HYPOXIA AND REPRODUCTIVE HEALTH

The role of hypoxia in the development and progression of endometriosis

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Abstract

Endometriosis is a benign gynecological disease that affects about 10% of women of reproductive age. Patients with endometriosis suffer from long-term coexistence with dysmenorrhea, dyspareunia, and even infertility, which severely reduces quality of life. So far, surgical removal and hormonal medication are the major treatment options; however, high recurrence and severe adverse effects hamper the therapeutic efficacy. Hypoxia is an inevitable cellular stress in many diseases that regulates the expression of a significant subset of genes involved in pathophysiological processes. A growing body of evidence demonstrates that hypoxia plays critical role in controlling the disease phenotypes of endometriosis, such as increasing adhesion ability, causing dysregulation of estrogen biosynthesis, aberrant production of proinflammatory cytokines, increasing angiogenic ability, and suppression of immune functions. In this review, we summarize the findings of the most recent studies in exploring the underlying mechanisms of hypoxia involved in endometriosis. Potential therapeutic options for targeting HIF and downstream effectors will also be discussed.

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Introduction

Endometriosis is a common gynecological disease characterized by endometriotic lesions growing outside of the uterine cavity. Endometriotic lesions are commonly seen on the wall of the peritoneum and the surface of the ovaries and cause clinical symptoms, such as pelvic pain during menstruation, dyspareunia, and infertility. The overall prevalence of endometriosis is around 6–10% in women of reproductive age; however, it is generally believed the number is underestimated due to diagnostic limitations of laparoscopy and a proportion of asymptomatic women. Although endometriosis is considered to be a nonlethal disease, clinical symptoms, together with the heavy financial burden, amounting to an average of thousands of dollars per person per year (Soliman et al. 2016), severely reduces patients' life quality. Surgical removal is the gold-standard treatment for patients with endometriosis; however, a report has shown that the recurrence rate after surgical removal is unsatisfactorily high (Cea Soriano et al. 2017). Hormonal-based medication is an alternative for treating endometriosis, but the main purpose of this is to ease endometriosis-associated pain instead of curing it.

These clinical facts suggest that treatments available so far are not able to thoroughly cure the disease and there are unmet medical needs to develop more efficacious treatment regimens.

The etiology of endometriosis is enigmatic. It is reported that the development of endometriosis is contributed by multiple gene–gene and gene–microenvironmental factors. According to Sampson’s theory, the presence of endometriotic tissues in the peritoneal cavity is associated with retrograde menstrual dissemination (Sampson 1927). Sampson hypothesized that endometriotic lesions are originated from the shed endometrial tissues of the uterus, which, along with the retrograde flow, move to the ectopic sites. However, retrograde menstruation happens in over 90% of women during their cycles (Halme et al. 1984) but only 10% of them develop endometriosis, suggesting there must be other critical factors that determine the disease progression.

Hypoxia is the first stress that these retrograde tissues encounter. Without enough oxygen and nutrients supplied, most of the cells die. However, some cells in the endometriotic lesions undergo epigenetic changes through unclarified mechanisms, and not only show...
good vitality under hypoxic stress, but also exhibit a capability for further growth (Wu et al. 2019). Despite originating from the endometrium, it is considered that those migrated endometrial cells undergo large phenotypic changes that benefit their own survival and adapt themselves to the microenvironment. Many studies have indicated that the unexpected alteration is due to dysregulation of hormones and impairment of the immune system, yet there is still no comprehensive explanation for the etiology, since endometriosis is not a disease of mutation.

Hypoxia-inducible factors (HIFs), which are heterodimeric complexes constituting α and β subunits, work as transcriptional factors to regulate the expression of their target genes under hypoxia. The β subunit (HIF-1β), also known as aryl hydrocarbon nuclear translocator, constitutively expresses under both normoxic and hypoxic conditions, while α subunits (HIF-1α and HIF-2α) only exist under hypoxia. Under ambient oxygen, HIF-α undergoes 26S proteasome degradation due to hydroxylation modification on its oxygen-dependent degradation domain, while under the low-oxygen condition, accumulated HIF-α dimerizes with HIF-1β and further regulates the genes with hypoxia response element (Semenza 2007). CREB binding protein and p300 are co-activators that bind to the transactivation domain of HIF-1α, and they are also found to be essential for HIF transcriptional activity during hypoxia. It has been reported that endometriotic stromal cells showed elevated HIF-1α expression (Wu et al. 2007), and HIF-1α directly regulates a number of genes at the transcriptional level (Wu et al. 2011, Lin et al. 2012, 2017), indicating there is a high possibility that the involvement of hypoxia in endometriosis is through HIF-1α regulation.

Through mediating gene expression, hypoxia regulates a broad range of biological processes such as cell proliferation, angiogenesis, apoptosis, immunology, and tumor metastasis as well as drug resistance. Herein, we summarize papers published to date to discuss the regulatory role of hypoxia in the development and progression of endometriosis.

