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Relationship between the change of ATP level controlled by ectonucleotidases and endometriosis-associated infertility and pain

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ABSTRACT

Background The dysfunction of the immune system is one of the pathogeneses of endometriosis. Immune cells can not only affect the microenvironment of the endometrium by secreting cytokines and defensins but also promote angiogenesis, growth and invasion of endometrial stromal cells.

Result ATP is a key mediator in the immune mechanism of endometriosis and plays a crucial role in endometriosis. While ATP acts as a purinergic signalling molecule, it has a close relationship with the pain of endometriosis via activating ATP receptors, including P2X3, P2X4, P2X7 and P2Y receptors, after being activated by the immune system. Besides, ATP levels reflect the impairment of mitochondrial function in granulosa cells, which could lead to infertility. The modulation of ATP expression levels is controlled by ectonucleotidases. The content of ectonucleotidases is altered in endometriosis which may be emerging non-invasive biomarkers.

Conclusion In the present review, we briefly introduce the relationship between the change of ATP level controlled by ectonucleotidases and endometriosis-associated infertility and pain, and illustrate our prospects for future research.

INTRODUCTION

Endometriosis, a common gynaecological disease among women of reproductive age, is characterised by the growth of endometrial-like tissue in ectopic locations.[1] It is frequently associated with chronic pelvic pain and infertility, which seriously undermine the quality of life of patients.[2 3] However, the aetiopathogenic mechanisms underlying endometriosis-related symptoms have not yet been fully understood, leading to a lack of effective biomarkers and treatment strategies.[4 5] This has created an urgent need for high-quality studies to define the underlying pathophysiological mechanisms that contribute to endometriosis (figure 1). An aberrant immune response in the peritoneal environment seems to be crucial for the proliferation of ectopic endometrial cells, and ATP may be a key mediator. Recent research has explored the relevance of ATP to the immune mechanisms of endometriosis.[1]

Endometriosis is an immune-related disease. After implantation into the peritoneal cavity, endometrial cells activate various immune cells to release various proinflammatory cytokines,[6–8] leading to the release of ATP.[9] ATP plays diverse roles in different conditions. Intracellular ATP, a critical factor in maintaining cellular homeostasis, exerts biological effects in energy-requiring processes, DNA/RNA biosynthesis and enzyme regulation.[10] However, extracellular ATP is mostly a proinflammatory molecule released under a variety of pathological situations, such as necrosis or apoptosis, hypoxia and inflammation.[11 12] It is also a kind of purinergic signalling factor[13] which is defined as the group modulating biological effects by extracellular nucleotides and nucleosides. The resulting cellular responses mediated by purinergic signalling depend on the consequence of the ratio of extracellular ATP and ADP, which is regulated by a network of ectonucleotidases.[14]

Here, we review the current immunological knowledge about ATP and ATP receptor-induced endometriosis-associated pain symptoms. Then, we demonstrate the mechanism of infertility in the case of endometriosis. The alteration of ectonucleotidases in endometriosis is also discussed briefly. Lastly, new therapeutic horizons of patients with endometriosis are discussed.

ATP IS INVOLVED IN THE OCCURRENCE OF ENDOMETRIOSIS-ASSOCIATED PAIN

The pain of endometriosis is considered as a kind of immune-related pain, for the proinflammatory cytokines, such as interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)-α, are increasingly released in ectopic lesions.[6–8] These changes are believed to

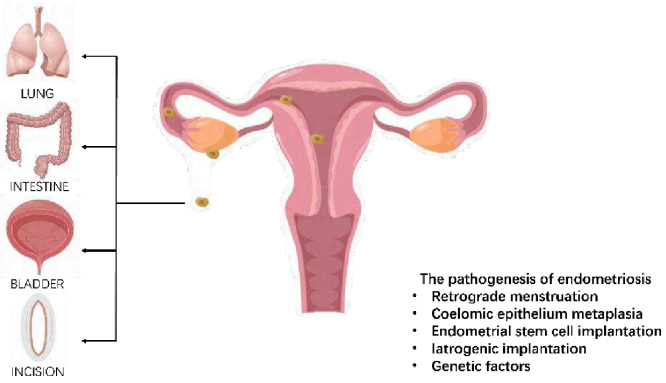

Figure 1 The pathogenesis of endometriosis. At present, there are several theories about the pathogenesis of endometriosis. Retrograde menstruation: during menstruation, endometrial fragments are regurgitated through the fallopian tube and implanted on the surface of the pelvic and abdominal organs. Coelomic epithelium metaplasia: ovarian germinal epithelium, pleural pelvic peritoneum, etc originated from coelomic epithelium. When they are subjected to inflammation, oestrogen and other repeated stimulation will be transformed into endometrioid tissue. Endometrial stem cell implantation: endometrial epithelial progenitor cells and mesenchymal stem cell-like cells are shed into the peritoneum via retrograde menstruation. Iatrogenic implantation: the surgical procedure brings the endometrial tissue directly to the incision. Genetic factors: endometriosis has a certain degree of familial clustering.

