Hypoxia-Induced MicroRNA-20a Expression Increases ERK Phosphorylation and Angiogenic Gene Expression in Endometriotic Stromal Cells

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Context: Aberrant activation of MAPK has been implicated to play important roles in pathological processes of endometriosis. However, how MAPK are constitutively activated in endometriotic tissues remains largely unknown. microRNA are small noncoding RNA that regulate the stability or translational efficiency of target mRNA by interacting with the 3’ untranslated region. Thus, miRNA are thought to be modulators of the transcriptional response, fine-tuning gene expression.

Objective: The aim of this study was to evaluate the functional roles of microRNA-20a (miR20a) in MAPK activation and the pathogenesis of endometriosis.

Design: miR20a expression was analyzed in nonpaired (endometrium = 17; endometriosis = 37) and paired (n = 12) endometriotic tissues by quantitative RT-PCR. Overexpression of miR20a in eutopic endometrial stromal cells or inhibition of miR20a in ectopic endometriotic stromal cells was used to evaluate its impact on ERK phosphorylation and subsequently angiogenesis- and proliferation-related gene expression.

Results: Levels of miR20a were up-regulated in endometriotic stromal cells. Elevation of miR20a was up-regulated by hypoxia inducible factor-1. The up-regulation of miR20a causes the down-regulation of dual-specificity phosphatase-2, which leads to prolonged ERK phosphorylation and an increase in the expression of several angiogenic genes. Furthermore, the up-regulation of miR20a enhances the prostaglandin E2-induced expression of fibroblast growth factor-9, a potent mitogen that stimulates both endothelial and endometrial cell proliferation.

Conclusion: Our findings provide the novel mechanism that not only functionally links together hypoxic stress, miR20a expression, aberrant ERK phosphorylation, and angiogenesis but also demonstrates that miR20a is an important modulator in the development of endometriosis. (J Clin Endocrinol Metab 97: E1515–E1523, 2012)
tion of ectopic endometrial cells must also escape apoptosis (3), adhere to the peritoneum (4), degrade the underlying extracellular matrix (5), generate neovascularity (6), acquire steroidogenic capacity (7, 8), and evade the immune surveillance system (9–12). Thus, there must be additional factors that increase susceptibility to endometriosis and that remain to be identified.

Aberrant activation of MAPK has been implicated to play important roles in pathological processes of disease progression (13, 14). For example, aberrant activation of ERK has been shown to be involved in IL-1β and macrophage migration inhibitory factor-induced cyclooxygenase-2 expression (15–17), prostaglandin E2 (PGE2)-induced fibroblast growth factor-9 (FGF9) expression (18), migration inhibitory factor-stimulated production of angiogenic factors (19), basal endometrial cell survival (20), and estrogen-stimulated cell proliferation (13). However, the mechanisms responsible for the aberrant activation of ERK remain largely unknown.

In an attempt to investigate the mechanisms responsible for the aberrant ERK activation and its impact on endometriosis, we have recently reported that expression of the ERK-specific phosphatase, dual-specificity phosphatase-2 (DUSP2), was down-regulated in endometriotic stromal cells due to hypoxia-inducible factor (HIF)-1α-mediated gene silencing (21). The suppression of DUSP2 expression by HIF-1α results in prolonged ERK activation. However, a close examination of the molecular mechanism reveals that the HIF-1α-inhibited DUSP2 promoter activity is not well correlated with the down-regulation of DUSP2 protein, which suggests that posttranscriptional regulation may also contribute to the down-regulation of the DUSP2 protein.

microRNA (miRNA) are 21–23 nucleotide RNA molecules that regulate the stability or translational efficiency of target mRNA by interacting with the 3′ untranslated region (UTR). Many miRNAs are evolutionarily conserved, from worms to humans, implying that these miRNA control essential processes during development and in the adult body. miRNA have been shown to exert critical functions in diverse physiological processes, including cellular differentiation, proliferation, and apoptosis. In contrast, aberrant expression of specific miRNA has been described in a variety of human malignancies including endometriosis (22). The expression profile of miRNA in normal endometria and endometriotic tissues has been evaluated by several groups (23–26). However, results are not consistent among groups. More importantly, most studies only superficially report the expression data without detailed information of the functional roles of miRNA.

