

# Hypoxia: The force of endometriosis

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## Abstract

**Aim:** Summarize recent findings of how hypoxia regulates numerous important processes to facilitate the implantation, proliferation and progression of ectopic endometriotic lesions.

**Methods:** Most up-to-date evidences about how hypoxia contributes to the disease pathogenesis of endometriosis and potential therapeutic approaches were collected by conducting a comprehensive search of medical literature electronic databases. Quality of data was analyzed by experienced experts including gynecologist and basic scientists.

**Results:** Uterus is a highly vascularized organ, which makes endometrial cells constantly expose to high concentration of oxygen. When endometrial tissues shed off from the eutopic uterus and retrograde to the peritoneal cavity, they face severe hypoxic stress. Even with successful implantation to ovaries or peritoneum, the hypoxic stress remains as a critical issue because endometrial cells are used to live in the well-oxygenated environment. Under the hypoxia condition, cells undergo epigenetic modulation and evolve several survival processes including steroidogenesis, angiogenesis, inflammation and metabolic switch. The complex gene regulatory network driven by hypoxia ensures endometriotic cells can survive under the hostile peritoneal microenvironment.

**Conclusion:** Hypoxia plays critical roles in promoting pathological processes to facilitate the development of endometriosis. Targeting hypoxia-mediated gene network represents an alternative approach for the treatment of endometriosis.

**Key words:** angiogenesis, epigenetics, gene regulatory network, hypoxia, steroidogenesis.

## Introduction

Endometriosis, manifested by the growth of endometrial tissues outside of the uterine cavity, is one of the most common gynecological disorders in women of reproductive age with complex etiologies. Symptoms of endometriosis include chronic pelvic pain, dysmenorrhea, dyspareunia and infertility, which significantly reduce life quality and economic productivity of affected women. The etiology of endometriosis remains largely unknown; however, retrograde menstruation has been proposed and well accepted to be a crucial constituent for the development of this

disease.<sup>1</sup> Although Sampson's theory is supported by clinical observations in human<sup>2–4</sup> and in baboons,<sup>5</sup> it is insufficient to explain why 90% women of reproductive age have retrograde menstruation but only 10–15% of them develop endometriosis.<sup>6</sup> Therefore, there must be some critical factors playing important modulatory roles in the pathogenesis of endometriosis for retrograded tissues to successfully survive and implant in the pelvic cavity.

According to the retrograde menstruation theory, shed-off endometrial tissues first lost blood supply and face hypoxic stress. Under such circumstance, it is necessary for these tissues to develop some sort of

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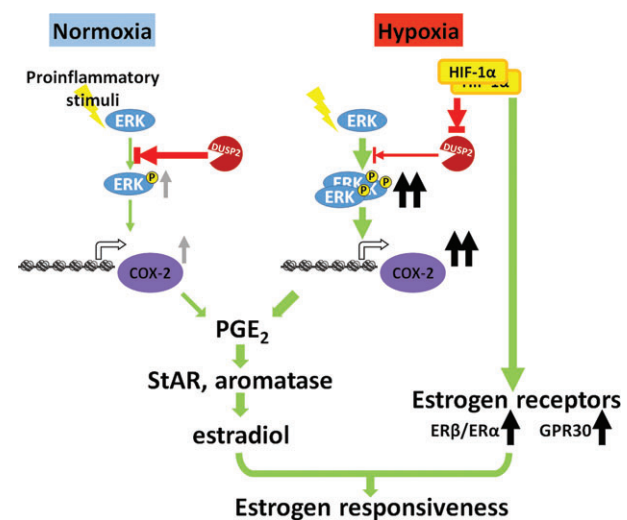
systems in order to survive in the hostile microenvironment. Hypoxia is a master regulator that controls numerous physiological and pathological processes, most of which are mediated via transcriptional regulation by hypoxia-inducible factors (HIF). HIF is consisted of two subunits,  $\alpha$  and  $\beta$ , to form the heterodimeric complex. HIF-1 $\beta$ , also known as aryl hydrocarbon nuclear translocator, is stably present under normoxia and hypoxia conditions. In contrast, the  $\alpha$  subunit is rapidly degraded under ambient oxygen concentration but is accumulated in the nucleus under hypoxic condition due to lack of enzymatic activity of prolyl hydroxylases, which prevents HIF-1 $\alpha$  from hydroxylation and thus degradation by 26S proteasome.<sup>7</sup>

