohistochemical analysis. Figure 4 shows that PD-L2 was not expressed or only weakly expressed in endometrium of both control group(Fig.4 E&F) and ADE-EU(Fig.4 C&D). Notably, it was expressed at

significantly higher levels in endometrium of ADE-EC(Fig.4 A&B) compared with control group, both in the proliferative and secretory phases (Fig.4 J both P<0.01). Moreover, ADE-EC showed higher PD-L2 expression than ADE-EU in the secretory but not the proliferative phase. Nevertheless, there was no significant difference of PD-L2 expression between ADE-EU and control group (Fig.4 J). The data also showed that in ectopic endometria of adenomyotic samples, PD-L2 was primarily expressed in glandular epithelial cells. During menstrual cycles, no periodic changes of the endometrial PD-L2 expression was found neither in adenomyotic group nor in the control group.

5. Decreased expression of B7-H2, B7-H3, B7-H4 and PD-L2 in patients with adenomyosis after mifepristone treatment

Immunohistochemical staining was used to detect and compare the changes of B7-H2, B7-H3, B7-H4 and PD-L2 expression in eutopic and ectopic endometrium of patients with adenomyosis treated with and without mifepristone. The results showed that the eutopic and ectopic endometrium of adenomyosis treated with mifepristone showed significantly lower expression of B7-H2, B7-H3, B7-H4 and PD-L2 protein compared with adenomyosis without mifepristone treatment, both in proliferative phase and in secretory phase (Fig.1,2,3,4 G&H&I&J all P < 0.01).

Discussion

The activation of T lymphocytes plays an essential role in the process of immunity. While this activation requires two signals simultaneously[10]: 1) the first stimulus signal was provided by T cell receptor recognized by MHC-antigen complex; 2) the key second signal was delivered by ligation of T cells and co-modulatory molecules expressed on antigen-presenting cells (APCs) belonging to the B7 or other families. In recent years, a breakthrough about the three main members of B7 family: CTLA-4, PD-1 and PD-L1 (B7-H1) has been made in the immune checkpoint treatment of cancer[10]. B7 family is the most important immunomodulatory molecule. Since there is little research on the correlation between B7 family and adenomyosis, our study explored the expression of B7-H2, B7-H3, B7-H4 and PD-L2 in adenomyosis treated with and without mifepristone.

B7-H2 is expressed on professional APCs and it binds to inducible costimulator molecule (ICOS) expressed on T cells. The ICOS/B7-H2 signal was engaged in various aspects of T-cell responses. This engagement plays an essential role in the differentiation of $CD4^+$ T cells into effector subsets, including Th1, Th2, Th17 and regulatory T cells (Tregs)[11, 12]. In acute myelocytic leukemia (AML)，strongly-activating B7-H2 exhibits an inhibitory function by which enabled AML cells to facilitate immune escape[13]. Recently, over-expression of B7-H2 was found in a variety of solid cancers by maintaining the function of immunosuppressive Tregs subset, which is associated with tumor progression and poor overall survival[14]. Th17/Tregs imbalance has been shown to be present in adenomyosis[15]. Considering the effect of B7-H2 on differentiation of Th17 and Tregs from $CD4^+$ T cells, it is reasonable to postulate that B7-H2 was involved in pathogenesis of adenomyosis. While further studies are needed to elucidate the definite mechanism. Furthermore, the engagement of B7-H2 with ICOS can stimulate IFN-

gamma, IL-4, IL-5 and IL-10 production by T cells and it most effectively induces IL-10[4, 16, 17]. Moreover ICOS also stabilizes IL-10R expression on T cells, rendering them sensitive to IL-10[18]. In this context, this may supply further interpretation to what we found in previous studies that up-regulated expression of IL-10[19] and IL-10R[20] was found in eutopic and ectopic endometrium of adenomyosis. B7-H2 plays a primary role in a regulatory capacity promoting Th2 immune response. In B7-H2-deficient mice, production of Th2 cytokines such as IL-4 and IL-10 by primed T cells is reduced[21]. Shifts towards Th2 immune response have been found being involved in endometriosis with relative predominance of IL-4 and IL-10[22]. In this study, we found the endometrial expression of B7-H2 was higher in patients with adenomyosis than the control group. Moreover, the adenomyotic ectopic expression of B7-H2 was much more intense than the adenomyotic eutopic endometrium. It implies that aberrant expression of B7-H2 involves in the pathogenesis of adenomyosis.