contribute to serious symptoms of pain such as chronic pelvic pain, dysmenorrhoea and dyspareunia. According to Sampson's theory, during menstruation, viable endometrial tissue is refluxed into the peritoneal cavity through the fallopian tubes where it subsequently implants into the peritoneal tissue and/or pelvic organs.[15] The implantation of ectopic endometrial cells will result in a strong immunological response in the body, recruiting various types of immune cells and provoking the secretion of proinflammatory cytokines. IL-1, a member of the inflammatory cytokine superfamily, is secreted by activated peritoneal macrophages.[16] It plays a role in regulating immunomodulation[17] in vivo mainly by secreting cytokines, B cells and antibodies as well as matrix metalloproteinases and prostaglandins to initiate the inflammatory response cascade.[18 19] Activated macrophages can also secrete IL-6 to increase haptoglobin at the lesion site. Haptoglobin may protect endometrial implants from clearance by the immune surveillance system by reducing the phagocytic activity of immune cells. The resulting positive feedback pathway favours the survival of ectopic endometrial tissue and further promotes its development.[20] It is worthy of noting that the increased IL-6 can inhibit natural killer (NK) cell cytotoxicity in peritoneal fluid of patients with endometriosis by downregulating the cytolytic granule component of NK cells,[21] which can also lead to dysfunctional clearance of ectopic endometrial stromal cells (ESCs).

These proinflammatory cytokines induce the release of ATP from intracellular to extracellular milieus in ectopic ESCs in endometriosis lesions via allowing Ca^{2+} influx.[9] Thus, the ATP content of ectopic endometrium was significantly higher than that in eutopic endometrium in patients with endometriosis. Increased extracellular ATP has been considered a key signalling transmitter of neuropathic pain, which could activate selective ATP receptors and play a critical role in inducing nociceptive sensitisation.[13] Patients suffered from more severe endometriosis pain with higher ATP content.[22]

ATP receptors could be divided into different families in terms of structure and function, including the P1 family (A1, A2A, A2B, A3), the P2X family (P2X1–7) and the P2Y family (P2Y1, P2Y2, P2Y4, P2Y6, P2Y11–14).[23]

P2X3 receptor (purinergic receptor P2X ligand-gated ion channel 3) is exclusively distributed in epithelial cells and non-peptidergic small-diameter sensory neurons, which could mediate neuropathic, nociceptive and inflammatory pain.[24] Research showed that inflammatory mediators such as IL-1β can lead to higher levels of mRNA and protein of the P2X3 receptor via activating mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase/cAMP response element-binding protein pathway, which could mediate nociceptive sensitisation via regulating protein phosphorylation.[9] Meanwhile, the P2X3 receptor plays a vital part in mediating reflex activities and pain in nociceptive C-fibres and Aδ-fibres (small and medium-sized neurons) of the dorsal root ganglia (DRG).[25 26] The expression levels of both ATP and P2X3 receptors released in endometriotic DRG tissue were significantly increased in comparison with the non-endometriotic rats and were positively correlated with the severity of hyperalgesia induced by endometriosis.[22] These findings show that elevated expression and activation of ATP and P2X3 receptors in endometriotic lesions and eutopic endometrium may lead to nerve sensitisation and signal transduction in endometriosis-associated pain.

Except for P2X3 receptors, other ATP receptors also play an important role in endometriosis-associated pain signal cascade triggering.[27] P2X4and P2X7 receptors are expressed in neural crosstalk between inflammatory cells and microglia.[28 29] Stimulation of P2X4 receptors in macrophages is related to the release of cyclo-oxygenase-dependent release of prostaglandin E2 (PGE2), which contributes to pain hypersensitivity,[30] thus playing a role in maintaining the immunological state of endometriosis after activating by ATP.

Purinergic receptors P2Y1 and P2Y2 are expressed in primary sensory and promote the pain-related symptoms of endometriosis through a variety of ways after activating transient receptor potential vanilloid-1 (TRPV1) channels.[31] The clinical research results of using TRPV1 inhibitors to relieve pain in patients with endometriosis are disappointing. Studies have reported that high temperature and hypothermia are the main side effects, weakening the potential use of TRPV1 inhibitors.[32] The activation of P2Y1 may result in the upregulation of P2X3 in endometriosis via the activating transcription factor 3 (ATF3)/activator protein (AP)-1 pathway[22] and

p38/MAPK pathway.[33] However, research by Chen *et al* stated that P2Y1 reduces P2X3 expression level with the p38/MAPK signalling pathway in DRG neurons.[34] More researches are needed to explain the exact effects of P2Y1. P2Y12 and P2Y13 are expressed in microglial in endometriosis, which is also involved in the release of proinflammatory mediators and the upregulation of the P2X3 receptor mediated by ADP.[35–37]

The development of painful symptoms of endometriosis is also related to the following factors.[38 39] Endometriosis is a kind of oestrogen-dependent disease with markedly elevated levels of oestrogen.[1] Increased oestradiol (E2) can regulate the release of bioactive granules from macrophages, thereby stimulating neural activity, and act as the key bridge between nerve tissue and macrophage in the peritoneal fluid of patients with endometriosis.[32 39] Increased numbers of macrophages and nerve fibres in the peritoneal fluid of patients with endometriosis promote the occurrence and development of the disease,[40] and their interaction can also promote the occurrence of painful symptoms of endometriosis. There is evidence that E2 increases the expression of nociceptive ion channels such as TRPV1 in lesions in a mouse model.[38 39] Mast cells are also associated with endometriosis-related pain.[41] Their numbers are significantly increased at the site of lesions in ovarian endometriosis.[42] At present, mast cell stabilisers have received widespread attention in the prospect of treatment of painful endometriosis.[43]

Based on published research results, E2, macrophages and mast cells, as well as ATP and ATP receptors, which are expressed at high levels in the ectopic endometrium, act together to cause hyperalgesia in endometriosis.[27] Abnormal innervations are observed in most endometriotic lesions: an increased number of total intact nerve fibres, increased sensory and decreased sympathetic nerve fibre density, the occurrence of cholinergic and unmyelinated nerve fibres, etc.[27 28] These changes are also closely related to pain.