Hypoxia-inducible factors play many important functions in endocrine and reproductive organs, including the uterus.<sup>8</sup> The presence of HIF in the endometrial cells was first discovered earlier in this century.<sup>9,10</sup> HIF-1 $\beta$  expresses constantly through the cycle and reaches its maximal levels in the glandular cells during the proliferative phase, while hypoxia inducible factor-1 alpha (HIF-1 $\alpha$ ) mainly expresses in secretory and menstrual phases in the functional layer.<sup>9</sup> These findings suggest that HIF may play some important roles in maintaining normal endometrial functions. The first piece of evidence that clearly demonstrates the pathological function of HIF-1 $\alpha$  in endometriosis was reported in 2007. Wu *et al.*<sup>11</sup> showed levels of HIF-1 $\alpha$  mRNA and protein are markedly increased in ectopic endometriotic lesions as compared to paired eutopic endometrial tissues. Elevation of HIF-1 $\alpha$  stimulates the expression of leptin,<sup>11</sup> which was previously shown to promote endometrial cell proliferation.<sup>12</sup> Following this pioneer observation, numerous papers subsequently reported the function and regulation of HIF-1 $\alpha$  during the development of endometriosis (see<sup>13</sup> for reference). In this review, we will focus on three most critical processes, namely, steroidogenesis, angiogenesis and epigenetic regulation to illustrate the regulatory roles of hypoxia (and HIF-1 $\alpha$ ) in the pathological processes of endometriosis.

## Hypoxia Regulates Estrogen Axis in Endometriotic Cells

The establishment and development of endometriosis is highly dependent on estrogen. Besides the estradiol of ovarian origin, it was later reported that ectopic

endometriotic stromal cells are able to de novo synthesize estradiol using cholesterol due to aberrant expression of steroidogenic acute regulatory protein (StAR)<sup>14</sup> and other steroidogenic enzymes,<sup>15</sup> especially aromatase.<sup>16</sup> Interestingly, expression of StAR and the other steroidogenic enzymes are upregulated by prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)<sup>15,17,18</sup> due to overexpression of cyclooxygenase-2 (COX-2) in endometriotic stromal cells<sup>19</sup> and peritoneal macrophage.<sup>20</sup> It has been known that ectopic endometriotic stromal cells are at least 100 times more sensitive to cytokines (such as interleukin-1 $\beta$ ) stimulation in terms of COX-2 overexpression.<sup>19</sup> In 2011, Wu *et al.*,<sup>21</sup> reported that hypoxia is a critical factor for the increase of COX-2 promoter sensitivity. They demonstrated that hypoxia is the driving force causing the prolonged activation of extracellular signal-regulated kinase (ERK) and p38 mitogen activated protein kinase (MAPK) via suppressing dual specificity phosphatase 2 (DUSP2), an ERK/p38 MAPK-specific phosphatase. Because ectopic endometriotic stromal cells have elevated HIF-1 $\alpha$ <sup>11</sup> and thus a reduced DUSP2 level, therefore, dephosphorylation of phospho-ERK and phospho-p38 MAPK is inefficient in these cells. As a result, the duration and magnitude of ERK and p38 MAPK signaling are enhanced upon exogenous stimulation. This leads to prolonged activation of ERK and p38 MAPK



**Figure 1** Hypoxia promotes steroidogenesis and estrogen responsiveness. COX-2, cyclooxygenase-2; DUSP2, dual specificity phosphatase 2; ER $\alpha$ , estrogen receptor alpha; ER $\beta$ , estrogen receptor beta; ERK, extracellular signal-regulated kinase; GPR30, G protein-coupled receptor 30; HIF-1 $\alpha$ , hypoxia inducible factor-1 alpha; PGE<sub>2</sub>, StAR.

downstream signaling and ultimately results in an increase of *COX-2* gene activity (Fig. 1). Taken together, these data demonstrate that hypoxia promotes the de novo biosynthesis of estradiol in ectopic endometriotic stromal cells.