B7-H3 was initially thought to co-stimulate the immune response, but recent studies have shown that it is predominantly a T-cell co-inhibitory molecule contributing to immune evasion[23, 24]. B7-H3 is widely expressed in both lymphoid and nonlymphoid organs at the RNA level, but the expression of B7-H3 protein is more restricted to cell types such as activated dendritic cells, monocytes, T cells, B cells, and NK cells. Aberrant expression of B7-H3 has been shown to be associated with poor outcome of various human malignancies[25] and autoimmune diseases[26]. Overexpression of B7-H3 was displayed in 60-93% of tumor tissues in the vast majority of cancer types by immunohistochemical assay, while very limited expression is seen in normal healthy tissue[25]. This was consistent with what we observed in this study that only weak or no expression of B7-H3was observed in normal endometrium, but stronger intensity of B7-H3 immunostaining was shown in adenomyosis. It implied the over-expression of B7-H3 might participate in the genesis of adenomyosis. Diverse studies have proved that up-regulation of B7-H3 is associated with impaired T-cell stimulated function[27,28], suppressed NK-mediated cell lysis[29], increased IL-10 secretion[30], modulation of the Jak/Stat pathway[31] which contributes to immune suppression and evasion by tumors. These results showed that B7-H3 was involved in the process of tumor cells by acting as a negative regulator of T cells and facilitating evade tumor immunity. Similar to that in tumors, B7-H3 also displays co-inhibitory properties in some immune diseases. Independent studies utilizing either protein blockade or gene-knockout mice have reported that B7-H3 ameliorates graft-versus-host-disease, prolongs cardiac allograft survival, reduces airway hypersensitivity, and delays experimental autoimmune encephalomyelitis onset, especially by down-regulating Th1 responses[32-34]. These examples provide further evidence for the co-inhibitory properties of B7-H3. Our study found B7-H3 was overexpressed in adenomyotic endometria than in control group. Moreover, the adenomyotic ectopic endometria expressed even much higher B7-H3 in comparation with eutopic tissues. We postulate the immunologic function of B7-H3 in adenomyosis was similar to that in malignancies and autoimmune diseases acting as a co-inhibitory immunomodulator. The over-expression of B7-H3 enable the endometrium to create an immunosuppressive microenvironment to facilitate the eutopic and ectopic endometrium to escape host immunosurveillance before infiltrating and after infiltrating into myometrium, thus led to the origination and progression of adenomyosis.

B7-H4 is a vital B7 ligand that acts as a negative regulator in the T cell mediated immune response. B7-H4 mRNA is widely distributed in human peripheral tissues. However, B7-H4 protein expression is more restricted in most normal tissues and can be induced on APCs after in vitro stimulation[35]. Recent studies found that the negative immunomodulatory role of B7-H4 on a wide range of tumors[36], autoimmune diseases[37], viral infection[38] and transplantation rejection[39]. In endometrium the expression of B7-H4 has been estimated in Miyatake T's study. They showed the staining of B7-H4 is faint or moderate in the apex of cytoplasmic membrane in normal or hyperplasic endometrium, but that is strong in circumferential membrane and cytoplasm in most endometrioid carcinomas[40]. A significant inverse correlation has been observed between the high expression of B7-H4 in majority endometrioid carcinomas and tumor infiltrating T cells, particularly the number of tumor-associated $CD3^+$ and $CD8^+$ lymphocytes[40]. High expressed B7-H4 in tumor microenvironment exerts negative immunomodulatory effects through several pathways, including arresting the cell cycle at the G0/G1 stage, promoting T cell apoptosis, inhibiting T cell growth, cytokine secretion and development of cytotoxicity[35], thereby affects the biological behavior of tumor cells, assists tumor immune escape, and leads to seriously poor prognosis of patients[41]. Similar to that, our results showed the endometrial expression of B7-H4 protein in the control group was extremely weak, almost no expression. While the expression of B7-H4 in the eutopic and ectopic endometrium of adenomyosis was significantly higher than that of control group. We hypothesize that over-expressed B7-H4 in adenomyosis participated in the formation of immunotolerant environment of the uterus through negatively regulating T cell proliferation, facilitating the ectopic endometrial lesions to evade host immunity and failure to be eliminated effectively, thus led to the development of uterine adenomyosis.