In this study, we sought to test the hypothesis that miRNA is involved in the hypoxia-mediated DUSP2 down-regulation and to further investigate the impact of miRNA in the pathogenesis of endometriosis. Herein we report the identification of miRNA-20a (miR20a) as a hypoxamir that targets and inhibits DUSP2 expression in endometriotic stromal cells. Our data demonstrate that hypoxia-induced miR20a expression plays an important role in the development of endometriosis.

**Materials and Methods**

**Patients**

Tissue specimens were obtained from 60 patients who were treated at the Department of Obstetrics and Gynecology at the National Cheng Kung University Hospital. The stages of endometriosis were determined visually according to the revised American Society of Reproductive Medicine classification (1997). During the procedure of laparoscopy and laparotomy, normal endometria from endometriosis-free patients (n = 17) and endometriotic lesions including pelvic endometriosis and ovarian endometrioma from patients with endometriosis (n = 31), were collected. In addition, another 12 sets of paired eutopic and ectopic endometrial tissues were also obtained from the patients with endometriosis. Patient data were reported previously (21). All samples were histologically confirmed by pathologists. All patients had regular menstrual cycles and none received any hormone treatment within 6 months before gynecological surgery. This study was approved by the Institutional Review Board of National Cheng Kung University Medical Center, and informed consent was obtained from each patient.

**Endometrial and endometriotic stromal cells**

The procedure used to isolate stromal cells from the eutopic endometria and ectopic endometriotic lesions was as described previously (8). In brief, after the tissues were minced and digested with type IV collagenase, stromal cells were separated from epithelial glands by nylon meshes. Filtrated stromal cells were then cultured in culture medium (DMEM/F12) at 37 °C until 70% confluence was reached. After serum starvation, the cells were subjected to different treatments. For mRNA isolation, cells were directly lysed in the well using a TRizol reagent. The concentration and quality of mRNA was determined by absorption at both 260 and 280 nm. For protein isolation, cells were lysed and collected in the dish using TRizol reagent according to the manufacturer’s instructions. Purity of the cell was immunostained with vimentin (stromal cell specific) and cytokeratin (epithelial cell specific) as described before (27).

**Western blotting**

Procedures used for Western blotting was as described previously (28). Dilutions of antibodies were: DUSP2, 1:200; phosphorylated (p)-ERK1/2, 1:2000; total (t)-ERK1/2, 1:2000. The DUSP2 antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA; catalog sc-32776). The p-ERK1/2 and t-ERK1/2 antibodies were purchased from Cell Signaling Technology (Beverly, MA; catalog no. 9101S and no. 4696, respectively). Band intensity was quantified by AlphaImager software (Alpha Innotech Corp, San Leandro, CA) and normalized against β-actin (for DUSP2) or t-ERK (for p-ERK).
Pre-miRNA and anti-miRNA inhibitor treatment

Pre-miR-20a (PM10057), negative control pre-miR (AM17110), anti-miR-20a inhibitor (AM10057), and anti-miR inhibitor negative control (AM17010) were purchased from Applied Biosystems (Foster City, CA). A final concentration of 100 nm pre-miR or anti-miR inhibitor was transfected by Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and incubated for 48 h. After incubation, cells were collected for subsequent experimental analysis.

Quantitative RT-PCR (RT-qPCR) and miRNA quantification

Total RNA (500 ng) was subjected to RT-qPCR. Transcripts were quantified by an ABI StepOne Plus thermocycler (Applied Biosystems). Each reaction contained 50 ng of reverse transcriptase products, 0.3 μl of specific primers, and 10 μl of SYBR Green mix. miRNA were quantified by TaqMan miRNA assay kits (catalog no. 4427975) according to the manufacturer’s instructions (Applied Biosystems). Total RNA (100 ng) was subjected to a reverse transcriptase reaction with primers specific for the mature miRNA. Transcripts were quantified by TaqMan assay-based real-time PCR.

