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MicroRNAs and Endometriosis: Distinguishing Drivers from Passengers in Disease Pathogenesis

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Abstract

Endometriosis is a disease common in women of reproductive age, characterized by pelvic pain and infertility. Despite its prevalence, the factors and mechanisms which contribute to the development and survival of ectopic lesions remain uncertain. MicroRNAs (miRNAs) are small RNA molecules that regulate posttranscriptional gene regulation which have been proposed to contribute to the pathogenesis of many diseases including that of endometriosis. This review summarizes the results of initial studies describing differentially expressed miRNAs between endometriotic lesion tissue and eutopic endometrium. Focus then moves toward discussion of studies on examining function of differentially expressed miRNAs to determine if they play a permissive role (driver of the disease) in events conducive to endometriosis progression/survival. Included in this discussion are the potential targets of these miRNAs and how their mis-expression may contribute to the disease. Limitations and challenges faced in studying miRNAs and endometriosis pathogenesis and recommendations to overcome these hurdles are presented at the end.

Keywords

endometriosis; pathogenesis; miRNA

Endometriosis Etiology and Pathogenesis

Endometriosis is a debilitating disease that affects as many as 10 to 15% of all women of reproductive age and as many as 40 to 50% of all women with infertility. The disease occurs in women of reproductive age (i.e., menstruating females) and is characterized by the major complaints of pelvic pain, dysmenorrhea, and infertility. Endometriosis is defined as the presence of both ectopic endometrial stromal and glandular tissue. This disease is thought to develop via reverse menstruation of viable endometrial tissue into the peritoneal cavity.

However, because almost all women of reproductive age exhibit some degree of retrograde menstruation,^{1,2} it is postulated that some other factors must contribute to the development and progression of the disease. Over the course of the past 30 years, additional factors which

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may predispose this subset of women to developing endometriosis have been proposed. Alterations in the immune system, eutopic endometrium, and stem cell population have also been demonstrated to be altered in women with endometriosis.^{3–5} While these alterations may contribute to the development and/or progression of endometriosis, a limiting factor is the difficulty in assessing cause and effect. This holds true for examining the potential role of any factor, including mRNAs, proteins, or microRNAs. Once a factor is identified as “mis-expressed” in endometriotic tissue, studies must be conducted to determine if this mis-expression is a cause for (driver of the disease) or a result of endometriosis (passenger).

MicroRNA Biogenesis and Function

MicroRNA (MiRNAs) are a class of small noncoding regulatory RNAs that posttranscriptionally regulate gene expression.^{6,7} The genes which encode miRNAs reside primarily between genes (intergenic) or within introns (intronic) of genes. MiRNAs also make up the entire introns as well as reside with exons,^{8,9} but this occurrence is less common compared with intergenic or intronic miRNAs. MiRNA transcription is mediated by polymerase II or III as the initial long primary RNA transcript (pri-miRNA) is capped (MGpppG) and polyadenylated.¹⁰ Pri-miRNA transcript then binds with the RNA-binding protein DGCR8 (DiGeorge syndrome critical region 8) and the pri-miRNA–DGCR8 complex is then cleaved by Drosha (an RNase III enzyme), yielding a stem-loop precursor miRNA (pre-miRNA). The pre-miRNA is then exported from the nucleus via exportin 5 and RAN-GTP into the cytoplasm. Once in the cytoplasm, a second RNase III enzyme, DICER, cleaves the pre-miRNA into a transient RNA duplex of approximately 18 to 25 nucleotides. Processing of all miRNAs, except miR-451,^{11,12} is thought to be DICER dependent.

Once processed, the miRNA duplex is cleaved, generating two strands. One strand is degraded while the remaining strand is preferentially loaded onto the RNA-induced silencing complex (RISC). The RISC is composed of DICER, a TAR RNA-binding protein (referred to as TRBP) and one of the four different Argonaute (Argo) proteins.¹³ Once within the RISC, miRNAs bind to the 3′-untranslated region (3′-UTR) of the target mRNA transcript. If the binding of the miRNA to the 3′-UTR “seed sequence” exhibits perfect base-pairing, the mRNA transcript is degraded and mRNA translation does not occur.¹⁴ If base pair binding homology between miRNA and 3′-UTR of the mRNA is imperfect, mRNA translation is inhibited. This most likely occurs via an inhibition of translation after initiation¹⁴ or through an RISC interaction with eukaryotic translation initiation factor 6 (EIF6) preventing assembly of 80S ribosomes.¹⁵ More recently, miRNA regulation of transcript stability and protein translation has also been proposed to be mediated at the level of the 5′-UTR^{16–18} as well as the DNA-coding sequence.^{17,18}