**Hypoxia as the force of endometriosis**

The involvement of hypoxia during the development of endometriosis was not studied until 2007, when Wu et al., first reported the upregulation of HIF-1α mRNA and protein in endometriotic lesions and its transcriptional activity in inducing leptin expression (Wu et al. 2007). It is conceivable that the retrograde tissues originating from the endometrium may encounter many challenges in the peritoneal cavity, including lack of oxygen, immune attack, mechanical damage, or other environmental stress. Despite so many difficulties, it seems that endometriotic tissues are able to overcome these hurdles in the microenvironment by utilizing hypoxia as the driving force. In the following sections, we will briefly classify the processes during the development of endometriosis into three sections, namely early-stage lesion implantation and survival, the immune clearance system, and disease progression, in order to discuss the role of hypoxia (Fig. 1). Moreover, we will also focus on the effect of hypoxia-mediated epigenetic modification in the pathogenesis of endometriosis in the last part.

**Role of hypoxia during early-stage implantation of endometriosis**

During the early period of endometriosis development, acquiring abilities of migration, adhesion, and invasion into the peritoneum or other sites is a prerequisite for the retrograded cells to implant and survive. In addition, as energy utilization is also extremely important at this difficult time, so as a result, the regulation of apoptosis, autophagy and metabolic changes are all crucial for maintaining cell viability. A number of studies have emphasized the importance of hypoxia involvement in mediating the pathophysiological processes during early endometriosis development. Herein, we summarize the previous findings to discuss the regulatory mechanisms of hypoxia involved in cell adhesion, migration/invasion, apoptosis, autophagy, and metabolism in endometriosis.

**Cellular adhesion**

Cells with higher adhesive ability have advantages in early-stage development of endometriosis (Choi et al. 2017). Recent studies showed that hypoxia induces several key genes to enhance the adhesive ability of endometriotic cells (Lin et al. 2018, 2019). According to Sampson’s retrograde menstruation theory, endometriotic lesions in the peritoneal cavity are brought by the retrograde flow (Sampson 1927). Theoretically, the floating tissues, without nutrient supply and physical support from the extracellular matrix, are not able to survive under these conditions. However, studies have revealed that endometriotic tissues show increased adhesive ability (Lin et al. 2019), which has been suggested as one of the critical factors to sustain survival in the early stage. In fact, severe adhesion in the peritoneal cavity is a condition that is commonly seen in clinical patients, and is one of the leading causes of pelvic pain and infertility (Stout et al. 1991, Cheong et al. 2001), and this provides a hint that cellular adhesion is involved not only in the early stage, but throughout the entire course of endometriosis. Certain kinds of adhesion molecules are found to be highly expressed in endometriotic lesions, such as integrins (Lessey et al. 1994), CD44 (Griffith et al. 2010), cell adhesion molecules (Vigano et al. 1998, Kuessel et al. 2017), and other adhesion-related molecules such as proprotein/matrix metallopeptidases (MMPs) (Pino et al. 2009). Chronic inflammation and

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Hypoxia are regarded as two major factors responsible for better cell adhesive ability in endometriotic stromal cells. For instance, transforming growth factor-β1 (TGF-β1), which mainly participates in tissue remodeling, wound healing, and fibrotic responses, is found to be elevated in peritoneal fluid from patients with endometriosis (Kupker et al. 1998). TGF-β1 is reported to induce peritoneal adhesion through increasing αV, α6, β1, and β4 integrins in an endometrial cell line (Choi et al. 2017) and in a mouse model of endometriosis (Soni et al. 2019). More interestingly, Lin et al. reported TGF-β1-stimulated integrin expression is a result of the hypoxic effect, finding that HIF-1α stabilization could enhance the level of SMAD2/SMAD3 phosphorylation in primary endometrial stromal cells (Lin et al. 2018). Recently, membrane-expressed anthrax toxin receptor 2 (ANTXR2) was shown to be a novel adhesion-related molecule (Bell et al. 2001, Lin et al. 2019). ANTXR2 is highly expressed in endometriotic stromal cells and is mediated by hypoxia. Hypoxia decreases enhancer of zeste homolog 2 (EZH2), a histone H3 lysine 27 (H3K27) methylating enzyme, leading to the reduction of trimethylated H3K27 in ANTXR2 promoter and resulting in transcriptional upregulation of ANTXR2 in endometriotic stromal cells. Furthermore, treatment with 1, 2, 3, 4, 6-penta-O-galloyl-β-D-glucopyranose, an ANTXR2 inhibitor, abolishes hypoxia-induced increased cell migration and adhesion. An animal model also demonstrated that blocking ANTXR2 signaling not only prevents endometriotic lesion development but also causes the established lesion to be regressed (Lin et al. 2019). These findings suggest that targeting ANTXR2 can be used in both preventive and therapeutic regimens for endometriosis.