PD-L2 as one of the two receptors for PD-1, plays crucial roles in the immune checkpoint pathways responsible for the suppression of T-cell activation[42]. PD-L2 expression can be induced on a diverse variety of other immune cells and non-immune cells depending on microenvironmental stimuli[42]. PD-L2 was shown to be moderately or strongly expressed in most tumor cells, interact with PD-1 and dramatically inhibits TCR-mediated proliferation, cytokine production by $CD4^+$ T cells and T-cell cytolysis[43]. Via utilization of immune checkpoint molecules, tumor cells exert immunomodulatoty function to tumor microenvironment and escape host immune surveillance. In this study, we found a higher level of PD-L2 expression in ectopic adenomyotic tissue than in normal endometria and eutopic adenomyotic tissues. In endometrial tissues, it is reported that PD-L2 expression was present in 47% of 15 cases of normal endometria and in 40% of 30 cases of endometrial cancer[44]. Expression of the PD-1/PD-L1/PD-L2 axis is associated with moderately and poorly-differentiated endometrial cancer and type II endometrial cancer in which more frequent expression of PD-1, PD-L1 and PD-L2 may cause immunosuppression to favor tumor growth and negatively affect patient's survival. In the same way, our results indicate the abnormal increased expression of PD-L2 in adenomyosis may repress T-cell activation and alter the immune microenvironment of ectopic endometrium This may enable ectopic endometrial cells to evade normal immunological surveillance and to initiate adenomyosis.

In this study, B7-H4 expression showed cyclic variation in women of the control group and eutopic endometria of adenomyosis, with elevated expression in the proliferative phase. It suggests that B7-H4 expression may be regulated by steroid hormones in normal endometrium. Consistent with this, Papenfuss TL's results[45] showed that estriol can up-regulate the expression of B7-H4 on the surface of dendritic cells, indicating that estrogen can up-regulate the expression of B7-H4. Furthermore, the cyclic change of B7-H4 expression was altered in ectopic endometria of patients with adenomyosis, with lowered expression of B7-H4 in the proliferative phase. These data suggest aberrant hormonal sensitivity of B7-H4 in adenomyotic foci may participate in the establishment of this disease.

Compared with no-treated adenomyosis, down-expressed B7-H2, B7-H3, B7-H4 and PD-L2 was observed in adenomyosis treated with mifepristone, both in eutopic and ectopic endometria. As far as we know, this is the first study to address the effect of mifepristone on the B7-H2, B7-H3, B7-H4 and PD-L2 expression. Mifepristone (RU486) is an antiprogestin with a high affinity for progesterone and glucocorticoid receptor. Studies concerning pregnant women showed that mifepristone can alter the endometrial immune balance and result in implantation failure. It is shown that mifepristone exerts effects through shifting immunological elements by enhancing the expression of cytotoxic lymphocytes[46], increasing cytotoxicity of peripheral blood NK cells[47] and uterine NK cells[48], and enhancing antigen-specific $CD4^+$ and $CD8^+$ T cell inflammatory cytokine (IFN-γ) and cytotoxic molecule release (granzyme B)[49]. Mifepristone regulates Tregs function mediated by dendritic cells through inhibiting the expression of TGF-β[50]. Our current study implies that mifepristone may play a therapeutical role in adenomyosis by inhibiting the expression of these four immunomodulatory molecules, improving the immune microenvironment status of the uterus and effectively inhibiting or clearing ectopic endometrial cells. However, further studies are needed to explore the mechanism refer to possible signaling underlying the finding.

Conclusions

Taken together, the results described in this study are the first to show immunostaining expression of B7-H2, B7-H3, B7-H4 and PD-L2 in patients with adenomyosis. The differential expression of these proteins in endometrium with or without adenomyosis suggests that the altered immunomodulatory molecules participate in the pathogenesis of adenomyosis. We postulate that these powerful immunomodulators are key in providing the eutopic and ectopic endometrium with a suitable immunological environment, leading to ineffective elimination of abnormal eutopic and ectopic endometria, and resulting in the initiation and maintenance of adenomyosis. These findings enrich our comprehension about the local immune status involved in the pathogenesis of adenomyosis, and provide potential targets for immunotherapy. The results of the study further investigated the possible mechanism of mifepristone treatment on adenomyosis, and may provide a theoretical data for future studying adenomyosis treatment. However, the primary limitation of present study is that we only examined the expression and location of B7-H2、B7-H3、B7-H4 and PD-L2 in adenomyosis treated with or without mifepristone by

immunohistochemistry. In next study, we will further investigate the involvement of these B7 family immunomodulators in adenomyosis using multiple experimental techniques.

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all participants prior to biopsy procedure and was approved by Shandong Provincial Hospital Affiliated to Shandong First Medical University.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

The datasets used and analyzed during the present study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

HZ conceived of the study, participated in its design and implementation, helped to draft and edit the manuscript for submission. XQ collected tissues and carried out the immunohistochemistry process, participated in the analysis and the interpretation of data and drafted the manuscript. ML participated in the design of the study, supervised the study and critically helped to revise the manuscript. XZ contributed to the design of the study, assisted in data analysis and revised the manuscript. CL helped to revise the manuscript. XQ, WL, CW and HZ performed the statistical analysis. All authors read and approved the final manuscript.