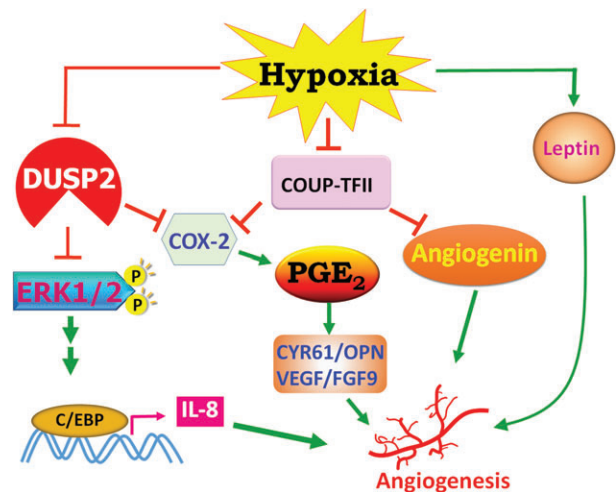
Hypoxia not only regulates estrogen biosynthesis, it also modulates estrogen responsiveness. There are two isoforms of nuclear receptor (estrogen receptor alpha ( $ER\alpha$ ) and estrogen receptor beta ( $ER\beta$ )) and one G protein-coupled receptor (GPR30) for estradiol.<sup>22,23</sup> All of three estrogen receptors involve in the development and maintenance of endometriosis. In a mouse uterine fragment-implanted model, knock-down of either  $ER\alpha$  or  $ER\beta$  impedes endometriotic-like lesions growth,<sup>24</sup> while treatment with a GPR30-selective ligand promotes proliferation of eutopic endometrial stromal cells.<sup>25</sup> In human, aberrant expression of  $ER\alpha$ ,  $ER\beta$  and GPR30 have been reported in women with endometriosis.  $ER\alpha$  downregulation and/or  $ER\beta$  upregulation are found in endometriotic tissue, causing the higher ratio of  $ER\beta/ER\alpha$ ,<sup>26,27</sup> whereas GPR30 is overexpressed in endometriotic tissues.<sup>28,29</sup> Interestingly, hypoxia was found to be a critical factor regulating the expression of  $ER\alpha$ ,  $ER\beta$  and GPR30. Treatment of eutopic endometrial stromal cells with hypoxia induces  $ER\beta$  but inhibits  $ER\alpha$  expression leading to a marked increase in  $ER\beta/ER\alpha$  ratio. The suppressive effect on  $ER\alpha$  and the induction of  $ER\beta$  by hypoxia are regulated at the transcriptional level in an HIF-1 $\alpha$  dependent manner.<sup>30</sup> Similarly, GPR30 is also upregulated by hypoxia in an HIF-1 $\alpha$ -dependent manner, which contributes to the antiapoptotic effect of estrogen.<sup>31</sup> More interestingly, estrogen has been shown to induce nuclear accumulation of HIF-1 $\alpha$  and this effect can be repressed by an antiestrogen antagonist.<sup>32</sup> Taken together, the vicious circle between estrogen/ $ER$  and hypoxia/HIF-1 $\alpha$  may favor the establishment of endometriotic lesions.

## Hypoxia Regulates Angiogenesis

One of the greatest challenges to the retrograde endometrial tissues in peritoneal cavity is to establish a functional vascular network for exchanges of oxygen, nutrients and metabolites. To overcome this constrain of limited oxygen level, the cells have to acquire the ability to recruit and to modulate the assembly of endothelial cells, smooth muscle cells into pre-existing vessels, namely, angiogenesis.