Migration/invasion

It was hypothesized that the destruction of the mesothelium layer is a consequence of invasion and migration of endometriotic cells; however, the underlying mechanism remained to be elucidated. Later, it was indicated that the damaged mesothelial basement membrane at the peritoneum surface is the site of preference for the attachment of endometriol...
fragments (Burney & Giudice 2012). Despite the fact that endometriosis is a benign gynecological disease, many studies have indicated that endometriotic cells shared the characteristics of malignant tumors. Some reports have indicated that the malignancy-like abilities of these refluxed endometrial stromal cells lead to tissue remodeling in the mesothelial layer (Ishimaru et al. 2004, Sotnikova et al. 2010), facilitating the implantation of endometriotic lesions. Taking enzyme proteins as an example, the ratio of inactivated and activated MMPs, as well as the imbalance between the levels of MMPs and tissue inhibitor of metalloproteinase, have been found to be important for the regulation of cellular migration and invasion in endometriosis (Bruner-Tran et al. 2002). It has been reported that both MMP2 and MMP9 are elevated in endometriotic stromal cells, and they can be regulated by a variety of factors, including tumor necrosis factor-α (TNF-α) (Pino et al. 2009) and prostaglandin (PG) E2 (Jana et al. 2016). Hypoxia is regarded as the upstream regulator that strengthens endometriotic cells to be more invasive. Wu et al. showed hypoxia could induce the levels of leptin (Wu et al. 2007) and PGE2 (Wu et al. 2011), two modulators shown to enhance cell invasion through mediating the expression of MMP2 in stromal cells. Additionally, hypoxia could also promote cell migration through causing the loss of CD26/dipeptidyl peptidase IV, which is also a peptidase complex involved in enzymatic reactions in cellular remodeling (Tan et al. 2014). As mentioned previously, Lin et al., reported that hypoxia-induced ANTXR2 upregulation in endometriotic stromal cells also contributes to cell migration (Lin et al. 2019).

**Cell survival anti-apoptosis**

Apoptosis is a type of programmed cell death commonly seen in many physiological conditions, which is important for maintaining cellular homeostasis during embryonic development (Fuchs & Steller 2011), organ formation (Lindsten et al. 2000), tissue remodeling (Gosden & Spears 1997), and so on. Reduced apoptosis is observed in endometriotic stromal cells (Delbandi et al. 2020) and hypoxia was reported to play some roles in this biological process, which may explain why endometriotic lesions in patients showed better survival. B-cell lymphoma 2 (Bcl-2), which serves as a blocker for apoptotic deaths, was the most studied apoptosis-related protein in early research (McLaren et al. 1997, Watanabe et al. 1997). It has been reported that Bcl-2 is cyclically expressed in human endometrium (Watanabe et al. 1997); however, the level of Bcl-2-positive cells in endometriotic stroma showed significantly higher levels than that in paired endometrial tissue, and also had no connection with menstrual phases (Harada et al. 1996, McLaren et al. 1997, Jones et al. 1998). Note that the ‘Bcl-2-positive cells’ described here include tissue macrophages (McLaren et al. 1997) and non-leukocytic stromal cells (Jones et al. 1998) in ectopic lesions, and both of these two cell types are enrolled in the anti-apoptotic function in endometriosis.

The involvement of the Fas/Fas ligands (FasL) system in endometriosis is still under debate. Harada et al. and Watanabe et al. considered that Fas, the receptor, may be less important in regulating the development of human endometriosis due to there being no expression change between eutopic and ectopic tissues (Harada et al. 1996, Watanabe et al. 1997). On the other hand, increased soluble FasL was found in both serum and peritoneal fluids from patients with endometriosis, which suggested that differentially expressed ligand is important in endometriosis. To investigate the key drivers in dysregulating apoptosis in endometriosis, most reports focus on the enriched protein factors that have been found in the peritoneal fluid of women with endometriosis. IL-8, chemokine ligand 2 (CCL2), and extracellular matrix molecules such as laminin, fibronectin, and collagen IV, are the inducers of FasL in endometriosis (Selam et al. 2006), stimulating the apoptosis reaction in cytotoxic T cells in the peritoneal cavity, and thus further enhancing the implantation of endometriotic lesions. Additionally, estrogen receptor (ER)-β is reported to inhibit caspase-8 and caspase-9 activation to prevent the extrinsic and intrinsic apoptosis signaling in a mouse model of endometriosis (Han et al. 2015). In fact, hypoxia is a potent modulator of IL-8 (Hsiao et al. 2014) and ER-β (Wu et al. 2012), which suggests that hypoxia may also be important in mediating the apoptosis reaction in endometriotic lesions. Moreover, it has been demonstrated that cyclooxygenase (COX)-2-derived PGE2 could activate the EP2/EP4 receptor to prevent cells from undergoing apoptosis in human endometriotic stromal cells (Buru et al. 2008). As COX-2 is upregulated by hypoxia (Wu et al. 2011), it is possible that endometriotic cells are more apoptosis-resistant due to a hypoxia-mediated gene expression. Other lines of evidence also showed that hypoxia mediates the expression of IL-6 via dual-specificity phosphatase 2 (DUSP2) suppression, which further enhances STAT3 activation to act against cell apoptosis in endometriotic stromal cells (Hsiao et al. 2017a).