Identification of hypoxia response element (HRE) and promoter activity assays

Human miR20a promoter sequence was analyzed using an in-house bioinformatic platform, The BEST (http://www.binfo.ncku.edu.tw/thebest/), to predict HIF-1 binding sites (Supplemental Fig. 1, published on The Endocrine Society’s Journals Online web site at http://jcem.endojournals.org). Sequence homology across several mammalian species was analyzed by multiple sequence alignment. A fragment of human miR20a 5' flanking region (~1452 to +133 bp) and serial deleted constructs were cloned into a pGL3-basic vector containing the luciferase reporter system. Site-directed mutagenesis of putative HRE constructs was generated using PCR amplification. A commercial plasmid containing a cytomegalovirus-driven β-galactosidase (β-gal) reporter system (Promega, Madison, WI) was used as an internal control as described before (29). Cells were plated in 24-well plates for the luciferase/β-gal assays. Plasmids were transfected using Lipofectamine 2000 (Invitrogen) for 6 h. After the medium was changed, cells were allowed to recover for 16 h and then exposed to hypoxic conditions in 10% DMEM/F12 for 24 h. Luciferase assays were performed using the dual-luciferase reporter assay system according to the manufacturer’s instructions (Promega). Each luciferase assay was performed in triplicate.

Gene reporter assay

A 645-bp fragment of 3'UTR of DUSP2 was amplified and cloned into a pBS-2 vector containing the luciferase reporter system. Site-directed mutagenesis of the miR20a recognition site construct was then generated using PCR amplification. A commercial plasmid containing a cytomegalovirus-driven β-gal reporter system was used as an internal control. Cells were plated in 24-well plates for the luciferase/β-gal assays. Plasmid transfection and luciferase activity assays were similar to that described in the previous paragraph. Each luciferase assay was performed in triplicate.

Chromatin immunoprecipitation-PCR assay

Protein (HIF-1α) and DNA from cells exposed to normoxia or hypoxia (1 × 10⁶ cells) were cross-linked by incubation for 10 min at 37°C with a final concentration of 1% formaldehyde. The chromatin immunoprecipitation assay was performed as previously described (28). DNA was released from the protein-DNA complex and subjected to 30 cycles of PCR amplification using a specific primer pair (Supplemental Table 1).

Statistical analysis

The data are expressed as mean ± SEM. A two-tailed Student’s t test was used to compare differences between two groups. Differences between groups were analyzed using one-way ANOVA followed by Bonferroni correction as posttest analysis using commercial statistical software (GraphPad Prism 4.02; GraphPad Software, San Diego, CA). Statistical significance was set at P < 0.05.

Results

miR20a down-regulated DUSP2 expression

Previous data demonstrated that expression of DUSP2 is down-regulated in ectopic endometriotic tissues and is driven by hypoxia (21). However, close examination revealed that HIF-1α-dependent inhibition of DUSP2 promoter activity accounts for only partial repression of the total mRNA and protein levels. Therefore, we aimed to investigate whether miRNA are involved in DUSP2 down-regulation. Using computational analyses (TargetScan; Whitehead Institute for Biomedical Research, http://www.targetscan.org, and The BEST, National Cheng Kung University Bioinformatics Center), miR20a was predicted to be a regulatory miRNA for DUSP2 and to contain a hypoxia response element in its promoter region. The 3'UTR seed sequence of DUSP2 targeted by miR20a was conserved across several mammalian species (Fig. 1A). To test whether miR20a can target DUSP2, the 3’UTR of DUSP2 that contains a miR20a recognition site was cloned into the pI-S-2 control vector creating a luciferase reporter system (Fig. 1B). Cotransfection of the DUSP2 3’UTR construct and the pre-miR20a into normal endometrial stromal cells yielded a lower relative luciferase activity as compared with that cotransfected with DUSP2 3’UTR and a control miRNA. In contrast, luciferase activity of a construct carrying mutated bases in the miR20a recognition sequence of DUSP2 3’UTR was not inhibited by cotransfection with the pre-miR20a (Fig. 1B).

Next, we examined whether expression of endogenous DUSP2 protein was affected by miR20a. Transfection of pre-miR20a into endometrial stromal cells reduced DUSP2 protein level (Fig. 1C). In contrast, transfection of anti-miR20a inhibitor into ectopic endometriotic stromal cells increased the DUSP2 protein level (Fig. 1D). Taken together, these data demonstrate that DUSP2 is a direct target of miR20a.
Up-regulation of miR20a in ectopic endometriotic stromal cells

We next determined the expression profile of miR20a in normal endometrial and endometriotic tissues. As shown in Fig. 2A, the level of miR20a was significantly up-regulated in endometriotic tissues. We also collected another 12 sets of paired eutopic endometrial tissues and ectopic endometriotic tissues from endometriosis patients and compared the expression level of miR20a. Levels of miR20a were greater in ectopic endometriotic tissues compared with eutopic endometria (Fig. 2B). Further analysis revealed that levels of miR20a were inversely correlated with levels of DUSP2 and positively correlated with levels of p-ERK (Fig. 2C). Because the endometrial tissues contain several types of cells and reduction of DUSP2 was observed only in stromal cells (21), we then compared the level of miR20a in purified endometrial and endometriotic stromal cells. As shown in Fig. 2D, levels of miR20a were up-regulated in ectopic endometriotic stromal cells compared with the eutopic endometrial counterparts.