While the majority of the literature supports the notion that miRNAs inhibit translation, there is some evidence that miRNAs can actually enhance translation through alterations in the Argo component of the RISC.⁷ Thus, while miRNAs appear to primarily regulate translation in an inhibitory manner, they may also enhance translation in certain biological scenarios.

miRNAs are Mis-expressed in Endometriotic Lesion Tissue

MiRNAs are postulated to play a role in normal biological processes while their mis-expression has been associated with numerous diseases.^{19–21} The study of miRNAs and endometriosis began nearly 10 years ago with the generation and comparisons of miRNA expression profiles between eutopic endometrium and endometriotic lesion tissue. One of the first assessments of miRNA expression comparing paired eutopic and ectopic endometrial tissue was conducted in 2009.²² Fourteen upregulated and eight downregulated miRNAs specific to endometriotic lesion were identified by microarray analysis (►Table 1). In silico analysis identified 3,851 mRNA transcripts as putative targets of these 22 miRNAs. Of these predicted targets, 673 were also differentially expressed in ectopic compared with eutopic endometrial tissue, as determined by microarray. Using Ingenuity Pathway Analysis (IPA) software, potential molecular pathways were assessed and determined to involve c-Jun, CREB-binding protein, protein kinase B (AKT), and cyclin D1 (CCND1) signaling, all of which have previously been associated with endometriosis pathogenesis.

Filigheddu and coworkers²³ conducted a similar profiling experiment and compared miRNA expression between ectopic and eutopic endometrium using tissue obtained during the proliferative stage of the menstrual cycle. Fifty miRNAs were considered differentially expressed between the two tissue types (►Table 1). Again, the mis-expression of these miRNAs was postulated to allow for dysregulation of several pathways previously proposed to contribute to the pathophysiology of endometriosis, including cytokines, enzymes, growth factors, receptors, and transcription regulators. In both of these studies,^{22,23} six common miRNAs (*miR-145*, *miR-143*, *miR-99a*, *miR-126*, *miR-100*, and *miR-29c*) were reported as upregulated. However, there were conflicting reports on whether or not their expression was influenced by the stage of the menstrual cycle.^{22,23}

The first transcriptome-miRNA analysis of endometriotic endometriomas (endometriotic cysts of the ovary) was conducted by Hawkins and colleagues in 2011.²⁴ MiRNAs expressed at the highest level in endometriomas and the corresponding abundance of transcript in non-endometriosis control endometrium was determined (►Table 1). Many of the miRNAs previously reported^{22,23} were again identified by Hawkins and colleagues.²⁴ This 2011 study²⁴ was the only one of the first three studies at the time to analyze the potential function of a differentially expressed miRNA. *miR-29c*, which exhibited the highest expression differential between tissue types, was evaluated using primary human endometrial stromal cells in vitro.²⁴ Putative extracellular matrix (ECM) protein gene targets of *miR-29c* (COL7A1, UPK1B, and TFAP2C) were downregulated in cells overexpressing *miR-29c* and the direct effect on the 3'-UTR of the genes was confirmed. Thus, mis-expression of *miR-29c* in endometriomas appears to functionally contribute to the aberrant expression of ECM proteins associated with the disease at least in isolated endometrial stromal cell culture.