**Cell survival autophagy**

The role of autophagy machinery in cell physiology is multifaceted. Autophagy is a constitutive process for energy conversion and will be induced particularly when cells encounter oxidative stress or other hostile conditions such as hypoxia (Yu et al. 2018). Mediated by a number of proteins that are involved in composing phagophores and lysosome fusion, phagosome and phagolysosome subsequently form for the purpose of obtaining more energy for survival by degrading some endogenous long-lived proteins. Moderate autophagy is beneficial
for cell survival, yet both excessive and insufficient autophagic activity can be harmful for maintaining cellular homeostasis. Phosphatidylinositol 3-kinase/Akt and mammalian target of rapamycin (mTOR) signaling are the dominant pathways in regulating autophagy which is mostly involved in activating autophagy-related proteins and further enhancing microtubule-associated protein light chain 3 (LC3) lipidation. Most studies have suggested that the activity of autophagy, with no cyclical difference, is repressed in both ectopic stromal and epithelial cells in endometriosis by the hormonal effect (Choi et al. 2014, Mei et al. 2015). In contrast, other studies showed upregulation of autophagy in endometriosis, in which they indicated this defense mechanism is induced in order to prevent endometriotic cells from dying (Liu et al. 2017). A series of studies done by Liu’s group not only demonstrated that the autophagic response in endometriosis is stimulated by hypoxia, but also found that HIF-1α is a critical factor that promotes LC3 lipidation in human endometriotic stromal cells (Liu et al. 2017, 2018). Moreover, hypoxia-induced autophagy is also a driver for epithelial-to-mesenchymal transition (EMT) that enhances cell migration and invasion abilities during the development of endometriosis (Liu et al. 2017, 2018).

**Cell survival metabolic changes**

The metabolic pattern of endometriotic cells is largely different from that in eutopic cells. It has been reported that endometriotic cells display a Warburg-effect-like phenotype, which showed a tendency of undergoing aerobic glycolysis. Indicators of glycolysis such as pyruvate dehydrogenase kinase 1 (PDK1) and lactate dehydrogenase A (LDHA) are found to be more highly expressed in endometriotic lesions than in the normal endometrium (Young et al. 2016, Lee et al. 2019). Moreover, lactate production is also increased in endometriotic stromal cells (Lee et al. 2019), which shows a high similarity with tumor cells. HIF-1α, the well-known modulator of cellular metabolism, was demonstrated to regulate the expression of genes in endometriotic stromal cells that are associated with glycolysis (Lee et al. 2019), suggesting hypoxia plays a critical role in transforming cellular characteristics in glucose metabolism.

**Hypoxia impairs immuno-clearance system in endometriosis**

Endometriosis is a chronic pelvic inflammatory disease that is characterized by high levels of proinflammatory cytokines in the peritoneal cavity. A variety of immune cells are found to be recruited to the endometriotic lesions, and the presence of these cells has been demonstrated to be beneficial for the growth of endometriotic tissues (Wu et al. 2002b, Li et al. 2014). More precisely, the functions of peritoneal leukocytes which are supposed to fight against invaders, the retrograded endometriotic tissues, are weakened due to some unclarified mechanisms which may be the cause and the effect of chronic inflammation in the peritoneal cavity (Raiter-Tenenbaum et al. 1998).

It has already been demonstrated that peritoneal macrophages are highly infiltrated in endometriotic implants (Wu et al. 2002b, Lin et al. 2006). Macrophages are regarded as the first-line defender during the primary immune response (Gordon 1998), showing the phagocytic ability to fight against pathogens and to eliminate cell debris for the maintenance of tissue integrity. Macrophages can be roughly classified into M1 and M2 phenotypes, which show a large difference in their immune functions, including the ability of phagocytosis and the types of cytokines released. Polarized macrophages exert distinct functions during inflammation and the development of disease pathogenesis (Mosser & Edwards 2008). For instance, the M1 macrophage is the classical phenotype of a macrophage which is characterized by the aggressive eliminating ability for the enhancement of proinflammation; meanwhile, the M2 macrophage is found to be incapable of getting rid of pathogens, and is usually considered as a brake during strong inflammation. The balance between M1 and M2 macrophages is important in order to maintain the normal immune function in the human body. However, without a clear mechanism, the phagocytes around ectopic tissues exhibit less aggressive characteristics as well as impaired function of recognition and engulfment (Raiter-Tenenbaum et al. 1998), a phenotype similar to M2 macrophages. It has been reported that the impairment of peritoneal macrophages is one of the main factors responsible for the presence of endometriotic lesions outside of the uterine cavity (Loh et al. 1999).

