Reproductive BioMedicine Online (2013) 27, 515–529

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SYMPOSIUM: IMPLANTATION
REVIEW

Tissue and circulating microRNA influence reproductive function in endometrial disease

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Abstract microRNA (miRNA) have emerged as important epigenetic modulators of gene expression in diverse pathological and physiological processes. In the endometrium, miRNA appear to have a role in the dynamic changes associated with the menstrual cycle, in implantation and in the pathophysiology associated with reproductive disorders such as recurrent miscarriage and endometriosis. This review explores the role of miRNA in endometrial physiology and endometrial disorders of reproduction and also raises the prospect that circulating miRNA may modulate endometrial function or reflect disordered endometrial activity. The clinical potential to use miRNA in diagnostic tests of endometrial function or in the treatment of endometrial disorders will also be discussed.

KEYWORDS: endometriosis, endometrium, implantation, microRNA, miscarriage

Introduction

microRNA (miRNA) are highly conserved 9–22-nucleotide-long non-protein-encoding RNA that represent only a small fraction (~0.01%) of the total RNA mass. However, since their initial discovery in *Caenorhabditis elegans* two decades ago (Lee et al., 1993), miRNA have been found to regulate more than 30% of the human genome (Bartel, 2009). The latest version (version 19) of miRBase sequence database lists 25,141 mature miRNA sequences in 193 species, including 2216 mature human miRNA (Griffiths-Jones et al., 2008).

The endometrium is a steroid-hormone-responsive tissue that undergoes highly organized cyclic structural changes in preparation for embryo implantation during the reproductive years. Large numbers of miRNA have been identified in the endometrium in both normal and disease states,

1472-6483/$ - see front matter
http://dx.doi.org/10.1016/j.rbmo.2013.07.012

and functional studies have demonstrated miRNA regulatory pathways in this dynamic tissue. More recently, circulating miRNA have been shown to influence cells at a site distant to their origin (Valadi et al., 2007). The possibility that circulating miRNA may reflect endometrial function or dysfunction is beginning to be explored, as is the possibility that circulating miRNA that reflect endometrial disease could provide noninvasive diagnostic information.

This review focuses on the emerging roles of miRNA in healthy endometrium and their aberrant expression in endometrial tissues and blood in pathological states. The most recent studies in this rapidly emerging field are emphasized, updating the information provided in the review by Pan and Chegini (2008). Reviews outlining the role of miRNA in ovarian function, pregnancy-related pathologies, endometrial cancer and leiomyoma have been undertaken (Carletti and Christenson, 2009; Gilabert-Estelles et al., 2012; Hawkins et al., 2011a; Nothnick, 2012) and are beyond the brief of this paper.

Biogenesis and function of miRNA

Most miRNA genes are intergenic or orientated antisense to neighbouring genes and are thought to be independently transcribed by polymerase II, although some lie in intronic regions of protein-encoding genes (Lee and Ambros, 2001). In the canonical miRNA biogenesis pathway, DNA is transcribed into primary miRNA in the nucleus and then cleaved to pre-miRNA by the Drosha enzyme. Alternatively, pre-miRNA hairpins can be generated through splicing and debranching by the lariat debranching enzyme (Ldbr) in the Drosha-independent mirtron pathway (Ruby et al., 2007; Westholm and Lai, 2011) (**Figure 1**).