Hypoxia is a well-known master regulator of angiogenesis. In normal endometrium, hypoxia treatment induces expressions of several vascular endothelial growth factor A (VEGF-A) isoforms in both epithelial and stromal cells,<sup>33</sup> suggesting that hypoxia and VEGF-A may modulate angiogenesis in endometriosis. In supporting this notion, levels of VEGF-A in the peritoneal fluids collected from women with endometriosis are actually higher than those in the control group.<sup>34,35</sup> Furthermore, inhibition of HIF-1 $\alpha$  expression in a mouse model of endometriosis suppresses VEGF-A expression and therefore blocking angiogenesis, which ultimately results in reducing the size of endometriotic-like lesions.<sup>36</sup> Of note, although blockage of hypoxia-induced VEGF-A expression impairs angiogenesis, the restoration of VEGF-A level by intraperitoneal injection does not rescue the development of endometriotic-like lesions, indicating that other angiogenic factors also contribute to the process of vascular remodeling in endometriotic tissues.<sup>37</sup>

Leptin is a potent angiogenic factor.<sup>38–40</sup> Blockage of leptin signaling either by treatment with leptin antagonist or by implantation of endometrial tissues derived from leptin receptor knockout mice results in reduced vascular lesions. As leptin is aberrantly expressed in endometriotic cells<sup>12,41</sup> in an HIF-1 $\alpha$ -



**Figure 2** Hypoxia stimulates angiogenesis through multiple routes. C/EBP, CCAAT/enhancer-binding protein; COUP-TFII, cyclooxygenase-2; COX-2, cyclooxygenase-2; CYR61, cysteine-rich protein 61 gene; DUSP2, dual specificity phosphatase 2; ERK1/2, extracellular signal-regulated kinase 1 and 2; FGF9, fibroblast growth factor-9; IL-8, interleukin-8; OPN, osteopontin; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; VEGF, vascular endothelial growth factor.

dependent manner,<sup>11</sup> it is reasonable to believe that hypoxia-induced angiogenesis may also be mediated, at least in part, via its stimulatory effect of leptin (Fig. 2).

Recent studies further advance our understandings in hypoxia-regulated angiogenesis as novel angiogenic factors are identified. Hsiao *et al.*<sup>42</sup> reported that interleukin-8 (IL-8) is a potent angiogenic factor in endometriosis. Production of IL-8 by endometriotic stromal cells induces tube formation of human umbilical vein endothelial cell in an *in vitro* assay system. They further demonstrate that expression of IL-8 is induced by hypoxia at the transcriptional level. However, it is not directly upregulated by HIF-1 $\alpha$ ; instead, hypoxia causes the downregulation of DUSP2 and thus the prolonged activation of ERK. Activation of ERK enhances the binding of its downstream transcription factor, CCAAT/enhancer-binding protein (C/EBP)  $\alpha$  and C/EBP $\beta$ , to IL-8 promoter to increase the transcription of IL-8, a mechanism similar to that occurs in cancer cells.<sup>43</sup> Treatment with IL-8 receptor antagonist, reparixin, not only inhibits hypoxia- or knockdown-of-DUSP2-induced angiogenesis *in vitro*, but also blocks blood vessel infiltration in mouse model of endometriosis. As a result, endometriotic-like lesion is shrunk.<sup>42</sup> Results from this study suggest that pharmacologically disrupt IL-8 signaling may inhibit angiogenesis and thus cause the regression of endometriotic lesions.

Fu *et al.*,<sup>44</sup> reports the unconventional angiogenic factor, angiogenin, is also upregulated in endometriotic cells and is regulated by hypoxia. Angiogenin is a potent angiogenic factor with unique features compared to VEGF.<sup>45</sup> Earlier study reveals that angiogenin exerts very weak RNase activity, which is required for its angiogenic activity.<sup>46</sup> It has been shown that angiogenic deficiency induced by loss of angiogenin cannot be rescued by administration of VEGF-A or basic fibroblast growth factors (bFGF),<sup>47</sup> suggesting angiogenin exerts a distinct and nonredundant function in regulating angiogenesis. Expression of angiogenin in normal endometrium was discovered in 2001,<sup>48</sup> but the function and mechanism of regulation were not characterized. Fu *et al.*<sup>44</sup> reported that angiogenin is upregulated by hypoxia in an indirect gene regulatory cascade. The expression of angiogenin is suppressed by chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII), an orphan nuclear receptor, in normal endometrial stromal cells. Treatment of endometrial stromal cells with hypoxia inhibits the expression of COUP-TFII and