Hormonal dysregulation is one of the major causes that worsen the immune surveillance system in patients with endometriosis. Estrogen stimulates endometrial stromal cells to produce monocyte chemotactic protein-1, which enhances macrophage infiltration and activation (Akoum et al. 2000). A number of studies have mentioned that estrogen impairs the immune system via affecting M1/M2 polarization and the phagocytic ability of macrophages (Veillat et al. 2012). aberrant expression of ER-β of endometriotic stromal cells is also found to affect the distribution of macrophages (Greaves et al. 2015). It has been demonstrated that the highly expressed ER-β in endometriotic stromal cells promotes CCL2 secretion via NF-κB signaling, which increases the recruitment of macrophages with M2 phenotype around endometriotic lesions (Gou et al. 2019). Apart from estrogen, prostaglandins are also reported to regulate the immune function of macrophages in endometriosis. A series of studies from our group demonstrates that

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PGE₂ suppresses the phagocytic ability of peritoneal macrophages by the inhibition of MMP-9 expression and activation (Wu et al. 2005a), CD36-dependent phagocytosis (Chuang et al. 2010), and Annexin A2-mediated phagocytosis (Wu et al. 2013), providing evidence to explain the dysfunction of immune cells during the development of endometriosis.

Many studies indicated that hypoxia is the master modulator that regulates the function of macrophages in endometriosis. It has already been demonstrated that the expression of estrogen receptors in endometriotic lesions are regulated by hypoxia (Wu et al. 2012). Hypoxia stimulates the expression of ER-β and knockdown of HIF-1α significantly increases the expression of ER-α and downregulates the level of ER-β in primary endometriotic stromal cells (Wu et al. 2012). Evidence has also indicated that hypoxia could upregulate PGE₂ via inducing the secretion of COX-2 in endometriosis (Wu et al. 2005b). Moreover, the abnormally expressed leptin in endometriotic cells (Wu et al. 2007), a kind of adipokine that also serves as an immunomodulator, is also reported to be upregulated by HIF-1α stabilization, and could mediate the function of peritoneal macrophages through affecting the expression of PGE₂ (Wu et al. 2010). Overall, hypoxia is a strong regulator that affects the immune function of peritoneal macrophages in endometriosis, which subsequently enhances the survival of endometriotic lesions in the peritoneal cavity.

Role of hypoxia in endometriotic lesion growth

To maintain a long-term survival, the retrograde endometriotic tissues equip themselves with some particular characteristics which improve their living conditions, such as capabilities of angiogenesis and proliferation. Both endometriotic lesions and the members in the microenvironment are found to release pro-survival factors to promote lesion growth. Hypoxia is known as a strong factor for promoting angiogenesis, cell proliferation, and even metastasis in cancer cells (Chen et al. 2020). Similarly, the growth of endometriotic lesions is also largely affected by the hypoxic force. The following part will further discuss the role of hypoxia in regulating factors involved in living maintenance.

Angiogenesis

The first problem that the temporarily survived endometriotic cells will encounter is how to get enough oxygen and nutrients to sustain further life. In addition to increasing autophagy to obtain more energy or transforming metabolic phenotype to change the energy utilization method, endometriotic tissues are also found to possess a higher level of angiogenesis activity to provide routes for blood transportation. Angiogenesis is a process that generates new vessels extending from the pre-existing vasculature structure, allowing the delivery of more nutrition and oxygen to neighboring tissues. The angiogenic activity is modulated by multiple factors, and a large proportion of them are found to be dysregulated in endometriosis, primarily due to hypoxic stress (Wu et al. 2019). Vascular endothelial growth factor (VEGF)-A and angiogenin, the widely known factors that mainly participate in angiogenesis, are highly expressed in endometriotic cells (Fasciani et al. 2000, Fu et al. 2018).

Immune factors involved in chronic inflammation also takes part in proangiogenic development. Elevated levels of proinflammatory cytokines in the peritoneal cavity such as IL-1β, TNF-α, and TGF-β, are all proved to induce angiogenic activity in endometriotic tissues. Additionally, angiogenesis in endometriosis could also be activated by certain kinds of chemokines, and IL-8, the most studied chemokine in endometriosis, serves as a strong inducer in the angiogenic process (Fasciani et al. 2000, Hsiao et al. 2014).