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Tables

Table 1: The characteristics of participants in each group

	N	age ranges(yrs)	Mean(yrs)	STD
control group				
Proliferative stage	47	38-52	46.9149	3.0132
Secretory stage	27	39-52	46.5556	3.0298
adenomyosis				
Proliferative stage	35	32-52	44.6286	4.2294
Secretory stage	23	40-52	45.6857	3.2813
adenomyosis treated with mifepristone	11	39-52	45.8182	3.8424

Abrreviations: N:number; yrs:years; STD: standard deviation

Figures

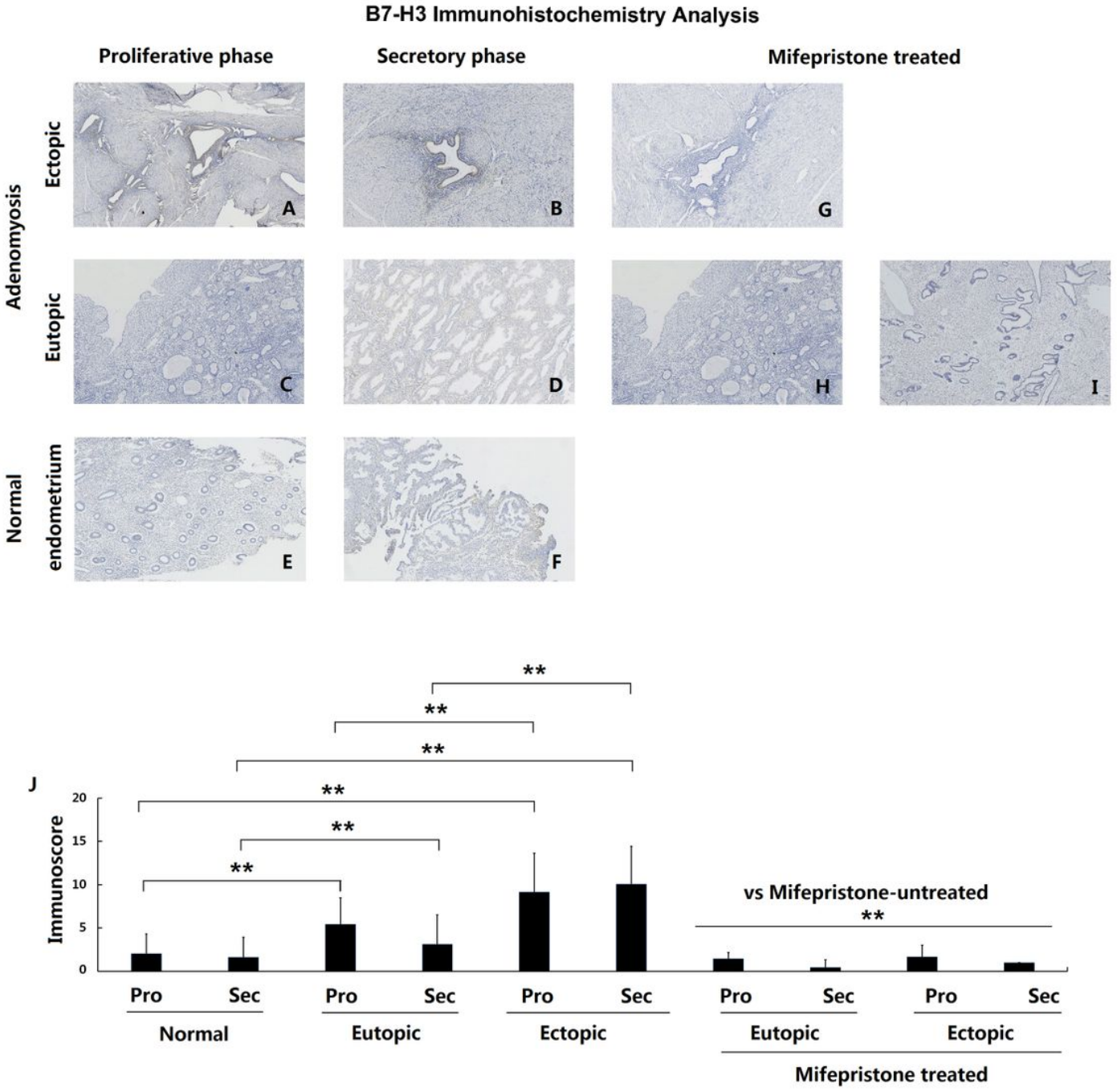

Figure 1

Immunoexpression and comparison of B7-H2 in normal, eutopic and ectopic endometrium of adenomyosis treated with and without mifepristone. A. Ectopic endometrium of proliferative phase in patient with untreated adenomyosis(n=35); B. Ectopic endometrium of secretory phase in patient with untreated adenomyosis (n= 23); C. Eutopic endometrium of proliferative phase in patient with untreated adenomyosis(n= 35); D. Eutopic endometrium of secretory phase in patient with untreated adenomyosis(n= 23); E. Normal endometrium of proliferative phase in patients without adenomyosis(n= 47); F. Normal endometrium of secretory phase in patients without adenomyosis(n= 27); G. ectopic