Pre-miRNA are exported into the cell cytoplasm, where they are further cleaved by the DICER enzyme to yield miRNA–miRNA* duplexes (reviewed by Bartel, 2004). These duplexes unwind, and the 'guide strand' (the mature miRNA) binds to Argonaute (Ago) proteins in the RNA-induced silencing complex (RISC). In mammals, targeted translational repression is effected by the RISC complex binding to the 3′-untranslated region of select messenger RNA (mRNA) (Filipowicz et al., 2008). Deactivated mRNA bound to the miRNA–RISC complex accumulate in cytoplasmic structures called P-bodies, where they are degraded or stored (Filipowicz et al., 2008). Generally, miRNA repress transcription of their targeted miRNA; however, in rare circumstances miRNA appear to be able to stimulate target mRNA translation (Vasudevan et al., 2007; **Figure 1**). There is evidence that miRNA-bound mRNA can also be released from P-bodies and become translationally active (Bhattacharyya et al., 2006). miRNA regulatory mechanisms are complex, with most miRNA able to regulate several hundred transcripts and several miRNA often regulating one mRNA target (Bartel, 2009). miRNA with a common mRNA target can be co-transcribed from a single miRNA gene cluster to amplify their repressive effect. Furthermore, miRNA and transcription factors are able to form reciprocal regulatory loops, through which gene expression is tightly controlled (Flynt and Lai, 2008).

miRNA can be transferred across the cell membrane into the systemic blood circulation, where they exist in a cell-free form. Circulating miRNA reside in microvesicles and other lipoprotein complexes, such as exosomes, microparticles and apoptotic bodies, which confer protection from endogenous RNase activity (Hunter et al., 2008; Pegtel et al., 2010; Taylor and Gercel-Taylor, 2008; Valadi et al.,

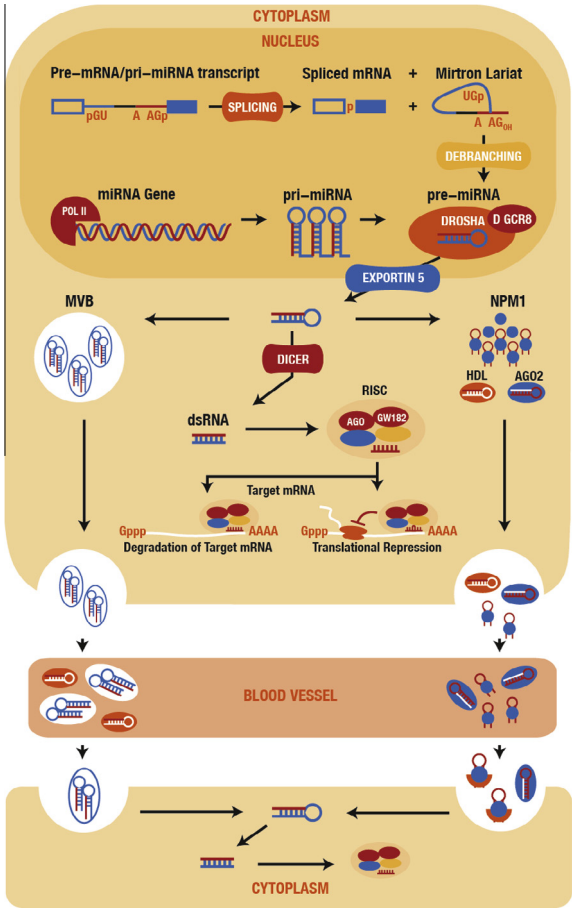

Figure 1 Biogenesis, mechanism of action and extracellular secretion of microRNA (miRNA). Pri-miRNA are transcribed by polymerase II (POL II) in the nucleus and processed by Drosha into pre-miRNA. An alternative non-canonical pathway is generated by certain debranched introns, called 'mirtrons', which undergo splicing and mimic the structural features of pre-miRNA, entering the miRNA-processing pathway without Drosha-mediated cleavage. Exportin transports pre-miRNA molecules to the cytoplasm, where DICER generates miRNA–miRNA* duplexes. These are converted into single-strand mature miRNA and incorporated into the RNA-induced silencing complex (RISC), which sequence-specifically binds to miRNA target sites on mRNA transcripts, effecting mRNA cleavage and degradation or, if the alignment if imperfect, repression of gene translation. Pre-miRNA are exported from cells via two mechanisms: (i) in multivesicular bodies (MVB), which release miRNA into the circulation via fusion with the cell membrane; and (ii) in association with RNA-binding proteins such as nucleophosmin 1 (NPM1), argonaute 2 (Ago2) or high-density lipoprotein (HDL). Circulating miRNA are taken up by the recipient cells either by endocytosis or, if protein bound, by receptor-mediated interactions at the cell surface. miRNA internalized by recipient cells can inhibit the expression of target protein-coding genes.