Expression of miR20a was up-regulated by HIF-1α

To investigate the underlying mechanism of aberrant expression of miR20a in endometriotic stromal cells, normal endometrial stromal cells were cultured under hypoxia or normoxia for 24 h, and levels of miR20a were quantified by RT-qPCR. Expression of miR20a was significantly increased in normal stromal cells cultured under hypoxia (Fig. 3A). The induction of miR20a by hypoxia was completely suppressed when HIF-1α was knocked down by small interfering RNA (Fig. 3B). Furthermore, forced expression of the oxygen-insensitive HIF-1α (HIF-1α-P420A/P564A) was able to induce miR20a expression under the normoxia condition (Fig. 3C).

HIF-1α transcriptionally up-regulates miR20a expression

To address the question of how HIF-1α induces miR20a expression, the promoter region of miR20a was cloned into the promoterless luciferase pGL3 vector. Luciferase activity was measured after transient transfection into endometrial stromal cells and exposure to normoxia or hypoxia for 24 h. As shown in Fig. 3D, exposure of normal stromal cells to hypoxia significantly induced miR20a promoter activity under the normoxia condition (Fig. 3C).

Expression of miR20a induces ERK phosphorylation via negatively regulating DUSP2 expression

We then aimed to address whether up-regulation of miR20a contributes to aberrant phosphorylation of ERK in ectopic endometriotic stromal cells. Pre-miR20a was
transiently transfected into normal endometrial stromal cells, and phosphorylation of ERK was detected by Western blot. As shown in Fig. 4A, transfection of pre-miR20a increased ERK phosphorylation in normal endometrial stromal cells, which is mirrored by the reduction of DUSP2. Forced expression of DUSP2 cDNA, which is not a target of miR20a, reversed the miR20a-mediated ERK phosphorylation (Fig. 4B). In contrast, transfection of anti-miR20a inhibitor into ectopic endometriotic stromal cells, which normally have a high level of phosphorylated ERK, induced DUSP2 expression and attenuated ERK phosphorylation (Fig. 4C). Taken together, these data demonstrate that levels of miR20a positively regulate ERK phosphorylation via inhibiting DUSP2 expression.

Hypoxia-induced miR20a expression results in up-regulation of ERK downstream target genes

To investigate the biological significance of miR20a up-regulation in endometriosis, we tested whether expression of several ERK target genes including early growth response protein-1 (EGR-1), cysteine-rich angiogenic inducer 61 (CYR61), and osteopontin, are regulated by miR20a. As shown in Fig. 5A, levels of EGR-1, CYR61, and osteopontin mRNA are up-regulated in normal endometrial stromal cells transfected with pre-miR20a. In contrast, levels of EGR-1, CYR61, and osteopontin mRNA were down-regulated in endometriotic stromal cells transfected with anti-miR20a (Fig. 5B). Furthermore, hypoxia-induced EGR-1, CYR61, and osteopontin expression was mediated by miR20a, as evidenced by the transfection of anti-miR20a suppressing the hypoxia-induced increase of these three mRNA (Fig. 5C). Consistent with these findings, levels of EGR-1, CYR61, and osteopontin were elevated in ectopic endometriotic stromal cells (Fig. 5D), in which miR20a are also elevated (Fig. 2C).

Discussion

Although the etiology of endometriosis remains largely unknown, emerging evidence suggests that dysregulation of miRNA may play a role in the pathological processes of endometriosis. However, the molecular mechanisms of how a particular miRNA is regulated and how it influences the development of endometriosis are not well characterized. Herein we provide evidence to show the regulation and function of miR20a in endometriosis. miR20a is overexpressed in endometriotic stromal cells and is transcriptionally induced by HIF-1α. Elevation of miR20a causes a reduction of its target protein, DUSP2, which results in aberrant ERK activation, ERK-regulated gene overexpression, and PGE2-induced FGF9 expression (Fig 6B).
cause these genes play important roles in angiogenesis and endometrial stromal cell proliferation, our data provide the first evidence to functionally link miRNA to endometriosis development and point to a new direction of endometriosis research.