results in derepression of angiogenin. In contrast, hypoxia-induced upregulation of angiogenin can be blocked by forced expression of COUP-TFII, providing direct evidence that hypoxia-induced angiogenin expression is mediated by COUP-TFII. Since the level of HIF-1 $\alpha$  is elevated<sup>11</sup> and COUP-TFII is downregulated<sup>49,50</sup> in endometriotic stromal cells, it is not surprised that angiogenin is overexpressed in endometriotic lesions. Interestingly, examination of normal endometria and endometriotic lesions by immunohistochemical staining demonstrates that CD31-positive staining (marker of endothelial cells) is colocalized with angiogenin-positive cells only in human endometriotic lesions but not in normal endometria. This result is clinically exciting as it implies that mechanisms of angiogenesis in normal endometria and endometriotic lesions are different, which highlights angiogenesis may be a potential molecular target for developing novel therapeutic regimens.

A group of angiogenic factors, including cysteine-rich protein 61 gene (CYR61), osteopontin, and FGF9<sup>51</sup> were identified to be upregulated by hypoxia.<sup>52</sup> CYR61, a CCN protein family, was originally found to express at sites where neovascularization occurs and a ligand to  $\alpha_v\beta_3$  integrin, which is involved in angiogenesis.<sup>53,54</sup> Similar to CYR61, osteopontin is another potent angiogenic factor that has been shown in both *in vitro* and *in vivo* models.<sup>55,56</sup> CYR61, FGF9 and osteopontin are aberrantly expressed in endometriotic tissues,<sup>52,57–63</sup> which can be recapitulated by hypoxia treatment in eutopic stromal cells.<sup>52</sup> Interestingly, elevation of CYR61, FGF9 and osteopontin is induced by hypoxia in an HIF-dependent epigenetic regulation manner (discuss below). Taken all together, these data demonstrate that microenvironmental hypoxic stress is a critical factor to trigger angiogenesis via multiple mediators/pathways to ensure the proper development of vessel networks in endometriotic lesions.

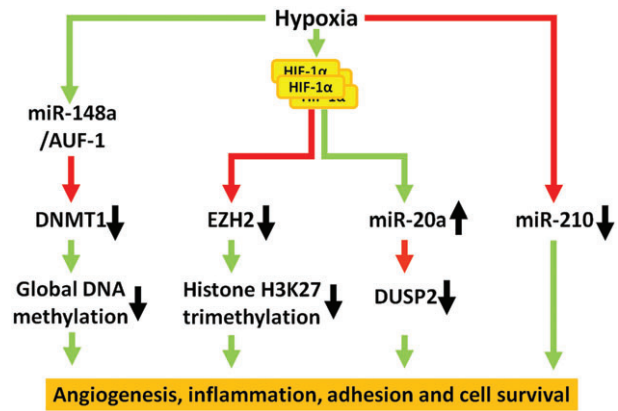
## Hypoxia-Mediated Epigenetic Regulation

Accumulating evidence indicates that endometriosis is an epigenetic disease.<sup>64</sup> Despite the identical genetic background between eutopic and ectopic endometrial cells in patients with endometriosis, numerous studies reported that significant differences in the biochemical properties of these two tissues exist. The distinct biochemical nature of ectopic endometriotic cells is due to epigenetic regulation of certain genes important for

the survival, proliferation, migration, differentiation and angiogenesis. Although recent studies employing genome-wide deep sequencing show there are some cancer driver gene mutations in noncancer endometriosis samples,<sup>65,66</sup> they are only limited to some but not all glandular epithelial cells and more importantly, they are somatic but not germline mutations, which means that endometriosis is not a genetic disease.