ATP IS INVOLVED IN THE OCCURRENCE OF ENDOMETRIOSIS-ASSOCIATED INFERTILITY

The ideal embryo implantation requires a good interaction between the blastocyst and the receptive endometrium.[44] Although abnormal folliculogenesis, increased oxidative stress, changes in immune function and the hormonal milieu in follicular and peritoneal environments, and decreased endometrial receptivity are all involved in the pathogenesis of endometriosis-associated infertility, the exact mechanism of the pathogenesis remains controversial.[45]

In the follicular microenvironment, granulosa cells (GCs) are in the immediate vicinity of oocytes and exert important effects on the function of oocytes through direct gap junctions.[46] The connections between oocytes and GCs could control the competency of oocytes and determine the outcomes of female fertility or assisted reproductive therapy.[47] The expression levels of mitochondria in mature oocytes may play a critical role in oocyte and embryo development; thus, ATP levels may be important parameters for pregnancy potential.[48] Studies have confirmed the senescence and the severe endoplasmic reticulum stress of GCs in endometriosis by RNA-sequencing and bioinformatics tools. There was a statistically significant decrease in the expression of mitochondrial membrane potential ratio and ATP production in GCs of women suffering from endometriosis. These data showed that patients with endometriosis may have abnormal mitochondrial functions, such as restricted nutrient synthesis, membrane transport and accumulation of ATP, which may damage follicular function and fertility, thus affecting embryogenesis.[49]

The implantation after embryo formation is mainly regulated by steroid hormones, but in order to successfully implant, the best proinflammatory and anti-inflammatory state must be maintained at the fetal–maternal interface. It has been proved that many local immune cells, cytokines, growth factors and adhesion molecules are involved in the attachment, adhesion and invasion of blastocysts.[50 51] Once the function of these immune cells and the production of cytokines change, it may lead to abnormal implantation, even poor oocyte quality and abnormal fertilisation.[52 53] Studies have shown that the higher concentration of inflammatory mediators in the peritoneal fluid in endometriosis has toxic effects on oocyte pickup by the fimbria, sperm oocyte interaction and embryo implantation, leading to an aberrant reproductive function in these women. These effects were reversed during hormonal treatment.[54]

The cytotoxic activity of peripheral and peritoneal NK cells was decreased with the severity of the disease.[55] However, when endometriosis is accompanied by infertility or recurrent abortion, the cytotoxicity of uterine NK cells (CD16/NKp46 is its cytotoxic marker) increases, producing cytotoxic factors, leading to infertility and/or abortion or abnormal placenta, which can be used as a marker associated with infertility or recurrent abortion.[56]

Regulatory T (Treg) cells are recognised by the expression of transcription factor FoxP3,[57] which secretes IL-10 and transforming growth factor (TGF)-β. IL-10 inhibits the synthesis of inflammatory factors and releases inflammatory cells to activate migration and adhesion; TGF-β inhibits the proliferation of immune cells and lymphocyte activation. These immunosuppressive cytokines can suppress the cytotoxicity of other immune cells, which is essential to prevent destructive immunity in all tissues.[58 59] The number of Treg cells in the endometrium of patients with endometriosis infertility during peri-implantation period and their positive correlation with the disease stage indicate that they are related to embryo implantation[45] and can be used as a potential target for infertility treatment. Moreover, polyclonal B cell activation, B-1 cell proliferation and autoantibody production may be associated with infertility in these patients.[60]

Table 1 Major ectoenzymes involved in endometriosis

Enzyme or enzyme family	Gene	Catalytic reaction
NTPDase family/ CD39	E-NTPD	ATP→ADP→AMP NTP→NDP→NMP
NPP family	ENPP	ATP→ADP→AMP ATP→AMP+PPi
Ecto-5'-nucleotidase family/CD73	NT5E	AMP→adenosine
Alkaline phosphatases	ALP	ATP→ADP→AMP→adenosine NTP→NDP→NMP PPi→Pi
Adenosine deaminase	ADA	Adenosine→inosine

ADA, adenosine deaminase; ALP, alkaline phosphatase; ENPP, ectonucleotide pyrophosphatase/phosphodiesterase; E-NTPD, ectonucleoside triphosphate diphosphohydrolase; NDP, nucleotide diphosphate; NMP, nucleotide monophosphate; NPP, nucleotide pyrophosphatase/phosphodiesterase; NT5E, ecto-5'-nucleotidase; NTP, nucleotide triphosphate; NTPDase, nucleoside triphosphate diphosphohydrolase; Pi, phosphonic acid; PPi, pyrophosphonic acid.