miRNA are potent negative regulators that destabilize mRNA and/or repress protein translation of their target genes. It has been suggested that miRNA are involved in controlling gene expression during the development of endometriosis. The expression of miRNA in

FIG. 3. Hypoxia-induced miR20a up-regulation is mediated by HIF-1α-dependent transcriptional up-regulation. A, Eutopic stromal cells were incubated under normoxia (Nor) or hypoxia (hypo) for 24 h and levels of miR-20a were quantified by RT-qPCR. Data show means and SEM from five independent experiments using different batches of cells. B, Eutopic stromal cells were transfected with scrambled (Scr) or two different small interfering RNA targeting HIF-1α and cultured under normoxia or hypoxia for 24 h. Data show results from four independent experiments using different batches of cells. C, Plasmids encoding oxygen-resistant HIF-1α (HIF-1α-P420A/P564A, HIF-1α-DM) or green fluorescent protein (GFP) only were transfected into eutopic stromal cells for 24 h. Levels of miR-20a were quantified by RT-qPCR. Data show means and SEM from four independent experiments using different batches of primary culture cells. Asterisks indicate significant difference at $P < 0.05$. D, Schematic drawing of serial deleted promoter of human miR20a (left panel) and data of promoter activity from three independent experiments using different batches of cells (right panel). Numbers above the drawing indicate different HRE. Asterisks indicate significant difference from normoxia treatment at $P < 0.05$. E, Activities of wild-type (WT) and HRE core sequence-mutated promoter construct from three independent experiments using different batches of cells. Constructs containing $-470/+133$ of miR20a promoter were used for site-directed mutagenesis. Asterisks indicate significant difference from normoxia treatment at $P < 0.05$. F, Schematic drawing of partial human miR20a promoter (upper panel). Primers were indicated by arrows. Lower panel, Representative gel pictures of chromatin immunoprecipitation-PCR results show enriched binding of HIF-1α to miR20a promoter under hypoxia. This experiment was repeated three times using different batches of cells. N, Normoxia; H, hypoxia.

FIG. 4. The level of p-ERK was up-regulated by miR-20a overexpression. A, Representative Western blots show levels of total ERK, p-ERK, and DUSP2 in nuclear extracts purified from eutopic stromal cells transfected with scrambled (Scr) or pre-miR20a (left panel). Right panel shows data quantitated from four independent experiments using different batches of cells. Asterisks indicate significant difference from control at $P < 0.05$. B, Representative Western blots show levels of total ERK and p-ERK in nuclear extracts purified from eutopic stromal cells transfected with scrambled or pre-miR20a in combination with empty vector (EV) or DUSP2. C, Representative Western blots show levels of total ERK, p-ERK, and DUSP2 in nuclear extracts purified from ectopic endometriotic stromal cells transfected with scrambled (Scr) or anti-miR20a inhibitor (left panel). Right panel shows data quantitated from three independent experiments using different batches of cells. Asterisks indicate significant difference from control at $P < 0.05$. 


the normal endometrium and in endometriotic tissues has been examined by several groups (23, 25, 26). However, the expression profiles of miRNA reported vary among studies, and most of them are inconsistent. For example, a differential expression of miR20a in the endometrium and in endometriotic tissue has been reported previously with conflicting results. Ohlsson Teague et al. (26) and Filigheddu et al. (25) showed a down-regulation of miR20a in endometriotic cells using a microarray-based screening method, whereas Pan et al. (23) did not see any difference in their microarray data set but demonstrated an up-regulation of miR20a by RT-qPCR. Ramon et al. (24) found that miR20a was down-regulated in ovarian endometrioma, showed no difference in pelvic adhesions, and was up-regulated in rectovaginal septum compared with the normal endometrium. The reason for this discrepancy might be due to the fact that array-based, large-scale screening of miRNA expression profiles, although effective, usually lacks the sensitivity and specificity to identify truly differentially expressed miRNA. Alternatively, it might be due to the use of whole-tissue RNA instead of using purified single-cell types in those studies. Different proportions of cell types in whole-tissue RNA preparations may result in different quantitative results. In this study, by thorough examination of endometrial and endometriotic tissues, we found that levels of miR20a were significantly up-regulated in both unpaired and paired endometriotic cells by RT-qPCR. Furthermore, cell type-specific expression was also assessed using purified stromal cells of endometrial and endometriotic origin. Results demonstrated that levels of miR20a were elevated in stromal cells derived from endometriotic tissues. To the best of our knowledge, this is the first report to show differential expression of miR20a in a cell type-specific manner in endometriotic tissues.