endometrium in patient with mifepristone-treated adenomyosis(n= 11); H&I. Eutopic endometrium in patient with mifepristone-treated adenomyosis(n= 11); J. Immunoscore comparation of B7-H2 between each groups,* P<0.05, ** P<0.01 A- I magnification: ×100;

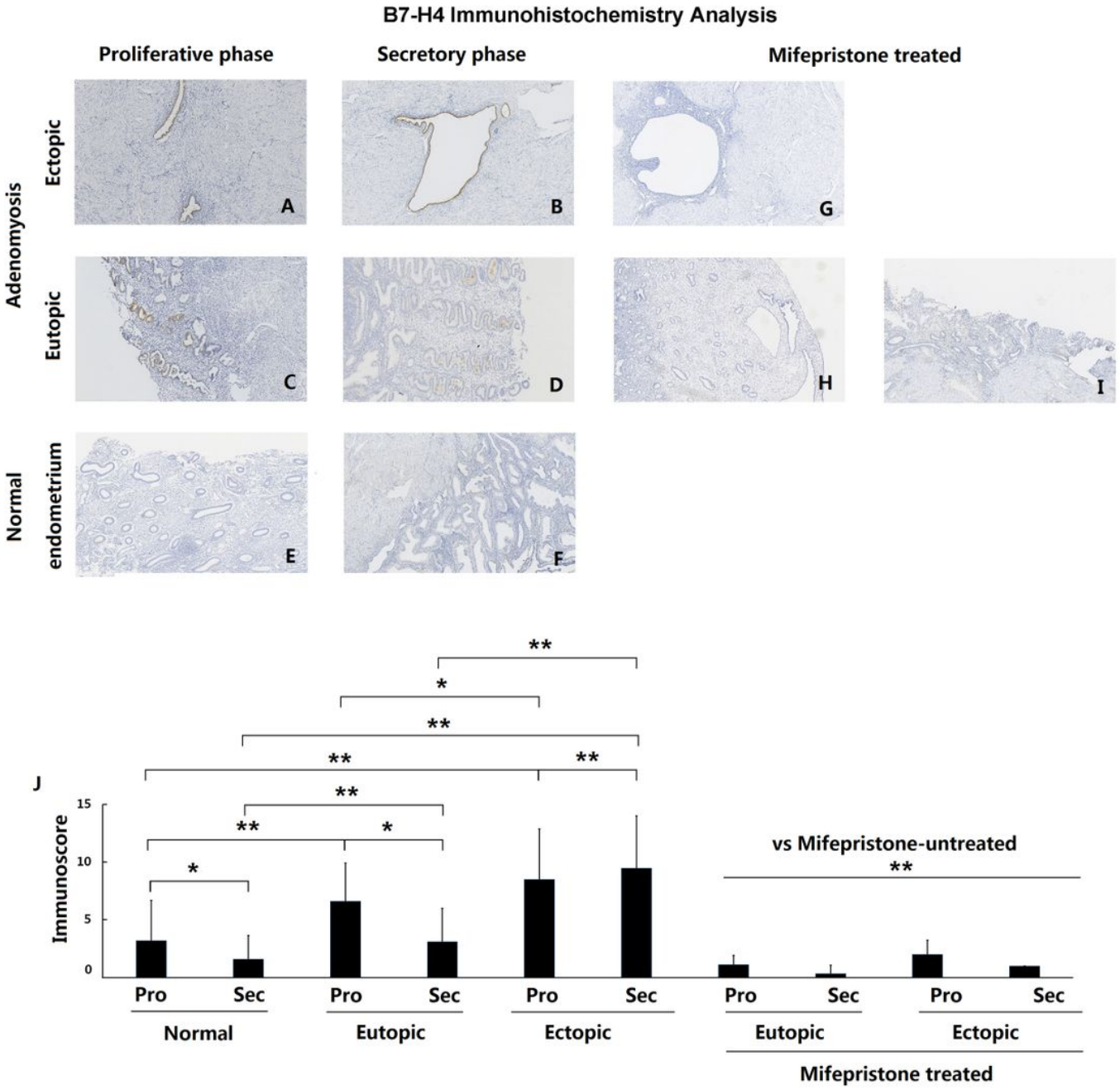

Figure 2

Immunoexpression and comparison of B7-H3 in normal, eutopic and ectopic endometrium of adenomyosis treated with and without mifepristone. A. Ectopic endometrium of proliferative phase in patient with untreated adenomyosis(n=35); B. Ectopic endometrium of secretory phase in patient with untreated adenomyosis (n= 23); C. Eutopic endometrium of proliferative phase in patient with untreated