2007; Wang et al., 2010). Exosomes and microparticles are formed through the inward budding of the endosomal or plasma membrane around miRNA, which are later released to the circulation via fusion with the cell membrane (Kosaka et al., 2010; Pegtel et al., 2010; Wang et al., 2010). Fluxes of calcium, cytoskeleton reorganization and sphingomyelinase 2 activity (a rate-limiting enzyme in ceramide biosynthesis) control the formation and release of exosomes and microparticles from cells (Kosaka et al., 2010; **Figure 1**). The expression profile of microvesicular miRNA and released miRNA do not correlate well with that of their cells of origin (Valadi et al., 2007), signifying that specific miRNA are likely to be selected either for intracellular retention or for release by exosomes. Moreover, different stimuli alter the number, composition and origin of microparticles and modify the release of miRNA (Pula et al., 2008), supporting the assumption that packaging and extracellular transport of miRNA is a highly regulated process.

Up to 90% of extravesicular plasma and serum miRNA co-fractionate with protein and lipoprotein complexes (Cortez et al., 2011) in blood. High-density lipoprotein (Vickers et al., 2011), the Ago2 component of the RISC complex (Arroyo et al., 2011; Turchinovich et al., 2011) and nucleophosmin (NPM1; Wang et al., 2010) are some of the proteins that complex with extravesicular miRNA during their intravascular transport.

The recipient cells take up circulating miRNA by endocytosis or through binding to receptors capable of recognizing miRNA-binding proteins at the cell membrane surface. Exosomal miRNA are processed by the same machinery used in miRNA biogenesis. Like cellular miRNA, exosomal miRNA promote gene silencing (Iguchi et al., 2010; Kosaka et al., 2010) and effect widespread changes in cell physiology (Cortez et al., 2011). In a similar fashion, miRNA—lipoprotein complexes alter the expression of target genes that can induce cellular responses. For example, miRNA bound to high-density lipoproteins were demonstrated to alter the cellular miRNA pool and repress miRNA targets through activation of a ceramide signalling pathway (Vickers et al., 2011; **Figure 1**).

Endometrial expression of miRNA across the menstrual cycle

The endometrial cycle comprises reproductive hormone-dependent morphological and biochemical changes. In the proliferative phase, ovarian oestradiol induces endometrial cell proliferation, whereas secretory-phase progesterone and oestradiol induce endometrial cell differentiation, before falling concentrations of both hormones in the case of no pregnancy results in endometrial menstrual shedding. Endometrial disorders such as impaired endometrial receptivity, dysfunctional uterine bleeding and endometriosis are thought to arise from aberrations in the cellular and molecular homeostasis of the endometrium. The role of steroids in regulating functions such as the onset of receptivity is reviewed in the accompanying article by Young (2013).

One of the first human studies to assess miRNA expression in endometrial tissues compared isolated stromal and epithelial primary cell cultures from women in the early to mid-secretory phase of the menstrual cycle and identified 32 differentially expressed miRNA (Pan et al., 2007). Endometrium-specific miRNA expression appeared to be cell-type-dependent, raising the possibility of miRNA-mediated cross-talk between the endometrial cellular compartments. The differentially expressed miRNA were predicted to target genes known to play critical roles in several endometrial functions, including transforming growth factor β (TGFβ), TGFβ receptors, oestrogen receptors, progesterone receptors and aromatase (CYP19A1) (Pan et al., 2007).