DUSP2 is a nuclear phosphatase that targets primarily phosphorylated ERK. Reduced expression of DUSP2 in endometriotic stromal cells leads to prolonged phosphorylation of ERK (21). Previously we showed that expression of DUSP2 was suppressed by HIF-1α/H9251 in endometriotic stromal cells (21). Because the reduction of DUSP2 by HIF-1α at the mRNA and protein levels was more pronounced than transcriptional inhibition, we reasoned that expression of DUSP2 may also be controlled by post-transcriptional regulation. Herein we provide compelling evidence to demonstrate that DUSP2 is a direct target of miR20a. Our data also demonstrate that miR20a is transcriptionally induced by HIF-1α. Taking our previous and current data together, we demonstrate that hypoxia can inhibit DUSP2 gene expression at both the transcriptional and posttranscriptional levels.

Hypoxia is an important factor that regulates numerous physiological and pathological processes. Surprisingly, its role in endometriosis has not so far been extensively studied. We have previously reported that the HIF-1α level is elevated in endometriotic stromal cells and that this induces the expression of leptin (30). Aberrant expression of leptin stimulates endometriotic stromal
cell proliferation in an autocrine fashion. Becker et al. (31) also reported that HIF-1α/H9251 stimulates vascular endothelial growth factor expression and angiogenesis in a mouse model of endometriosis. Recently we identified that elevation of HIF-1α/H9251 leads to the down-regulation of DUSP2, which ultimately results in prolonged ERK phosphorylation (21). In this study, we demonstrated that hypoxia up-regulates miR20a expression in endometrial stromal cells. Elevation of miR20a inhibited DUSP2 protein expression, leading to prolonged ERK phosphorylation and overexpression of ERK-target genes in endometriotic stromal cells. Taken together, our data demonstrate that elevation of HIF-1α-induced miR20a in endometriotic stromal cells represents a critical factor for DUSP2 down-regulation and consequently aberrant ERK phosphorylation.

Aberrant activation of ERK activity has been implicated in many pathological processes of endometriosis. One of the most novel findings in this study is that hypoxia induces miR20a expression to exert another layer of DUSP2 protein down-regulation and ERK activity up-regulation. The end point of this double-dose effect is to provide an ERK-activating cellular environment to facilitate the expression of angiogenic and cell-proliferating factors to secure the survival of hypoxic endometriotic cells. This finding suggests that the hypoxia induced angiogenesis in endometriotic tissue may be mediated via a miR20a-DUSP2-dependent pathway in addition to the well-characterized vascular endothelial growth factor signaling. Another example is the induction of FGF9, a potent mitogen for endothelial and endometrial stromal cells, by PGE2. Our previous report demonstrated that PGE2, via binding to the endogenous PGE2 receptor (EP3 subtype), induces FGF9 expression in an ERK-dependent manner (18). Because the up-regulation of miR20a leads to prolonged activation of ERK, we reasoned that this may augment PGE2 action in FGF9 up-regulation. Indeed, overexpression of miR20a resulted in increased PGE2-induced FGF9 up-regulation, suggesting that miR20a exerts a permissive effect in FGF9 overexpression. Because hypoxia is the first and most consistent stress that ectopic endometriotic cells will face, we hypothesize that it might be the critical factor in controlling endometriosis development. Further investigation in hypoxia-mediated pathological processes is an important issue in understanding the molecular mechanisms responsible for endometriosis pathology.

In conclusion, by using bioinformatics and molecular biology approaches, we show that hypoxia induces miR20a expression to exert another layer of DUSP2 protein down-regulation and ERK activity up-regulation. The end point of this double-dose effect is to provide an ERK-activating cellular environment to facilitate the expression of angiogenic and cell-proliferating factors to secure the survival of hypoxic endometriotic cells. Understanding this mechanism prompts us to reconsider that endometriosis is not just an endocrine disorder disease. The nature of endometrial cells striving to survive under stress, such as a lack of oxygen support, may need to be taken into account when characterizing the pathogenesis of endometriosis.

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