ECTONUCLEOTIDASES ALTERED IN ENDOMETRIOSIS

Studies showed that ectonucleotidases, which belonged to specialised membrane enzymes, governed the levels of extracellular ATP and adenosine by hydrolysing nucleotides and nucleosides individually or sequentially.[61] Ectonucleotidases could be divided into four families: (1) exonucleotide triphosphate diphosphate hydrolase family, called CD39 family, mainly hydrolyses ATP and ADP to AMP; (2) exonucleotide pyrophosphatase/phosphodiesterase family (ENPP) that mainly converts ATP into AMP; (3) the ecto-5'-nucleotidase family (CD73) dephosphorylates AMP to adenosine[62–64]; and (4) alkaline phosphatase family (ALP), which hydrolyses nucleoside triphosphate and nucleoside diphosphate to AMP, releasing inorganic phosphate.[14] Adenosine deaminase (ADA) is a soluble enzyme that often combines with dipeptidyl peptidase IV/CD26 to inactivate adenosine and produce inosine. As shown in table 1, the main exoenzymes involved in endometriosis have been described.[14]

Several proinflammatory cytokines, oxidative stress and hypoxia regulate the expression of CD39 through transcription factors Sp1, Stat3[65 66] and zinc finger protein growth factor independent-1.[67] When the body is exposed to hypoxia,[68 69] as well as proinflammatory mediators, such as TGF-β, interferons, TNF-α, IL-1β and PGE2, increase,[70 71] the expression and function of CD73 are upregulated. After having triggered the inflammatory response, extracellular ATP in normal adult women is degraded into AMP by CD39 and then converted to adenosine by CD73.[72] The end products can downregulate the intensity and duration of leucocyte activation, inhibit the activity of immune cells and play an effective anti-inflammatory role. They can be considered as 'immunological switches' that transform ATP-driven proinflammatory immune cell activity into an adenosine-mediated anti-inflammatory state (figure 2).

However, studies have found an alteration in CD39–CD73 pathway in ectopic lesions. CD39 expression was lost in stromal cells of eutopic endometrium in women with endometriosis. CD73 expression was lost in ovarian and deeply infiltrative endometriosis lesions. These findings indicate that the changes in the ATP hydrolysis pathway correlate with the severity of endometriosis since they are lost mostly in the deeply infiltrating lesions and stage IV pelvic endometriosis. Changes in endometriosis ectonucleotidases promote the accumulation of ATP in the tissue microenvironment.[73] The insufficient activity of the CD39/CD73 axis is related to the expansion and uncontrolled activation of neutrophils,[74 75] the expansion of chemotaxis[76 77] and the increased adhesion of vascular endothelium.[74 78]

Meanwhile, we should focus that in women with deeply infiltrating lesions, loss of ectonucleoside triphosphate diphosphohydrolase 3 epithelial expressions also happened.[73] These changes in ectonucleotidases would result in the accumulation of extracellular ATP, which in turn induces the release of cytokines from the intracellular to the extracellular milieus, in favour of an increase of the survival and development rates of endometriotic cells.[14] These studies are in accordance with the previous findings that indicated the level of ATPase Na+/K+ transporting family member beta 4 decreases with the

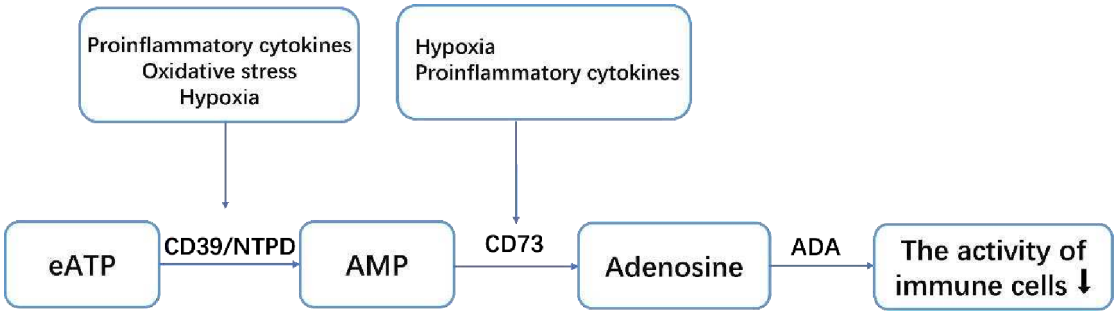

Figure 2 Mechanisms of immune cell activation in the microenvironment CD39–CD73 can be considered as 'immunological switches' that transform ATP-driven proinflammatory immune cell activity into adenosine-mediated anti-inflammatory state. ADA, adenosine deaminase; eATP, extracellular ATP; NTPD, nucleoside triphosphate diphosphohydrolase.

degree of ATP hydrolysis of endometriotic cells, and is differentially expressed between women with and without endometriosis.[79]

The increased expression of ENPP3 in stromal cells from patients with endometriosis is a special phenomenon.[73] ENPP3 may be a cellular tool to offset the decrease in ATPase resulting from the lack of CD39, which explains the high expression of ENPP3 in endometriotic stromal cells throughout the cycle. Thus, it might be an effective biomarker of the disease in the future. However, the activity of ENPP3 would not be able to replace the ATPase action, as CD39 has a higher affinity for ATP than ENPP3; moreover, the expression of ENPP3 in stromal cells is not in line with the expression CD73 throughout the cycle.[73]

Except for changes of ectonucleotidases in ectopic and eutopic endometrial tissue with endometriosis, there also exists changes of ectonucleotidases in the contents of ovarian endometriomas. Endometriosis can appear as different entities, including peritoneal endometriosis, ovarian endometriomas and deep infiltrating lesions.[80] Among them, endometriomas are also known as 'chocolate cysts' because the contents derived from the cysts are dark, internal fluid blood.[15] In endometriomas' contents' aspirates, the activation of enzymes is quantifiable, with significantly higher ectonucleotidase activity in endometriomas compared with simple cysts. Furthermore, the expression levels of ADA and ENPP1 are significantly higher than in simple cysts. Meanwhile, the levels of ALP and ENPP3 did not show huge differences between the two groups. Thus, the ectonucleotidases ADA and ENPP1 may well be candidates for endometriomas' histological markers.[81]

PROSPECTIVE

Currently, the drugs widely used to treat endometriosis have many defects in clinical application. Since most of the drugs used are contraceptives, women may have to choose between managing painful symptoms and trying to conceive. In addition, most women with endometriosis also purchase over-the-counter drugs to seek pain relief, such as paracetamol and non-steroidal anti-inflammatory drugs, which may inhibit ovulation.[82] The importance of the immune system in the pathogenesis of endometriosis supports the idea of using therapeutic strategies involving drugs that modulate specifically the function of immune cells.[83] Considering that ATP and P2X3 receptors play a critical role in the pain mechanism of endometriosis, there will be emerging therapy in endometriosis-associated pain.