More recently, Long et al²⁵ also investigated the expression of the *miR-29* family in the endometrium samples from women without endometriosis, as well as in paired ectopic and eutopic endometrium samples. As opposed to earlier studies,^{22–24} these authors observed a decrease in lesion expression of *miR-29c*. The investigators further examined the role of

miR-29c in endometrial cell proliferation, invasion, and apoptosis in vitro. *miR-29c* suppressed endometrial cell proliferation and invasion, promoting cell apoptosis. The authors concluded that *miR-29c* exhibits inhibitory action on endometrial cell proliferation and invasion by inhibiting the expression of c-Jun. It should be emphasized that while these investigators demonstrated a decrease in *miR-29c* expression in endometriotic lesion tissue which would suggest enhanced cell proliferation and invasion due to low *miR-29c* expression, all of the earlier reports demonstrated an increase in *miR-29c* expression.^{22–24}

Since these initial reports, additional studies have focused on differentially expressed miRNA between paired endometriotic lesion and eutopic endometrium. More recently, Shi and colleagues²⁶ profiled differentially expressed miRNAs in the normal, eutopic, and ectopic endometrium using miRNA microarray screening analysis (►Table 1). Of those differentially expressed, *miR-183-5p* was found to be downregulated in the ectopic and eutopic tissues from women with endometriosis compared with eutopic endometrium from women free of disease. Functional analysis indicated that *miR-183-5p* may contribute to endometrial stromal cell apoptosis (but had no influence on cell proliferation) and exhibits a negative regulatory impact on the invasive ability of these cells. Stromal cell *miR-183* expression was also examined in response to ovarian steroids (17 β -estradiol and progesterone) and inflammatory cytokines (tumor necrosis factor- α and interleukin-6). Administration of all these reagents decreased *miR-183* expression. It was concluded from this study that repressed levels of *miR-183* may modulate the growth and invasive potential of endometriotic endometrial stromal cells contributing to the development and progression of endometriosis.

Yang and colleagues²⁷ used miRNA microarrays to identify differentially expressed miRNAs between endometriotic lesion tissues from women with stage III/IV disease compared with eutopic endometrium from women without endometriosis. Lesion tissue expressed significantly higher levels of expression of seven miRNAs and significantly lower levels of expression of ten miRNAs (►Table 1). Of these miRNAs, two of the upregulated miRNAs (*miR-106-5p* and *miR-16-5p*) and six of the downregulated miRNAs (*miR-126*, *miR-146a-5p*, *miR-15a-5p*, *miR-19b-1-5p*, *miR-200b*, and *miR-200c*) were predicted to target key regulators of angiogenesis. Expression of VEGFA was significantly upregulated, whereas EGFR2, PTEN, and CXCR4 were markedly down-regulated in lesion tissue compared with endometrium from women without endometriosis. The authors concluded that the differentially expressed miRNAs could modulate VEGFA, EGFR2, PTEN, and/or CXCR4 expression and contribute to the pathogenesis of endometriosis.

Putative Targets of, and Functions for, Specific miRNAs in Endometriosis Pathogenesis

From the initial generation of miRNA profiles, later investigations began to focus on specific miRNAs and putative targets. The potential miRNA targets may be relevant to the pathophysiology of endometriosis and may aid in determining whether these miRNAs function as drivers of the disease. Ramón and colleagues²⁸ assessed the expression of miRNAs proposed to play a role in angiogenesis. In this study, the investigators correlated

the expression of *miR-15b*, *miR-16*, *miR-17-5p*, *miR-20a*, *miR-21*, *miR-125a*, *miR-221*, and *miR-222* with that of specific angiogenesis factors, vascular endothelial growth factor-A (VEGF-A), and thrombospondin-I (TSP-I). When analyzing paired specimens, ovarian endometriomas exhibited significantly lower levels of proangiogenic VEGF-A mRNA and protein and higher levels of *miR-125a* and *miR-222* compared with corresponding eutopic endometrium. In contrast, levels of the angiogenesis inhibitor (TSP-I) were significantly higher in endometriomas and this was associated with reduced levels of *miR-17-5p*. Significant inverse correlations were noted between *miR-222* and VEGF-A protein expression and *miR-17-5p* and TSP-I protein levels. These correlations suggest that the mis-expression of these miRNAs may at least in part contribute to the observed altered expression of the angiogenic factors and thus contribute to the pathogenesis of the disease.