adenomyosis(n= 35); D. Eutopic endometrium of secretory phase in patient with untreated adenomyosis(n= 23); E. Normal endometrium of proliferative phase in patients without adenomyosis(n= 47); F. Normal endometrium of secretory phase in patients without adenomyosis(n= 27); G. ectopic endometrium in patient with mifepristone-treated adenomyosis(n= 11); H&I. eutopic endometrium in patient with mifepristone-treated adenomyosis(n= 11); J. Immunoscore comparation of B7-H3 between each groups, * P<0.05, ** P<0.01 A- I magnification: ×100;

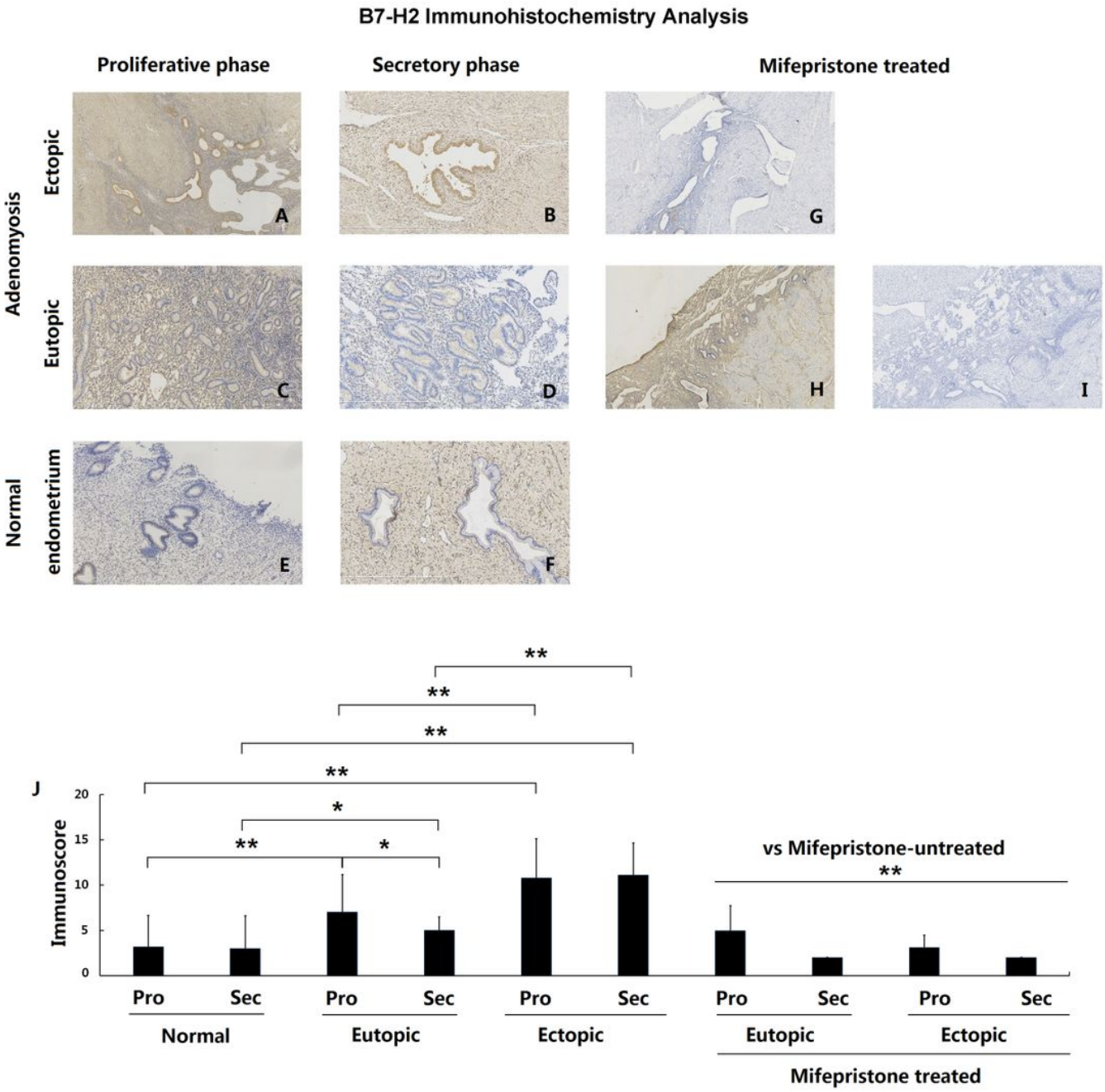

Figure 3

Immunoexpression and comparison of B7-H4 in normal, eutopic and ectopic endometrium of adenomyosis treated with and without mifepristone.. A. Ectopic endometrium of proliferative phase in