Studies found that intramuscular injection ATP could reduce the size of endometrial lesions in rat models which seems in contrast to the conclusion that ATP is closely linked to the genesis and development of endometriosis.[84] Further researches are needed to solve this problem. The use of inhibitors of ectonucleotidases does not seem to represent an appropriate strategy. On the contrary, increasing the ATPase activity would combat the eventual ATP accumulation in the endometrial microenvironment. A-317491, a selective P2X3 receptor antagonist, could lead to a reduction in mechanical and heat hyperalgesia in a rat model of endometriosis by blocking the Ca^{2+} influx induced by extracellular ATP in endometriosis rat models.[85] Although P2X3 is a noteworthy target, its antagonists usually have poor pharmacokinetics. At present, a new compound (BAY1817080) is being used in clinical trials to solve this problem. Another treatment worth mentioning is SP600125, a c-JUN N-terminal kinase, that could inhibit the ATF3/AP-1 signalling pathway thereby reducing endometriotic lesions and attenuating the upregulation of P2X3 in rat models.[22] Other antagonists targeting P2X4, P2X7 or P2Y receptors are needed to achieve clinical trial.[27]

The MAPK pathway can increase the clinical repercussions of inflammation and endometriosis by recruiting immune cells and amplifying the inflammatory response,[86] generating an anti-apoptotic signal,[87] increasing growth factor expression leading to angiogenesis,[88] and playing a role in the development of pain and hypersensitivity to pain.[89] It was shown that their inhibitors can control the progression of disease in vitro and animal models.[90] However, the use of MAPK inhibitors in the treatment of endometriosis remains limited owing to their teratogenic nature and specific adverse side effects.[91]

For diagnosis, ectonucleotidases may be valuable biomarkers. The emergence of ENPP3 is closely related to stromal cells of endometriosis. The expression levels of CD39 and CD73 are decreased in deep infiltrating endometriosis.[73] The presence of ADA and ENPP1 levels is significantly increased in endometriomas.[92] Lastly, restoring the activity of ectonucleotidases might be another effective therapy to deal with endometriosis-associated pain.

CONCLUSION

Current evidence indicates that ATP (and/or ATP receptors) and the immune system are associated with the mechanism of pain and infertility of endometriosis. It could serve as cytokines and energetic molecules, which are significantly involved in the pathogenesis of endometriosis. Through in-depth research on endometriosis, some new approaches to diagnosis and treatment have been found. Further studies are needed not only to have a better understanding of this disease but also to improve current therapeutic strategies.