One of the miRNAs evaluated by Ramón and colleagues²⁸ was also examined by Zhao and coworkers.²⁹ *miR-20a* expression was assessed in a total of 40 patients with ovarian endometriosis and 20 controls with benign ovarian tumors. qRT-PCR assessment revealed significantly increased *miR-20a* expression in ovarian endometriosis as compared with control ovarian cyst tissue. This increase in *miR-20a* expression is in contrast to the decrease detected by Ramón and colleagues²⁸ as well as earlier assessments^{22,23} which reported lower *miR-20a* expression in lesion tissue. However, the reason for these discrepancies was not discussed. Zhao and colleagues determined the pathways potentially targeted by *miR-20a*, and the cell cycle pathway was identified as one of the most relevant. Assessment of predicted *miR-20a* target gene, netrin-4 (NTN4), revealed a significant decrease in patients with ovarian endometriosis. From this study, it was concluded that the increased expression of *miR-20a* may play an important role in the pathogenesis of ovarian endometriosis by suppressing NTN4 expression.

In addition to the earlier-mentioned miRNAs, several miRNAs have been further examined in endometriotic lesion tissue and/or cells. *miR-199a* is downregulated in several types of cancers,^{30,31} but had yet to be reported in endometriotic tissue. Considering that the pathophysiology of endometriosis shares similar characteristics with cancer, Dai and coworkers³² evaluated *miR-199a* expression in matched ovarian endometriomas and eutopic endometrium as well as endometrium from women free of endometriosis. Compared with eutopic endometrium from women without endometriosis, *miR-199a* expression was lower in both the eutopic endometrium from women with endometriosis and ovarian endometriomas. Forced expression of *miR-199a* in endometrial stromal cells resulted in dampened cell adhesion, invasion, and migration. These cellular phenotypes were associated with suppression of the IKK/NFκB pathway and reduced interleukin-8 expression, suggesting a possible functional role for the mis-expressed levels of *miR-199a* in the pathogenesis of endometriosis.

More recently, the above group investigated the role of *miR-199a* in the angiogenic potential of endometrial stromal cells under hypoxic conditions.³³ Forced overexpression of *miR-199a* significantly attenuated its angiogenic potential under hypoxia and was also associated with a reduction in VEGF-A expression in these cells. Reporter gene assays targeting the 3'-UTR of VEGF-A and hypoxia-inducible factor (HIF)-1α were incorporated to demonstrate that *miR-199a* downregulation was mediated at the level of the 3' – UTR of

these target genes. These findings may suggest that *miR-199a* may attenuate the angiogenic potential of stromal cells under hypoxia partly through HIF-1 α /VEGF-A pathway suppression, thereby contributing to the pathogenesis of endometriosis.

miR-126 is also a proposed regulator of angiogenesis as well as growth, adhesion, and invasion.^{34,35} Earlier reports^{22,23} demonstrated increased expression of this miRNA in lesion tissue. Liu and colleagues,³⁶ in contrast, reported that *miR-126* expression was significantly reduced in ovarian endometriomas and eutopic endometrium from women with endometriosis compared with eutopic endometrium from women free from the disease. Associated with this reduction in *miR-126* was a significant increase in v-crk sarcoma virus CT10 oncogene homolog (CRK) mRNA and protein as well as a highly significant association between endometrioma and eutopic endometrial *miR-126* expression. More severe forms of endometriosis were associated with lower levels of *miR-126* expression in these tissues. Unfortunately, the ability of *miR-126* to modulate cellular events conducive to endometriosis pathogenesis, such as cellular proliferation, adhesion, and/or invasion, was not evaluated in this study. It should also be noted that a more recent array analysis²⁷ observed decreased levels of ectopic endometrial tissue expression of *miR-126* which is in agreement with the study by Liu and coworkers,³⁶ but in contrast to earlier array analysis reports.^{22,23}

There is considerable evidence that steroidogenic factor-1 (SF-1) expression is increased in stromal cells from endometriotic tissue and this may be due to multiple mechanisms.^{37–39} *miR-23a/b* expression is reduced in endometriotic and eutopic endometrium from women with endometriosis. As *miR-23a/b* is proposed to target *SF-1*, the potential mechanistic link between *miR-23a/b* and SF-1 expression in the pathogenesis of endometriosis was examined.⁴⁰ Reduced levels of *miR-23a/b* expression was confirmed in ectopic endometriotic tissue and eutopic endometrium from women with endometriosis compared with eutopic endometrium from women free of endometriosis and this reduction was associated with elevated transcript levels of *NR5A1* (SF-1), *STAR*, and *CYP19A1*. To confirm that *miR-23a/b* directly regulated *NR5A1* (*SF-1*) expression, luciferase reporter assays were conducted. Despite the fact that transfection with *miR-23a/b* precursor suppressed *NR5A1* (*SF-1*) transcript, *miR-23a/b* did not bind to the 3'-UTR for *NR5A1* (*SF-1*), suggesting that *miR-23a/b* mediation of SF-1 expression is via an indirect mechanism.