Pan et al. (2007) also demonstrated that oestradiol and progesterone, alone or in combination with their antagonists (ICI-182780 and RU486, respectively), cell-specifically altered the expression of miR-20a, miR-21 and miR-26a in stromal and epithelial human endometrial primary cell cultures. The expression of miR-17-5p, miR-23a, miR-23b and miR-542-3p and of their targets, which include CYP19A1, steroidogenic acute regulatory protein and cyclo-oxygenase 2 was oestradiol dependent and central to endometrial proliferation (Toloubeydokhti et al., 2008). Oestradiol-dependent up-regulation of miR-155, miR-429 and miR-451 and down-regulation of miR-181b and miR-204 was also seen in mouse uteri (Nothnick and Healy, 2010; Nothnick et al., 2010). There is evidence that oestradiol may affect miRNA activity by binding to the Drosha complex and inhibiting the processing of pri-miRNA (Cochrane et al., 2011; Klinge, 2012; Yamagata et al., 2009).

Oestradiol alters miRNA expression in the supra-physiological hormonal milieu created for in-vitro cell culture experiments. However, the physiological effect of reproductive steroids on miRNA signatures in human endometrial tissues is just starting to be delineated. Kuokkanen et al. (2010) performed a miRNA array analysis, using RNA extracted from human epithelial cells isolated from late-proliferative-phase and mid-secretory-phase endometrium (4 samples in each cycle phase). Twenty-four differentially expressed miRNA were identified when the cycle phases were compared, of which 12 miRNA were up-regulated in the mid-secretory phase (Kuokkanen et al., 2010). Predicted targets of menstrual-phase-specific miRNA were identified in a parallel mRNA microarray analysis and were associated with DNA replication, cell cycle progression and cell proliferation. Over-expression of miRNA that repress cell cycle progression was seen in the secretory phase and may represent a pathway for progesterone's ability to moderate the proliferative effects of oestradiol. Other mRNA profiling studies that have characterized the mRNA transcriptome at different phases of the menstrual cycle support these findings (Borthwick et al., 2003; Burney et al., 2007; Dennis et al., 2003; Petracco et al., 2012). Of note, miRNA profiling of endometrial tissues that include both epithelial and stromal components has not been performed in a menstrual-cycle-phase comparison experiment.

Smaller studies have identified individual miRNA with cycle-specific endometrial expression patterns. Petracco et al. (2011) demonstrated that miR-135a and miR-135b were down-regulated in early secretory endometrial tissues and directly suppressed expression of the transcription factor homeobox A10 (HOXA10). In human endometrium, HOXA10 peaks during the implantation window and regulates various cell adhesion molecules and signal transduction factors that are essential for endometrial receptivity (Zanatta et al., 2010). Ramon et al. (2011) reported

increased concentrations of the angiogenesis regulating miRNA miR-15b, miR-20a and miR-221 in secretory-phase eutopic endometrium from healthy women, indicating that a highly regulatory environment is likely to be required for angiogenesis at this vital time.

The evidence that endometrial miRNA exhibit dynamic expression throughout the menstrual cycle strongly suggests that they have a role in maintaining homeostasis in this complex tissue. In this scenario, changes in miRNA activity could impair endometrial function, potentially having a negative impact on implantation and menstrual bleeding patterns. It is also possible that iatrogenic miRNA manipulation in endometrial dysfunction could correct endometrial disorders; however, more knowledge regarding endometrial miRNA function and its safe manipulation is required before this becomes a clinical reality.

miRNA in implantation

Complex structural and biochemical changes occur in the endometrium to effect endometrial receptivity during the 'window of implantation', a limited period in the mid-secretory phase of the menstrual cycle (usually days 19—23 of a 28-day cycle). These changes, in conjunction with decidualization (stromal cell differentiation in response to progesterone), are prerequisites for establishing and sustaining a pregnancy (Harper, 1992). Impaired endometrial receptivity and/or decidual aberrations are thought to underpin infertility, fertility treatment failure and recurrent pregnancy loss (Macklon et al., 2006). Endometrial receptivity is still poorly understood and, although many attempts have been made to identify markers of a receptive endometrium, none are in clinical use (Horcajadas et al., 2007; Koot et al., 2012). The obstacles to the development of clinically useful biomarkers of receptivity are discussed in the accompanying article by Salamonsen et al. (2013). More recently, researchers have provided evidence of a role for epigenetic regulatory mechanisms in endometrial receptivity and implantation events (Munro et al., 2010; **Table 1**).