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REFERENCES

1 Vercellini P, Viganò P, Somigliana E, *et al*. Endometriosis: pathogenesis and treatment. *Nat Rev Endocrinol* 2014;10:261–75.
2 Vitale SG, La Rosa VL, Rapisarda AMC, *et al*. Impact of endometriosis on quality of life and psychological well-being. J Psychosom Obstet Gynecol 2017;38:317–9.
3 Laganà AS, La Rosa VL, Rapisarda AMC, *et al*. Anxiety and depression in patients with endometriosis: impact and management challenges. *Int J Womens Health* 2017;9:323–30.
4 Tian Z, Chang X-H, Zhao Y, *et al*. Current biomarkers for the detection of endometriosis. *Chin Med J (Engl)* 2020;133:2346–52.
5 May KE, Conduit-Hulbert SA, Villar J, *et al*. Peripheral biomarkers of endometriosis: a systematic review. *Hum Reprod Update* 2010;16:651–74.
6 Sikora J, Mielczarek-Palacz A, Kondera-Anasz Z. Imbalance in cytokines from Interleukin-1 family - role in pathogenesis of endometriosis. *Am J Reprod Immunol* 2012;68:138–45.
7 Zhang X, Yao H, Huang X, *et al*. Nerve fibres in ovarian endometriotic lesions in women with ovarian endometriosis. *Hum Reprod* 2010;25:392–7.
8 Kobayashi H, Yamada Y, Morioka S, *et al*. Mechanism of pain generation for endometriosis-associated pelvic pain. *Arch Gynecol Obstet* 2014;289:13–21.
9 Ding S, Zhu L, Tian Y, *et al*. P2X3 receptor involvement in endometriosis pain via ERK signaling pathway. *PLoS One* 2017;12:e0184647.
10 Bonora M, Patergnani S, Rimessi A, *et al*. ATP synthesis and storage. *Purinergic Signal* 2012;8:343–57.
11 Lazarowski ER. Vesicular and conductive mechanisms of nucleotide release. *Purinergic Signal* 2012;8:359–73.
12 Lazarowski ER, Sesma JI, Seminario-Vidal L, *et al*. Molecular mechanisms of purine and pyrimidine nucleotide release. *Adv Pharmacol* 2011;61:221–61.
13 Bodin P, Burnstock G. Purinergic signalling: ATP release. *Neurochem Res* 2001;26:959–69.
14 Yegutkin GG. Enzymes involved in metabolism of extracellular nucleotides and nucleosides: functional implications and measurement of activities. *Crit Rev Biochem Mol Biol* 2014;49:473–97.
15 Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. *Am J Pathol* 1927;3:93–110.
16 Wu MY, Ho HN. The role of cytokines in endometriosis. *Am J Reprod Immunol* 2003;49:285–96.
17 Herington JL, Bruner-Tran KL, Lucas JA, *et al*. Immune interactions in endometriosis. *Expert Rev Clin Immunol* 2011;7:611–26.
18 Hudelist G, Lass H, Keckstein J, *et al*. Interleukin 1Alpha and tissue-Lytic matrix metalloproteinase-1 are elevated in ectopic endometrium of patients with endometriosis. *Hum Reprod* 2005;20:1695–701.
19 Riccio L da G, Santulli P, Marcellin L, *et al*. Immunology of endometriosis. Best Pract Res Clin Obstet Gynaecol 2018;50:39–49.
20 Sharpe-Timms KL, Zimmer RL, Ricke EA, *et al*. Endometriotic haptoglobin binds to peritoneal macrophages and alters their function in women with endometriosis. *Fertil Steril* 2002;78:810–9.
21 Kang Y-J, Jeung IC, Park A, *et al*. An increased level of IL-6 suppresses NK cell activity in peritoneal fluid of patients with Endometriosis via regulation of Shp-2 expression. *Hum Reprod* 2014;29:2176–89.
22 Ding S, Yu Q, Wang J, *et al*. Activation of Atf3/AP-1 signaling pathway is required for P2X3-induced Endometriosis pain. *Hum Reprod* 2020;35:1130–44.
23 Burnstock G. Purine and Pyrimidine receptors. *Cell Mol Life Sci* 2007;64:1471–83.
24 Bernier L-P, Ase AR, Séguéla P. P2X receptor channels in chronic pain pathways. *Br J Pharmacol* 2018;175:2219–30.
25 Kobayashi K, Yamanaka H, Noguchi K. Expression of ATP receptors in the rat dorsal root ganglion and spinal cord. *Anat Sci Int* 2013;88:10–6.
26 Dunn PM, Zhong Y, Burnstock G. P2X receptors in peripheral neurons. *Prog Neurobiol* 2001;65:107–34.