patient with untreated adenomyosis(n=35); B. Ectopic endometrium of secretory phase in patient with untreated adenomyosis (n= 23); C. Eutopic endometrium of proliferative phase in patient with untreated adenomyosis(n= 35); D. Eutopic endometrium of secretory phase in patient with untreated adenomyosis(n= 23); E. Normal endometrium of proliferative phase in patients without adenomyosis(n= 47); F. Normal endometrium of secretory phase in patients without adenomyosis(n= 27); G. ectopic endometrium in patient with mifepristone-treated adenomyosis(n= 11); H&I. eutopic endometrium in patient with mifepristone-treated adenomyosis(n= 11); J. Immunoscore comparation of B7-H3 between each groups, * P<0.05, ** P<0.01 A-I magnification: ×100;

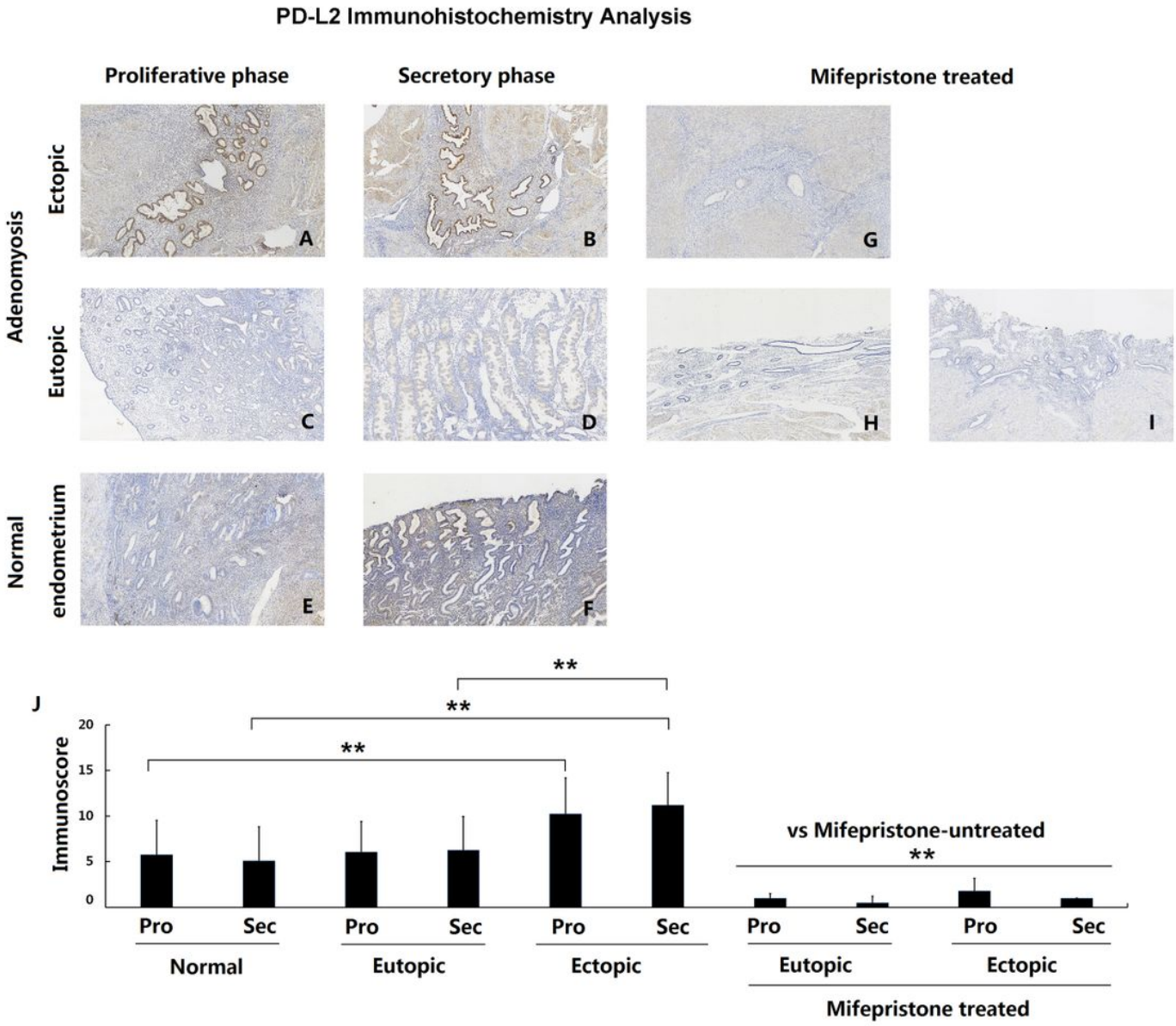

Figure 4