miR-145 expression has been shown to be mis-expressed in endometriotic tissue compared with eutopic endometrium.^{22,23,41,42} Coupled with the fact that *miR-145* putatively targets factors involved in cellular events⁴¹ conducive to endometriosis survival/progression, Adammek and colleagues⁴³ examined the expression and potential function of *miR-145* in the pathogenesis of endometriosis. Epithelial endometriotic 12Z cells were transfected with *miR-145* which resulted in reduced cell proliferation and invasion, while *miR-145* reduced cellular proliferation in transfected primary ectopic and eutopic endometrial stromal cells from endometriosis patients. In addition, it was discovered that *miR-145* induced posttranscriptional downregulation of targets FASCIN-1, PAI-1, and JAM-A at the transcript and protein level in 12Z cells. Direct binding of *miR-145* to the 3' – UTR of *JAM-A* was confirmed by luciferase reporter construct assays. In contrast, only FASCIN-1 was shown to be modulated by *miR-145* in primary ectopic and eutopic endometrial stromal cells from

endometriosis patients. Cytoskeleton proteins ACTG2, TAGLN, and MYL9 were shown to be differentially regulated by *miR-145* with overexpression of *miR-145* leading to decreased *ACTG2* transcript expression but increased *TAGLN* transcript expression, while *MYL9* expression was unaffected. Similarly, *miR-145* overexpression in 12Z and stromal cells from both eutopic and ectopic endometrial cells from women with endometriosis resulted in decreased transcript expression of pluripotency and stemness-related markers. Modulation of protein expression or verification of direct binding via 3'-UTR reporter assays was not performed to further validate these targets. Although initial results on *miR-145* expression in different endometriosis samples are conflicting,^{22,23,42} these data suggest that *miR-145* appears to inhibit endometriotic cell proliferation and invasion as well as regulation of stem cell properties. As discussed earlier, miRNAs are proposed to regulate steroid action within the endometrium to control the cellular events necessary for "normal" endometrial function. Mis-expression of miRNAs is in turn thought to contribute to the pathogenesis of diseases of endometrial tissue origin such as endometriosis. Within the field of endometriosis research, several miRNAs have emerged as potential players in modulating the events conducive to the establishment, survival, and progression of the ectopic implant. Of these, functional data exist for only a few. To truly understand which miRNAs are important in the pathogenesis of endometriosis and how they contribute to the disease, greater effort must be put forth to standardize what constitutes true "control" groups and what constitutes "endometriotic tissue." Reported and unpublished observations strongly suggest that not only does miRNA expression vary based on the "type" of implant (ovarian endometrioma vs. peritoneal implant) among study subjects but that this expression also varies by type of implant within study subject.

Building upon their earlier observations that *miR-183* is decreased in endometriotic lesion tissue,^{23,26} Chen and colleagues incorporated microarray analysis to identify *miR-183* targets that may be relevant to cellular functions associated with endometriosis pathophysiology.⁴⁴ Twenty-seven differentially expressed genes were identified of which four of those downregulated genes (*ITGB1*, *AMIGO2*, *VAV3*, and *PSEN2*) were further assessed based on potential function and relevancy to endometriosis. Assessment of protein expression by Western blots demonstrated that integrin $\beta 1$ (*ITGB1*), but not *AMIGO2*, was affected by *miR-183* over-expression. Expression of detectable levels of neither *VAV3* nor *PSEN2* proteins was observed. Luciferase reporter assay verified that *miR-183* targeted *ITGB1* and that *ITGB1* was overexpressed in the endometrium of endometriosis patients. Forced expression of *ITGB1* could also rescue the repressive effects of *miR-183* on endometrial stromal cell invasiveness. From these studies, the authors concluded that *miR-183* may be involved in the development of endometriosis by regulating stromal cell *ITGB1* expression and function.