Epigenetic regulation includes three different types of mechanisms, namely, DNA methylation, histone modification and noncoding RNA-mediated gene expression. Alteration of epigenetic codes leads to regulation of gene expression profiles without changing genetic composition, which is a dynamic and economic way of modulating cellular function. Methylation of cytosine at the 5'-position (5mC) of DNA often leads to gene silencing. DNA methylation is catalyzed by DNA methyltransferase (DNMT) and removed by ten-eleven translocation (TET) enzymes. There are four DNMT including DNMT1, DNMT3a, DNMT3b and DNA methyltransferase 3 like (DNMT3L). DNMT3L is specifically expressed in testis, while the others are ubiquitously expressed in all cell types. DNMT1 is an enzyme that catalyzes hemi-methylated DNA to become full-methylated DNA molecule and is referred as maintenance DNMT. The other three DNMT are de novo methyltransferases that add a methyl group to unmethylated cytosine. In contrast, TET proteins (TET1/2/3) catalyze the conversion of the 5mC into 5-hydroxymethylcytosine (5hmC),<sup>67</sup> which is further oxidized by TET proteins to form 5-formyl cytosine and 5-carboxylcytosine.<sup>68,69</sup> Aberrant methylation of promoter region in certain genes, such as estrogen receptor  $\beta$ ,<sup>70</sup> progesterone receptor,<sup>71</sup> steroidogenic factor-1,<sup>72</sup> homeobox A10,<sup>73</sup> and aromatase<sup>74</sup> had been reported. However, these are only sporadic studies without in-depth investigation of the underlying mechanism.

A comprehensive investigation of DNA methylation change in endometriosis was conducted by Hsiao *et al.*,<sup>75</sup> who reported that the expression of DNMT1 but not DNMT3a or DNMT3b was reduced in stromal cells derived from patients with endometriosis, an observation similar to what was reported by van Kaam *et al.*<sup>76</sup> As a result, global 5mC levels are reduced that results in global hypomethylation in endometriotic stromal cells.<sup>77</sup> The main factor that causes DNMT1 downregulation in endometriotic stromal cells is hypoxic stress. Hypoxia recruits miR-148a and AU-rich element binding factor 1 (AUF-1), an



**Figure 3** Hypoxic stress facilitates the development of endometriosis via multiple epigenetic mechanisms. AUF-1, AU-rich element binding factor 1; DNMT1, DNA methyltransferase 1; DUSP2, dual specificity phosphatase 2; EZH2, Enhancer of Zeste Homolog 2; HIF, hypoxia-inducible factor.

mRNA destabilizing protein, to the 3'untranslated region (UTR) of DNMT1 transcript to destabilize its mRNA. As a result, levels of DNMT1 protein and subsequently 5mC are reduced in endometriotic stromal cells (Fig. 3). This causes aberrant expression of genes in endometriotic stromal cells. For example, GATA6, HOXA3 and SLC16A5, genes that were known to be hypomethylated in endometriotic stromal cells by an independent genome-wide study,<sup>78</sup> are upregulated when normal endometrial stromal cells are cultured under hypoxia condition for 48 or 72 h.<sup>75</sup> Taken together, these data demonstrate that hypoxia inhibits DNMT1 in endometriotic stromal cells to modulate certain genes expression via passive demethylation of their promoter. A thorough characterization of hypoxia-mediated DNA hypomethylation-dependent gene expression and functions may provide a different view of the etiology of endometriosis.

Histones, the key components of nucleosome, pack the lengthy genomic DNA molecules into compact forms in the nuclei. Modifications of histones, including methylation, acetylation, phosphorylation, ubiquitination, SUMOylation, citrullination and adenosine diphosphate (ADP)-ribosylation, alter the structure of chromatin and subsequently change gene expression status. The majority of post-translational modification takes place on the amino terminal-tail of histones extruding from the nucleosome. Lysine, arginine, serine and threonine are four amino residues subjected for modifications. A recent review by Nasu *et al.*,<sup>79</sup> summarizes effects of aberrant modification of histones on