27 Trapero C, Martín-Satué M. Purinergic signaling in endometriosis-associated pain. *Int J Mol Sci* 2020;21:8512.
28 Oliveira-Fusaro MC, Gregory NS, Kolker SJ, *et al*. P2X4 receptors on muscle macrophages are required for development of hyperalgesia in an animal model of activity-induced muscle pain. *Mol Neurobiol* 2020;57:1917–29.
29 Janks L, Sprague RS, Egan TM. ATP-Gated P2X47 receptors require chloride channels to promote inflammation in human Macrophages. *J Immunol* 2019;202:883–98.
30 Ulmann L, Hirbec H, Rassendren F. P2X4 receptors mediate Pge2 release by tissue-resident macrophages and initiate inflammatory pain. *EMBO J* 2010;29:2290–300.
31 Liu J, Liu X, Duan K, *et al*. The expression and functionality of transient receptor potential vanilloid 1 in ovarian endometriomas. *Reprod Sci* 2012;19:1110–24.
32 Symons LK, Miller JE, Kay VR, *et al*. The immunopathophysiology of endometriosis. *Trends Mol Med* 2018;24:748–62.
33 Kwon SG, Roh DH, Yoon SY, *et al*. Acid evoked thermal hyperalgesia involves peripheral P2Y1 receptor mediated Trpy1 phosphorylation in a rodent model of thrombus induced ischemic pain. *Mol Pain* 2014;10:2.
34 Chen Y, Li G, Huang L-YM. P38 Mapk mediates glial P2X7R-neuronal P2Y1R inhibitory control of P2X3R expression in dorsal root ganglion neurons. *Mol Pain* 2015;11:68.
35 Xu F, Yang J, Lu F, *et al*. Fast green Fcf alleviates pain hypersensitivity and down-regulates the levels of spinal P2X4 expression and pro-inflammatory cytokines in a rodent inflammatory pain model. *Front Pharmacol* 2018;9:534.
36 Yu T, Zhang X, Shi H, *et al*. P2Y12 regulates Microglia activation and excitatory synaptic transmission in spinal Lamina II neurons during neuropathic pain in rodents. *Cell Death Dis* 2019;10.
37 Liu PW, Yue MX, Zhou R, *et al*. P2Y12 and P2Y13 receptors involved in adpbetas induced the release of IL-1Beta, IL-6 and TNF-alpha from cultured dorsal horn microglia. *J Pain Res* 2017;10:1755–67.
38 Greaves E, Grieve K, Horne AW, *et al*. Elevated peritoneal expression and estrogen regulation of nociceptive ion channels in endometriosis. *J Clin Endocrinol Metab* 2014;99:E1738–43.
39 Greaves E, Temp J, Esnal-Zufiurre A, *et al*. Estradiol is a critical mediator of macrophage-nerve cross talk in peritoneal endometriosis. *Am J Pathol* 2015;185:2286–97.
40 Tran LVP, Tokushige N, Berbic M, *et al*. Macrophages and nerve fibres in peritoneal endometriosis. *Hum Reprod* 2009;24:835–41.
41 Kirchhoff D, Kaulfuss S, Fuhrmann U, *et al*. Mast cells in endometriosis: guilty or innocent bystanders *Expert Opin Ther Targets* 2012;16:237–41.
42 De Leo B, Esnal-Zufiaurre A, Collins F, *et al*. Immunoprofiling of human uterine mast cells identifies three phenotypes and expression of Erbeta and glucocorticoid receptor. *F1000Res* 2017;6:667.
43 Zhu TH, Zou G, Ding SJ, *et al*. Mast cell stabilizer Ketotifen reduces hyperalgesia in a rodent model of surgically induced endometriosis. *J Pain Res* 2019;12:1359–69.
44 Carson DD, Bagchi I, Dey SK, *et al*. Embryo implantation. *Dev Biol* 2000;223:217–37.
45 Chen S, Zhang J, Huang C, *et al*. Expression of the T regulatory cell transcription factor Foxp3 in peri-implantation phase endometrium in infertile women with endometriosis. *Reprod Biol Endocrinol* 2012;10:Artn .
46 Albertini DF, Combelles CM, Benecchi E, *et al*. Cellular basis for paracrine regulation of ovarian Follicle development. *Reproduction* 2001;121:647–53.
47 Dumesic DA, Meldrum DR, Katz-Jaffe MG, *et al*. Oocyte environment: follicular fluid and cumulus cells are critical for oocyte health. *Fertil Steril* 2015;103:303–16.
48 Lin X, Dai Y, Tong X, *et al*. Excessive oxidative stress in cumulus granulosa cells induced cell senescence contributes to endometriosis-associated infertility. *Redox Biol* 2020;30:101431.