Building upon the initial report by Ohlsson Teague and colleagues which demonstrated decreased levels of *miR-142-3p* in endometriotic lesion tissue,²² Kästingschäfer and coworkers⁴⁵ investigated the functional effect of *miR-142-3p* expression in endometrial stroma cells in vitro. Increased expression of *miR-142-3p* via transient transfection resulted in a significant reduction in cell viability as well as reduced expression of steroid sulfatase, interleukin-6-coreceptor gp130, and interleukin-6-mediated activation of the STAT3 pathway. These data were interpreted to imply that *miR-142-3p* may modulate cell viability

and that this may involve both steroid hormone- and cytokine-mediated signaling events. While these results would be in agreement with reduced levels of *miR-142-3p*²² contributing to the survival of endometriotic lesion tissue, the reader should be aware that Yang and colleagues²⁷ observed increased levels of this miRNA in endometriotic lesion tissue.

miR-451 is one of the few miRNAs associated with endometriosis²⁴ which has been examined not only in human tissue/cells but also in animal models for the disease. Our laboratory reported the initial study examining both *miR451* expression and function in human tissue and cells.⁴⁶ *miR-451* is elevated in endometriotic lesion tissue compared with paired eutopic endometrium from women with the disease.²⁴ *miR-451* expression exhibited an inverse association with transcript and protein expression of putative target, macrophage migration inhibitory factor (MIF). Using luciferase reporter constructs for MIF, we demonstrated that *miR-451* specifically bound to the 3' – UTR to regulate its expression. Furthermore, forced expression of *miR-451* induced a significant downregulation of both MIF mRNA and protein in a human endometrial cell line which was associated with a significant reduction in cell survival.

An additional study by Joshi and colleagues⁴⁷ identified *miR-451* mis-expression in women with endometriosis as well as in baboons with induced disease. Induction of endometriosis in baboons results in altered expression of several miRNAs including *miR-451*. Reduction in eutopic endometrial *miR-451* expression was associated with increased expression of *miR-451* target, *YWHAZ*, and this was observed in both baboon and human tissue. Regulation of *YWHAZ* by *miR-451* was confirmed using 3'-UTR reporter assays in vitro. Lastly, overexpression of *miR-451* in an immortalized human endometriotic epithelial cell line (12Z) led to the decreased expression of its target *YWHAZ* which was correlated with decreased cell proliferation. Collectively, both our study⁴⁶ and that of Joshi and colleagues⁴⁷ demonstrated that *miR-451* is capable of modulating cell proliferation and this is associated with alterations in the expression of targets MIF and *YWHAZ*.

One of the major functional endpoints which have been examined with respect to miRNA function in endometrial/endometriotic cells has been angiogenesis. *miR-15a-5p* was reported to be suppressed in endometriotic lesion tissue and proposed to mediate angiogenic events associated with the disease.²⁷ In a follow-up to their initial study,²⁷ Liu and colleagues further examined *miR-15a-5p* expression in endometriotic lesion tissue as well as assessed its functional role.⁴⁸ Compared with control samples, *miR-15a-5p* was downregulated in patients with endometriosis, supporting their initial array analysis observation.²⁷ Forced expression of *miR-15a-5p* suppressed cell proliferation, migration, and invasion in endometriotic ectopic stem cells by targeting the 3'-UTR region of *VEGFA*. Thus, the suppressed levels of *miR-15a-5p* in endometriotic tissue coupled with the suppressive effects of this miRNA on cell proliferation in vitro may suggest that reduced *miR-15a-5p* levels may be conducive to lesion proliferation and survival.

Summary and Conclusion

In summary, early analysis of differentially expressed miRNAs between endometriotic tissue and eutopic endometrium has identified dozens of differentially expressed miRNAs. Subsequent studies have begun to dissect the role of these miRNAs to distinguish between drivers, those miRNAs which modulate key events conducive to the pathophysiology of endometriosis, and passengers, those miRNAs whose expression may change as a result of the disease pathogenesis. Driver miRNAs have been shown to modulate key components of biological pathways highly relevant to the pathophysiology of endometriosis including cellular proliferation, invasion and angiogenesis. While there is agreement among some studies with respect to which miRNAs are differentially expressed and the level of their expression compared with control tissue, there is also disagreement among these levels of expression. These discrepancies may be rooted in differences in the study groups, such as the types of samples that were included in their control groups as well as what “types” of endometriotic lesions were evaluated in their study groups. It is evident that for progress to be made in understanding the role of miRNAs in the pathogenesis of endometriosis, greater emphasis needs to be placed upon standardization of study designs and populations. By doing so, it will not only lead to more consistent experimental outcomes but also enhance experimental rigor and reproducibility. Nonetheless, the data that exist to date strongly suggest that miRNAs are mis-expressed in endometriotic lesion tissue where they are proposed to contribute to the pathogenesis of the disease. Future studies are expected to more clearly define the roles of these mis-expressed miRNAs in endometriosis pathophysiology by identifying the pathways and their components that are regulated by these miRNAs.