endometriosis. Arosh *et al.*,<sup>80</sup> reports that treatment with selective PGE<sub>2</sub> receptor, prostaglandin E2 receptor EP2 subtype (EP2) and prostaglandin E2 receptor EP4 subtype (EP4) antagonists, alters the expression levels of histone modifying enzymes in human endometriotic cells, suggesting that PGE<sub>2</sub> may influence histone modification patterns and thus modulate gene expression epigenetically. Among the histone modifying enzymes, Enhancer of Zeste Homolog 2 (EZH2), a subunit of the polycomb repressive complex 2 (PRC2) catalyzing trimethylation of histone H3 lysine 27 (H3K27), has been studied recently. Two reports<sup>81,82</sup> show the elevation of EZH2 in endometriotic cells, while the other two reports<sup>83,84</sup> show reduced expression of EZH2. Although the discrepancy of these reports is not known, the immunostaining result in Lin's paper clearly shows more and stronger nuclear staining of EZH2 in eutopic endometrial epithelial and stromal cells as compared to ectopic endometriotic counterparts. Furthermore, Lin *et al.*<sup>84</sup> shows that not only EZH2 but also other components of PRC2 such as SUZ12, embryonic ectoderm development (EED) and RB binding protein 4 (RBBP4) are all downregulated in ectopic endometriotic cells. Further investigation reveals that reduced EZH2 is due to hypoxia-mediated transcriptional suppression. Hypoxia, through HIF-1 $\alpha$ -dependent manner, inhibits EZH2 expression and reduces H3K27 trimethylation in a subset of genes leading to aberrant expression in ectopic endometriotic cells (Fig. 3). As a result, endometriotic cell adhesion, migration, proliferation and angiogenesis are all enhanced.

Noncoding RNA includes short noncoding RNA, such as microRNA (miRNA),<sup>85</sup> long noncoding RNA<sup>86</sup> and circular RNA.<sup>87</sup> MicroRNA are small noncoding RNA (usually less than 30 nucleotides) modulating the target gene expression through mRNA degradation or translational repression, while long noncoding RNA (>200 nucleotides) and circular RNA exert their functions by either upregulating or downregulating target genes expression. The biological function of miRNA in endometriosis has been implicated in inflammation, tissue repair, cell proliferation, apoptosis, extracellular matrix remodeling and angiogenesis.<sup>88</sup> Genome-wide analysis of miRNA expression profile demonstrated that dysregulated miRNA plays critical roles during the development of endometriosis.<sup>89,90</sup> Hundreds of miRNA, known as hypoxamiRs, have been shown to be regulated by hypoxia. Surprisingly, only a handful of hypoxamiRs are studied in endometriosis. The first studied miRNA is miR-20a. It has been reported that expression of miR-20a is

relatively higher in ectopic lesions compared to that in eutopic endometrial tissues.<sup>52</sup> By using bioinformatic and molecular biology approaches, Lin *et al.*<sup>84</sup> identified that promoter of miR-20a harbors a functional hypoxia response element. Further study demonstrated that expression of miR-20a is induced by hypoxia. Interestingly, one of the targets of miR-20a is DUSP2 (Fig. 3). Transient transfection of miR-20a mimics represses DUSP2 expression, while administration of anti-miR-20a abolishes hypoxia-induced DUSP2 downregulation. This finding indicates that hypoxia suppresses DUSP2 expression via two parallel pathways: a direct transcriptional repression as described in previous section and an indirect pathway by upregulating miR-20a, which highlights the importance of prolonging ERK phosphorylation in endometriosis. Hypoxia-induced miR-20a expression leads to downregulation of DUSP2 resulting in overexpression of downstream ERK-regulated genes, such as COX-2, angiogenic and mitogenic factors.<sup>52</sup>