49 Boucret L, Bouet P-E, Riou J, *et al*. Endometriosis LOWERS the cumulative live birth rates in Ivf by decreasing the number of embryos but not their quality. *J Clin Med* 2020;9:2478.
50 Norwitz ER, Schust DJ, Fisher SJ. Implantation and the survival of early pregnancy. *N Engl J Med* 2001;345:1400–8.
51 van Mourik MSM, Macklon NS, Heijnen CJ. Embryonic implantation: cytokines, adhesion molecules, and immune cells in establishing an implantation environment. *J Leukoc Biol* 2009;85:4–19.
52 Ulukus M, Cakmak H, Arici A. The role of endometrium in endometriosis. *J Soc Gynecol Investig* 2006;13:467–76.
53 Simón C, Gutiérrez A, Vidal A, *et al*. Outcome of patients with endometriosis in assisted reproduction: results from in-vitro fertilization and oocyte donation. *Hum Reprod* 1994;9:725–9.
54 Paul Dmowski W, Braun DP. Immunology of endometriosis. *Best Pract Res Clin Obstet Gynaecol* 2004;18:245–63.
55 Wilson TJ, Hertzog PJ, Angus D, *et al*. Decreased natural killer cell activity in endometriosis patients: relationship to disease pathogenesis. *Fertil Steril* 1994;62:1086–8.
56 Giuliani E, Parkin KL, Lessey BA, *et al*. Characterization of uterine NK cells in women with infertility or recurrent pregnancy loss and associated endometriosis. *Am J Reprod Immunol* 2014;72:262–9.
57 Takamura M, Koga K, Izumi G, *et al*. Simultaneous detection and evaluation of four subsets of Cd4+ T lymphocyte in lesions and peripheral blood in endometriosis. *Am J Reprod Immunol* 2015;74:480–6.
58 Corthay A. How do regulatory T cells work. *Scand J Immunol* 2009;70:326–36.
59 Sun J, Madan R, Karp CL, *et al*. Effector T cells control lung inflammation during acute influenza virus infection by producing Il-10. *Nat Med* 2009;15:277–84.
60 Chishima F, Hayakawa S, Hirata Y, *et al*. Peritoneal and peripheral B-1-cell populations in patients with endometriosis. *J Obstet Gynaecol Res* 2000;26:141–9.
61 Yegutkin GG. Nucleotide- and nucleoside-converting ectoenzymes: important modulators of purinergic signalling cascade. *Biochim Biophys Acta* 2008;1783:673–94.
62 Martín-Satué M, Lavoie EG, Fausther M, *et al*. High expression and activity of Ecto-5'-Nucleotidase/Cd73 in the male murine reproductive tract. *Histochem Cell Biol* 2010;133:659–68.
63 Fausther M, Lecka J, Soliman E, *et al*. Coexpression of Ecto-5'-nucleotidase/Cd73 with specific Ntpdases differentially regulates adenosine formation in the rat liver. *Am J Physiol Gastrointest Liver Physiol* 2012;302:G447–59.
64 Antonioli L, Pacher P, Vizi ES, *et al*. Cd39 and Cd73 in immunity and inflammation. *Trends Mol Med* 2013;19:355–67.
65 Deaglio S, Robson SC. Ectonucleotidases as regulators of purinergic signaling in thrombosis, inflammation, and immunity. *Adv Pharmacol* 2011;61:301–32.
66 Eltzschig HK, Köhler D, Eckle T, *et al*. Central role of Sp1-regulated Cd39 in hypoxia/ischemia protection. *Blood* 2009;113:224–32.
67 Chalmin F, Mignot G, Bruchard M, *et al*. Stat3 and Gfi-1 transcription factors control Th17 cell immunosuppressive activity via the regulation of ectonucleotidase expression. *Immunity* 2012;36:362–73.
68 Synnestvedt K, Furuta GT, Comerford KM, *et al*. Ecto-5'-nucleotidase (Cd73) regulation by hypoxia-inducible factor-1 mediates permeability changes in intestinal Epithelia. *J Clin Invest* 2002;110:993–1002.
69 Eltzschig HK, Ibla JC, Furuta GT, *et al*. Coordinated adenine nucleotide phosphohydrolysis and nucleoside signaling in posthypoxic endothelium: role of ectonucleotidases and adenosine A2B receptors. *J Exp Med* 2003;198:783–96.
70 Beavis PA, Stagg J, Darcy PK, *et al*. Cd73: a potent suppressor of antitumor immune responses. *Trends Immunol* 2012;33:231–7.
71 Regateiro FS, Howie D, Nolan KF, *et al*. Generation of anti-inflammatory adenosine by leukocytes is regulated by TGF-beta. *Eur J Immunol* 2011;41:2955–65.
72 Langer D, Hammer K, Koszalka P, *et al*. Distribution of ectonucleotidases in the rodent brain revisited. *Cell Tissue Res* 2008;334:199–217.
73 Trapero C, Vidal A, Fernández-Montolí ME, *et al*. Impaired expression of ectonucleotidases in ectopic and eutopic endometrial tissue is in favor of ATP accumulation in the tissue microenvironment in endometriosis. *Int J Mol Sci* 2019;20:5532.
74 Eltzschig HK, Thompson LF, Karhausen J, *et al*. Endogenous adenosine produced during hypoxia attenuates neutrophil accumulation: coordination by extracellular nucleotide metabolism. *Blood* 2004;104:3986–92.
75 Kukulski F, Bahrami F, Ben Yebdri F, *et al*. Ntpdase1 controls IL-8 production by human neutrophils. *J Immunol* 2011;187:644–53.
76 Corriden R, Chen Y, Inoue Y, *et al*. Ecto-nucleoside triphosphate diphosphohydrolase 1 (E-Ntpdase1/Cd39) regulates neutrophil chemotaxis by hydrolyzing released ATP to adenosine. *J Biol Chem* 2008;283:28480–6.
77 Reutershan J, Vollmer I, Stark S, *et al*. Adenosine and inflammation: Cd39 and Cd73 are critical mediators in Lps-induced Pmn trafficking into the lungs. *FASEB J* 2009;23:473–82.
78 Eltzschig HK, Weissmüller T, Mager A, *et al*. Nucleotide metabolism and cell-cell interactions. *Methods Mol Biol* 2006;341:73–87.
79 Zhao Y, Liu YN, Li Y, *et al*. Identification of biomarkers for endometriosis using clinical proteomics. *Chin Med J (Engl)* 2015;128:520–7.
80 Johnson NP, Hummelshoj L, Adamson GD, *et al*. World endometriosis society consensus on the classification of endometriosis. *Hum Reprod* 2017;32:315–24.
81 Trapero C, Jover L, Fernández-Montolí ME, *et al*. Analysis of the ectoenzymes ADA, ALP, Enpp1, and Enpp3, in the contents of ovarian endometriomas as candidate biomarkers of endometriosis. *Am J Reprod Immunol* 2018;79.
82 Norman RJ, Wu R. The potential danger of COX-2 inhibitors. *Fertil Steril* 2004;81:493–4.
83 Osuga Y, Koga K, Hirota Y, *et al*. Lymphocytes in endometriosis. *Am J Reprod Immunol* 2011;65:1–10.
84 Zhang C, Gao L, Yi Y, *et al*. Adenosine triphosphate regresses endometrial explants in a rat model of endometriosis. *Reprod Sci* 2016;23:924–30.
85 Hansen RR, Nasser A, Falk S, *et al*. Chronic administration of the selective P2X3, P2X2/3 receptor antagonist, A-317491, transiently attenuates cancer-induced bone pain in mice. *Eur J Pharmacol* 2012;688:27–34.
86 Kaminska B. Mapk signalling pathways as molecular targets for anti-inflammatory therapy--from molecular mechanisms to therapeutic benefits. Biochim Biophys Acta2005;1754:253–62.
87 Harada H, Quearry B, Ruiz-Vela A, *et al*. Survival factor-induced extracellular signal-regulated kinase phosphorylates bim, inhibiting its association with BAX and proapoptotic activity. *Proc Natl Acad Sci U S A* 2004;101:15313–7.
88 Hamden KE, Whitman AG, Ford PW, *et al*. RAF and VEGF: emerging therapeutic targets in Kaposi's sarcoma-associated herpesvirus infection and angiogenesis in hematopoietic and nonhematopoietic tumors. *Leukemia* 2005;19:18–26.
89 Ji RR, Suter MR. P38 MAPK, microglial signaling, and neuropathic pain. *Mol Pain* 2007;3:33.
90 Leconte M, Santulli P, Chouzenoux S, *et al*. Inhibition of MAPK and VEGFR by sorafenib controls the progression of endometriosis. *Reprod Sci* 2015;22:1171–80.
91 Santulli P, Marcellin L, Tosti C, *et al*. Map kinases and the inflammatory signaling Cascade as targets for the treatment of Endometriosis *Expert Opin Ther Targets* 2015;19:1465–83.
92 Texidó L, Romero C, Vidal A, *et al*. Ecto-nucleotidases activities in the contents of ovarian endometriomas: potential biomarkers of endometriosis. *Mediators Inflamm* 2014;2014:120673.