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Table 1

Over- and under-expressed endometriotic lesion miRNA identified by array or deep sequencing analysis

miRNA	Level of expression	Reference
miR-145, miR-143, miR-99a	Increased (lesion/eutopic)	22
miR-99b, miR-126, miR-100,		
miR-125b, miR-150, miR-125a,		
miR-223, miR-194, miR-365,		
miR-29c, miR-1		
miR-200a, miR-141, miR-200b	Decreased (lesion/eutopic)	22
miR-142-3p, miR-424, miR-34c,		
miR-20a, miR-196b		
miR-1, miR-100, miR-101	Increased (lesion/eutopic)	23
miR-126, miR-130a, miR-143,		
miR-145, miR-148a, miR-150,		
miR-186, miR-196b, miR-199a,		
miR-202, miR-221, miR-28,		
miR-299-5p, miR-29b, miR-29c,		
miR-30e-3p, miR-30e-5p, miR-34,		
miR-365, miR-368, miR-376,		
miR-379, miR-411, miR-493-5p,		
miR-99a		
miR-106a, miR-106b, miR-130b	Decreased (lesion/eutopic)	23
miR-132, miR-17-5p, miR-182,		
miR-183, miR-196b, miR-200a,		
miR-200b, miR-200c, miR-20a,		
miR-25, miR-425-5p, miR-486,		
miR-503, miR-638, miR-663,		
miR-671, miR-768-3p, miR-768-5p		
miR-93		
let7d, let7i, miR-202, 193a-3p	Increased (lesion/eutopic)	24
miR-29c, miR-708, miR-509-3-5p,		
miR-574-3p, miR-193a-5p, miR-451,		
miR-485-3p, miR-100, miR-720		
let7a, b, c, e, f, g, h	Decreased (lesion/eutopic)	24
miR-504, miR-141, miR-429,		
miR-203, miR-10a, miR-200a,		
miR-873, miR-200b, miR-200c,		
miR-449b, miR-375, miR-34c-5p		
miRPlus-F1038, miR-1915, miR-637	Increased (lesion/eutopic)	26

miRNA	Level of expression	Reference
miR-518e*, miR-519a*, miR-519b-5p,		
miR-519c-5p, miR-522*, miR-523*,		
miR-574-5p, miR-615-3p, miR-1909,		
miR-224*, miR-133b, miR-622,		
miR-628-3p, ebv-miR-BHRF1-2, miRPlus-F1215,		
miRPlus-F1221, miR-1470, miR-1469,		
miR-520d-5p, miR-551b*, miR-361-3p,		
miR-941, miRPlus-F1223, miR-202,		
miR-663b, miRPlus-F1042, miR-381,		
miR-412		
miR-203, miR-425, miR-183	Decreased (lesion/eutopic)	²⁶
miR-92a, miR-196b , miR-363*,		
let-7i, miRPlus-E1031, miR-200b ,		
miRPlus-F1231, miR-215, miR-362-3p,		
miR-342-3p, miR-200c , miR-93,		
miR-24-1*, miR-25 , miR-106b*		
miR-106-5p, miR-142, miR-145-5p	Increased (lesion/eutopic)	²⁷
miR-16-5p, miR-181a, miR-205,		
miR-663		
miR-126 , miR-146a-5p, miR-148a	Decreased (lesion/eutopic)	²⁷
miR-150, miR-15a-5p, miR-19b-1-5p,		
miR-200b , miR-200c , miR-423,		
miR-675		

Note: miRNAs which have been identified as mis-expressed in more than one study are indicated in bold text.