The second miRNA that was shown to be associated with hypoxia-mediated gene expression in endometriosis is miR-148a.<sup>75</sup> MiR-148a is overexpressed in endometriotic stromal cells but is not induced by hypoxia. Under hypoxia condition, miR-148a is recruited to the 3'UTR of DNMT1, where it coordinates with AUF-1 to promote the degradation of DNMT1 mRNA. As a result, level of DNMT1 protein is reduced, which leads to passive global hypomethylation of endometriotic stromal cells. Recently, the function of another hypoxamiR, miR-210 was studied. Level of miR-210 is elevated in ectopic endometriotic stromal cells, likely mediated by hypoxia-mediated increased HIF-1 $\alpha$  transactivation.<sup>91</sup> Hypoxia induces miR-210 expression to promote autophagy and enhance cell survival (Fig. 3). It is worth noted that not all miRNA are upregulated in endometriotic cells. Dai *et al.*<sup>92</sup> reports that miR-199a is downregulated in endometriosis. Two important target genes of miR-199a are HIF-1 $\alpha$  and VEGF-A. Forced overexpression of miR-199a reduces HIF-1 $\alpha$  and VEGF-A levels and thus inhibits angiogenesis.<sup>93</sup> Thus, reduction of miR-199a in endometriotic cells may stabilize HIF-1 $\alpha$  and induce angiogenesis. These two studies provide a different angle to illustrate how miRNA contributes to hypoxia-mediated pathophysiology of endometriosis.

Consider the number of hypoxamiRs in mammalian cells and the critical function of hypoxia in the pathogenesis of endometriosis: one would reason that effects of many hypoxamiRs on endometriosis remain

uninvestigated. Since endometriosis is considered an epigenetic disease, more studies are needed to unravel the regulation and function of hypoxia-regulated epigenetic modulators in the pathogenesis of endometriosis.

## Targeting Hypoxia-Mediated Gene Network as Potential Therapeutic Approach

Endometriosis is a complex disease that involves gene–gene and gene–microenvironment interactions, which highlights the inefficacy of current treatment regimens. As discussed above, hypoxia regulates estrogen biosynthesis/responsiveness axis and blood vessel developing system, two most important processes in endometriosis. Not to mention that there are many other processes such as alteration of glucose metabolism<sup>94,95</sup> and immune dysfunction<sup>96–98</sup> are either directly or indirectly regulated by hypoxia and its downstream gene regulatory network. Therefore, it is reasonable to propose that targeting hypoxia-mediated gene network would simultaneously block several processes and exert a better outcome for treating endometriosis. To achieve this goal, one needs to employ bioinformatics and systems biology approaches to identify the most upstream targets directly regulated by HIF-1 $\alpha$ . Recently, we combined the analytic results of several large-scale databases and found that Yes-associated protein 1 (YAP1) is a likely candidate.<sup>99</sup> YAP1 is a transcription coactivator of TEA domain family member (TEAD),<sup>100</sup> which is famous (or notorious) for its role in cell fate determination and cancer development. YAP1 protein is abundant in cytosol as a phosphorylated form, of which is inactivated. Under certain circumstances, the kinase that phosphorylates YAP1, large tumor suppressor kinase 1 (LATS1), is degraded and results in YAP1 dephosphorylation. Unphosphorylated YAP1 then translocates to nucleus and binds to TEAD to induce target genes expression. We discover that level of LATS1 is downregulated in ectopic endometriotic cells, which leads to increased nuclear translocation of YAP1. Further investigation reveals that LATS1 is suppressed by hypoxia. Treatment of normal endometrial stromal cells with hypoxia results in LATS1 downregulation and YAP1 nuclear translocation. More strikingly, administration of verteporfin, a YAP1 inhibitor, simultaneously suppresses numerous

pathological processes of endometriosis including cell proliferation, angiogenesis, estrogen biogenesis, prostaglandin biosynthesis, cell migration, innervation, inflammation and antiapoptosis. As one of the side effects of hormone-based therapy is suppression of reproduction, we tested whether treatment with verteporfin would affect female mice's reproductive function. Encouragingly, not only female reproductive function is not reduced by verteporfin but the offspring development is also unaffected.<sup>99</sup> Results from this study provide a proof-of-concept that targeting YAP1 may have therapeutic potential without obvious adverse effects on reproductive organs and fertility. It also points out a new avenue for designing alternative therapeutic regimens for endometriosis. Future investigations can focus on identifying more novel druggable targets like this for treating endometriosis.

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## Disclosure

The authors declare no conflicts of interest.

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