Rodent implantation models have provided insights into miRNA activity in the implantation window. A next-generation sequencing study identified 563 dysregulated mRNA, 62 dysregulated miRNA and an increase in mature miRNA editing in mouse endometrium that exhibited an active implantation state when compared with endometrium displaying delayed implantation (Su et al., 2010). The oestradiol-dominant pre-receptive-phase murine endometrium was compared with that from the progesterone-dominant receptive phase and 37 dysregulated miRNA were identified (Chakrabarty et al., 2007). miR-101a and miR-199a regulated cyclo-oxygenase 2 expression, an enzyme required for implantation that controls the rate-limiting step in prostaglandin synthesis (Chakrabarty et al., 2007).

miR-21, one of the implantation-specific miRNA in rodents (Xia et al., 2010), was down-regulated by progesterone in ovariectomized mice, which may explain the detrimental effect of prematurely elevated progesterone on endometrial receptivity in IVF programmes. miR-21 regulates blood vessel remodelling through its suppressive regulation of a membrane-anchored metalloproteinase inhibitor, reversion-inducing-cysteine-rich protein with kazal motifs (RECK) (Chandana et al., 2010; Hu et al., 2008). RECK may facilitate angiogenic implantation events as it is usually down-regulated at endometrial implantation sites. In a prematurely progestagenic environment, reduced miR-21 concentrations were associated with increased RECK concentrations, which would be predicted to lead to inhibition of vascular development (Shulman et al., 1996).

The translation of the established effects of miR-21 in murine implantation to human pathophysiology is questionable as women undertaking IVF with a premature progesterone rise did not demonstrate down-regulation of miR-21 in the endometrium (Li et al., 2011). It was also speculated that miR-21 can be regulated by the endometrial receptivity marker leukaemia inhibitor factor, via signal transducer and activator of transcription 3; however, this premise requires experimental validation (Carletti and Christenson, 2009).

Decidualization of primary human endometrial stromal cell cultures can be induced *in vitro* to create a human model of this critical implantation process. miRNAome analysis comparing decidualized to nondecidualized human endometrial stromal cells identified a panel of differentially expressed miRNA (Estella et al., 2012; Qian et al., 2009; **Table 1**). These miRNA were predicted to alter transcripts for actin filaments, which are key protein markers of the cellular differentiation that occurs during decidualization. Other transcript targets known to influence the decidualization process included epidermal growth factor receptor, vascular endothelial growth factor A (VEGF-A), TGFβ2 and HOXA10 (Estella et al., 2012; Giudice et al., 2002; Haouzi et al., 2009; Popovici et al., 2000). Loss-of-function manipulation of some of these miRNA revealed that miR-222 and miR-29b altered the expression levels of genes important in adhesion and extracellular matrix formation (Qian et al., 2009), whereas an over expression of miR-29b effected a blunted decidualization response (Hawkins et al., 2011b).

Two studies have profiled miRNA in endometria from fertile (Altmäe et al., 2012a) and infertile women (Sha et al., 2011), comparing the mid-secretory window of implantation with endometrium from the early secretory phase to identify implantation-associated miRNA (**Table 1**). The intersection of the predicted and experimentally validated targets of these miRNA included a panel of transcripts previously suggested as important for endometrial receptivity, including calpastatin, cystic fibrosis transmembrane conductance regulator, fibroblast growth factor receptor 2, leukaemia inhibitor factor (Altmäe et al., 2012a) and osteopontin (Sha et al., 2011). These miRNA and their targets make up networks that have functional relevance to implantation, such as axon guidance, Wnt/β-catenin, extracellular signal-regulated kinase/mitogen-activated protein kinase, TGFβ and tumour protein 53 pathways (Altmäe et al., 2012b; Horcajadas et al., 2007). An altered miRNA profile consistent with a shift in endometrial maturation was observed when natural and stimulated cycles were compared, suggesting that miRNA signatures could be reliable markers of endometrial receptivity and maturation (Sha et al., 2011).