Hypoxia was demonstrated to be the upstream factor that regulates angiogenesis in endometriosis (Fig. 2). Upregulation of VEGF-A (Sharkey et al. 2000), IL-6 (Hsiao et al. 2017a), and IL-8 (Hsiao et al. 2014) are reported to be associated with the hypoxic stress or HIF-1α stabilization. Two regulators that are involved in hypoxia-mediated angiogenesis in endometriosis are DUSP2 and chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII). DUSP2 is a phosphatase that negatively regulates ERK signaling, an important signaling pathway showing an extensive effect on cellular functions. Therefore, it is not surprising to find that the downregulation of DUSP2 in endometriosis enhances the disease progression (Lin et al. 2012).

Figure 2 Hypoxia-regulated signaling pathways leading to angiogenesis in endometriosis. Adapted with permission from Fig. 2 from the article ‘Hypoxia: The force of Endometriosis’ published in ‘J. Obstet. Gynaecol. Res. Vol. 45, No. 3: 532–541, March 2019’.

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Levels of DUSP2 are markedly suppressed by hypoxia in endometriotic stromal cells (Wu et al. 2011). Angiogenesis-related genes such as IL-6 (Hsiao et al. 2017a), IL-8 (Hsiao et al. 2014), early growth response protein-1 (EGR-1), cysteine-rich angiogenic inducer 61 (CRY61), and osteopontin (Lin et al. 2012) are stimulated by hypoxia through DUSP2 downregulation. COX-2, the rate-limiting enzyme for PGE₂, is also induced by hypoxia-suppressed DUSP2 (Wu et al. 2011), which further promotes angiogenesis in endometriosis. On the other hand, COUP-TFI1 is an orphan nuclear receptor that is critically involved in embryo development and cardiovascular-related functions. Fu et al. demonstrated hypoxia suppressed the expression level of COUP-TFI1 in endometriosis, which further drives angiogenesis through the increasing level of angiogenin (Fu et al. 2018). Moreover, suppression of COUP-TFI1 could also promote the COX-2 expression in endometriotic stromal cells (Lin et al. 2014) to induce the PGE₂ production for tissue angiogenesis.

Cell proliferation

Endometrial cells from patients with endometriosis showed a more persistent proliferative capacity than that from disease-free women (Klemmt et al. 2007). It is known that the disequilibrium between cellular apoptosis and proliferation may lead to the cell overgrowth or tissue damage. In endometriosis, the retrograde endometrial tissues are featured with increased proliferation and decreased apoptosis (Wing et al. 2003, Lee et al. 2019), which consequently contributes to the tissue growth. Certain genes related to cell proliferation or survival are found to be increased in endometriotic implants when compared to paired eutopic tissues. Differentially expressed estrogen receptors in endometriotic cells, which show higher ER-β than ER-α, are found to have a higher proliferative ability (Han et al. 2015). The attachment to specific ECM components such as laminin, fibronectin, and vitronectin, significantly induces DNA synthesis in endometriotic stromal cells, suggesting adhesion could further enhance cell proliferation in endometriosis (Klemmt et al. 2007). Besides those, peptide growth factors are supposed to mediate the proliferation of endometriosis. Several peptide growth factors, such as EGF (Huang et al. 1996), insulin-like growth factor (Sbracia et al. 1997), leptin (Wu et al. 2002a), and fibroblast growth factor (FGF) (Wing et al. 2003) are proposed to be good candidates to stimulate endometriotic lesion growth. Among these, FGF-9 is the best characterized and least argued one. FGF-9 is a potent endometrial stromal cell growth factor and its expression fluctuates with the menstrual cycle with a peak at the late proliferating phase, which is correlated with the concentration of estrogen (Tsai 2002). Indeed, FGF-9 is an estromedin that mediates estrogen-stimulated endometriotic stromal cell proliferation (Wing et al. 2003). In endometriosis, FGF-9 and its cognate receptors are all expressed in endometriotic stromal cells and are functional (Wing et al. 2003). FGF-9 binds to FGFR2IIIc and FGFR3IIIc in endometrial stromal cells to stimulate protein expression, an essential step for cell differentiation and proliferation, through mammalian target of rapamycin (mTOR) and extracellular signal-regulated kinase pathways (Wing et al. 2005). More interestingly, FGF-9 is not only upregulated by PGE₂-induced estrogen (Wing et al. 2003) but also directly by PGE₂ through a different PGE₂ receptor-mediated signaling pathway in endometriotic stromal cells (Chuang et al. 2006). Since the aberrant production of PGE₂ is a result of hypoxia-mediated COX-2 overexpression, it is likely that overexpression of FGF-9 in endometriotic stromal cells is initiated by hypoxic stress. Indeed, it has been shown that hypoxia can upregulate the FGF-9 protein level via DUSP2-COX-2-PGE₂ cascade (Wu et al. 2011), microRNA-20a-COX-2 pathway (Lin et al. 2012) and in Yes-associated protein 1 (YAP1)-dependent pathway (Lin et al. 2017). These data clearly indicate that hypoxia-induced, FGF-9-mediated cell proliferation is a major factor for endometriosis development and progression; further studies focusing on disrupting FGF-9/FGFR signaling may lead to the discovery of alternative endometriosis treatment strategies.