Immunoexpression and comparison of PD-L2 in normal, eutopic and ectopic endometrium of adenomyosis treated with and without mifepristone. A. Ectopic endometrium of proliferative phase in patient with untreated adenomyosis(n=35); B. Ectopic endometrium of secretory phase in patient with untreated adenomyosis (n= 23); C. Eutopic endometrium of proliferative phase in patient with untreated

adenomyosis(n= 35); D. Eutopic endometrium of secretory phase in patient with untreated adenomyosis(n= 23); E. Normal endometrium of proliferative phase in patients without adenomyosis(n= 47); F. Normal endometrium of secretory phase in patients without adenomyosis(n= 27); G. ectopic endometrium in patient with mifepristone-treated adenomyosis(n= 11); H&I. eutopic endometrium in patient with mifepristone-treated adenomyosis(n= 11); J. Immunoscore comparation of B7-H3 between each groups, * P<0.05, ** P<0.01 A-I magnification: ×100;

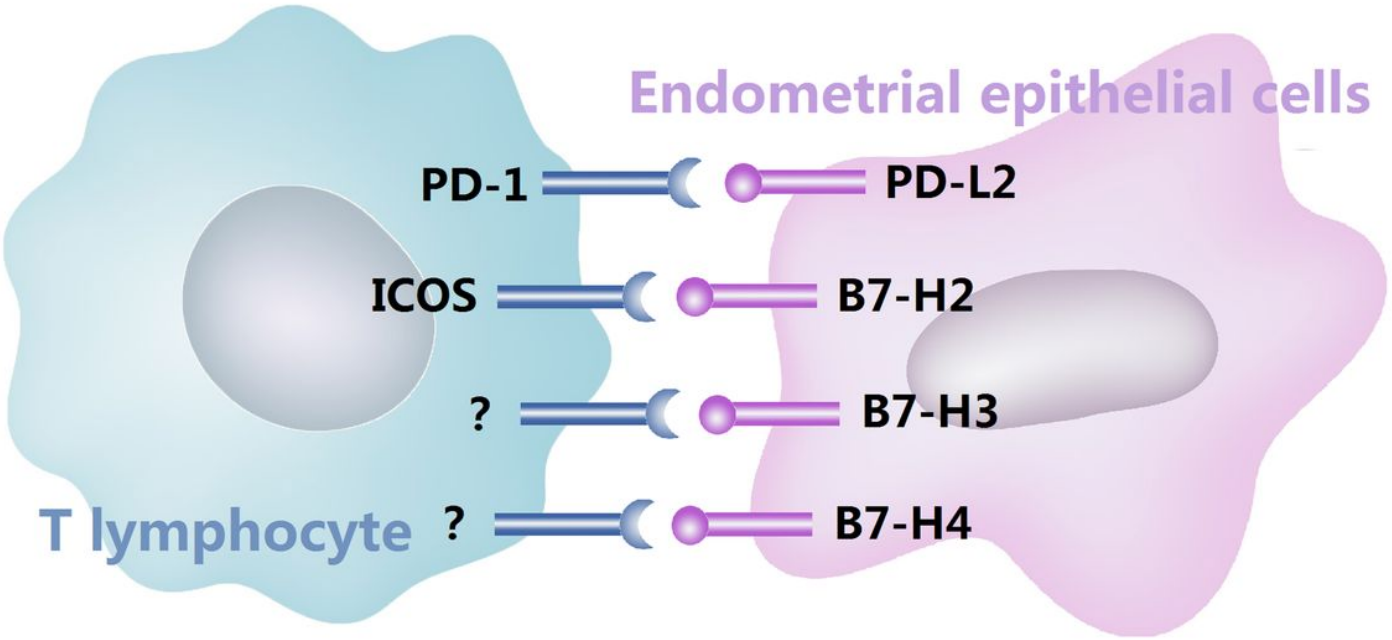

Figure 5

Schematic representation of B7-H2, B7-H3, B7-H4 and PD-L2 with their respective receptor