Epigenetics and noncoding RNA

Epigenetic regulations such as DNA methylation and ncRNA expression result in distinct gene expression patterns in cells, which may explain why the same DNA sequence in a lineage of cells can generate different phenotypes in normal and abnormal cell populations. Through modifying the structure of DNA or RNA, the stringently controlled process is reported to be powerful; indeed, studies have indicated the modifications at the molecular level play critical roles in regulating cellular homeostasis, and even disease progression. Hypoxic stress is a strong force that drives a number of gene expression changes, and its role in modulating the molecular structure of both DNA and RNA in disease pathogenesis has been greatly emphasized. Since there is no evidence demonstrating that endometriosis is caused by germline mutation, it is suggested that endometriosis is an epigenetic disease (Guo 2009). The following section will summarize how hypoxia regulates the pathogenesis of endometriosis via epigenetic modulation.

DNA methylation

Modulation of DNA methylation plays an important role in chromatin remodeling and transcriptional regulation. The major form of DNA methylation in mammalian cells is 5-methyl cytosine, of which the
formation and maintenance is mainly controlled by DNA methyltransferases (DNMTs)-1, -3a, and -3b. DNMT1 favors hemi-methylated DNA as a substrate and transmits the methyl markers from passage to passage. Both DNMT3a and 3b generate new DNA methylation sites using the unmethylated templates as substrates. In endometriosis, there are studies demonstrating that certain genes such as ER-β ([Xue et al. 2007a]), aromatase ([Izawa et al. 2011]), and steroidogenic factor-1 ([Xue et al. 2007b]) are hypomethylated at their promoters, leading to aberrant expression of these genes in endometriotic cells. In contrast, hypermethylation of ER-α, leading to reduced expression of ER-α in ovarian endometrioma, was also reported ([Maekawa et al. 2019]). A genome-wide DNA methylation study shows that 403 genes were found significantly different in CpG island methylation when comparing the modification status between endometriotic and normal endometrial stromal cells ([Dyson et al. 2014]). A recent study shows that endometriotic stromal cells express less DNMT1 and have a lower 5-methylcytosine level than normal endometrial stromal cells ([Hsiao et al. 2015]). The molecular mechanism responsible for global downregulation of DNMT1 was also revealed (Fig. 3). Hypoxia recruits AU-rich element binding factor 1, a mRNA destabilizing RNA-binding protein, and microRNA-148a (miR-148a) onto the 3’ UTR of DNMT1 mRNA to cause the degradation of DNMT1 mRNA. The authors further demonstrated that suppression of DNMT1 by hypoxia for 3 days results in aberrant gene expression in normal endometrial stromal cells ([Hsiao et al. 2015]). These data provide solid evidence to support the notion that hypoxia can regulate gene expression via altering the DNA methylation pattern during the development of endometriosis.

**Histone modification**

Histone modifications and DNA methylation are two dominant regulatory pathways in cellular epigenetics. Covalent modifications of histones are differentially expressed in endometriotic lesions ([Samartzis et al. 2013, Monteiro et al. 2014]). Monteiro et al. showed acetylation of histones in endometriotic tissues is globally decreased particularly at the site of histone 3 ([Monteiro et al. 2014]). Concomitantly, histone deacetylase 1 (HDAC1) is found to be increased both in endometriotic epithelial and stromal cells ([Samartzis et al. 2013]). Treatment with HDAC inhibitor (HADCi) successfully enhances the acetylation of histones on the promoter of genes related to cell cycle checkpoints in endometriotic stromal cells, which further induces cell cycle arrest and cellular apoptosis ([Kawano et al. 2011]). In contrast, by reanalyzing microarray data deposited in Gene Expression Omnibus ([Hever et al. 2007]), Lin et al., discovered that the groups of polycomb repressive complex 2 proteins including EED, RBBP4, SUZ12 and EZH2, were all decreased in the endometriotic tissues compared to their eutopic counterparts ([Lin et al. 2019]). Experimental characterization also showed that EZH2 is downregulated in endometriotic stromal and epithelial cells, which results in a global decrease of trimethylated H3K27, a suppressive mark for gene expression, in endometriotic cells (Fig. 3). Hypoxia is the driving force to cause the downregulation of EZH2 and concomitantly overexpression of a group of genes involved in cell adhesion, proliferation, migration, and angiogenesis ([Lin et al. 2019]).

**Non-coding RNAs**

Noncoding RNAs are single-strand RNA which can be roughly classified by the length: small ncRNA and
long ncRNA. In the category of small ncRNA, miRNAs are widely known to be involved in many pathological processes by modulating their target mRNAs. Hypoxia has been shown to regulate the biogenesis of miRNAs (Liao et al. 2014). Generally, miRNA is able to mediate mRNA stability through binding to the seed region of 3’UTR in a post-transcriptional manner. As for the long ncRNA (lncRNA), which is defined as having a transcript’s length over 200 bases, the major function is to serve as a sponge for miRNA or regulator of transcription. The third kind of ncRNA is circular RNA (circRNA), of which length can be shorter or longer than 200 bases. The functions of ncRNAs as epigenetic regulators are multifaceted. For instance, circRNAs can serve as regulators of transcription, as intermediates in RNA processing reactions, or as sponges for targeted miRNAs (Hsiao et al. 2017b). In recent decades, due to the advance of techniques, both miRNAs and lncRNAs have emerged as novel regulators in endometriosis (Teague et al. 2010). However, most papers report aberrant expression (up- or downregulation) of them and subsequent impacts on target genes’ expression without investigating how these noncoding RNAs are regulated. Recently, hypoxia has been considered as one of the major factors to modulate the expression of noncoding RNA in endometriosis. For instance, it has been reported that hypoxia promotes autophagy activity in endometriotic stromal cells via direct association between HIF-1α and miR-210 (Xu et al. 2016). In addition, a report showed that hypoxia-regulated autophagy can also be mediated via long ncRNA (Liu et al. 2019). Angiogenesis is an important process in endometriotic lesion development and is found to be mediated by hypoxia-induced miRNAs. MiR-20a (targets DUSP2), miR-302a (targets COUP-TFII), miR-199a (targets VEGF-A), miR-148a (targets DNMT1), and miR-210-3p (targets BARD1) are all regulated by hypoxia (Lin et al. 2012, 2014, Hsu et al. 2014, Hsiao et al. 2015, Dai et al. 2019) and play critical roles in endometriosis pathophysiological processes. Interestingly, hypoxia regulation of miRNAs can be via upregulation (miR-20a, miR-210-3p), downregulation (miR-199a), or recruiting it to the targeted mRNA 3’UTR (miR-148a).

Targeting hypoxia-mediated gene regulatory network as therapeutic strategies

As hypoxia is a potent regulator in the pathogenesis of endometriosis, blocking the hypoxic effect is considered to be an ideal therapeutic strategy for treating this disease. Some studies reported that targeting HIF-1α protein in endometriosis mice could successfully suppress the lesions growth via the inhibition of vascular permeability (Becker et al. 2008). Using HADCi could also show antiangiogenic effect in endometriotic cells by reducing the expression of both HIF-1α and VEGF (Imesch et al. 2011). Additionally, signaling blockers such as Soraenib and Rapamycin are effective to reduce the level of HIF-1α, and further restricting the development of endometriosis (Moggio et al. 2012). However, HIF-1α is critical not only for the pathological abnormalities but also for normal physiological functions; thus, targeting HIF-1α may not work for benign diseases such as endometriosis. Besides targeting HIF-1α, interventions in downstream target genes of HIF-1α are also alternative ways for treating endometriosis. As mentioned previously, elevated YAP1 expression has been found in endometriotic stromal cells (Joshi et al. 2016, Song et al. 2016, Lin et al. 2017), and this is a promising target for future clinical therapy. Increased YAP1 is found to promote cell proliferation and prevent cells from undergoing apoptosis in endometriosis (Song et al. 2016, Lin et al. 2017). Verteporfin, an YAP1 inhibitor, could successfully block steroidogenesis, cell proliferation, angiogenesis, proinflammatory cytokine production, and invasion in an endometriosis mouse model, without compromising the fertility of female mice (Lin et al. 2017). Blocking hypoxia-induced ANTXR2 also showed a beneficial effect on lesion regression in an endometriosis mice model (Lin et al. 2019).

Conclusion

A growing body of evidence suggests that the development of endometriosis is considerably affected by hypoxic stress. Owing to the effect of stabilized HIF-1α under hypoxia, many genes involved in the pathological conditions of endometriosis are found to be directly regulated at the transcriptional level. In addition, hypoxia could also affect certain genes indirectly through epigenetic regulation such as DNA methylation, histone modification, or miRNA-mediated post-transcriptional mRNA destabilization. So far, major clinical options for treating patients with endometriosis are surgery and hormonal drug medication. However, the high recurrence rate and severe adverse effects nevertheless continue to be problems. Since endometriosis is a disease of complicated etiology, a single treatment method may not be an effective cure. Based on the findings so far, it is believed that hypoxia strongly modulates the pathological processes in endometriosis. Hence, considering targeting the hypoxia-mediated gene regulatory network in clinical therapy as a combinatorial treatment may be a promising strategy in the future.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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