A genome-wide analysis revealed four down-regulated miRNA (miR-451, miR-424, miR-30b and miR-125b) and 22 dysregulated mRNA transcripts in women undertaking IVF with decreased endometrial receptivity due to elevated

progesterone concentrations on the day of human chorionic gonadotrophin compared with women with normal progesterone concentrations (Li et al., 2011). Osteopontin and VEGF were predicted and validated targets of these miRNA, promoting tissue remodelling and angiogenesis respectively. Both osteopontin and VEGF transcripts are significantly up-regulated in endometrium during the implantation window and high VEGF concentrations are positively correlated with implantation rates in IVF—intracytoplasmic sperm injection cycles (Haouzi et al., 2009).

miRNA have been implicated as causal factors in implantation failure, which is defined as repeated intrauterine transfers of high-quality embryos without pregnancy during IVF. A miRNA array study of mid-secretory endometrium from women with repeated implantation failure identified a panel of 13 dysregulated miRNA (Revel et al., 2011). Two miRNA, miR-23b and miR-145, were confirmed as over-expressed, whereas their predicted targets, a group of cell adhesion molecules involved in implantation, (N-cadherin, H2A histone family, member X, netrin-4 and secreted frizzled-related protein 4) were down-regulated in samples from an independent cohort of patients with implantation defects (Revel et al., 2011).

Although endometrial receptivity is imperative, successful implantation also depends on oocyte and embryo quality (Cakmak and Taylor, 2011). miRNA involved in oocyte and embryo development have been described in association with a range of pregnancy-related disorders; however, this literature is beyond the scope of this review (Choudhury and Friedman, 2012; Hossain et al., 2012; Morales Prieto and Markert, 2011; Novakovic and Saffery, 2012).

There is ample evidence that miRNA are important in endometrial receptivity and decidualization although further work is required to determine their precise roles in these events. In this early phase of endometrial miRNA exploration, it is still too soon to determine if miRNA can be viewed as diagnostic markers of implantation disorders or as novel therapeutic agents to correct implantation defects.

The role of miRNA in recurrent miscarriage

Endometrial alterations have been previously reported in women with a history of recurrent miscarriage, including aberrant expression of immune cell populations (Quenby et al., 1999) and proinflammatory, anti-inflammatory and angiogenic cytokines and growth factors (Banerjee et al., 2012). Transcriptome alterations have also been identified by messenger RNA microarray studies comparing secretory-phase endometrium from women suffering recurrent miscarriage and that from fertile subjects (Mokhtar et al., 2012). As far as is known, there are no miRNA expression studies assessing the endometrium from women afflicted by recurrent miscarriage.

Wang et al. (2012b) demonstrated a 30-times greater expression of miR-133a in chorionic villi from women with recurrent miscarriage compared with women who had an induced abortion at 7 weeks of gestation. miR-133a was shown to repress the human leukocyte antigen HLA-G (Wang et al., 2012b), a protein which in increased in chorionic villi at 6—7 weeks gestation and is known to have an important role in maternal immune tolerance to the fetus (Jiang et al., 2012). The authors suggested that miR-133 is involved in pathogenesis of recurrent miscarriage, via interference with fetal—maternal tolerance.

miRNA may play a role in progesterone support of early pregnancy. In addition to endometrial support, progesterone protects the conceptus from maternal immune attack, inhibiting T-lymphocyte activity and decreasing production of inflammatory cytokines (Miyaura and Iwata, 2002). The mechanism for progesterone's immune suppressive affects appears to be via inhibited nuclear factor kappa B (NFκB) activation in macrophages, which suppresses lipopolysaccharide-induced miR-155 expression. Low concentrations of miR-155 resulted in the up-regulation of suppressor of cytokine signalling 1 which inhibits interleukin (IL)-6 and interferon γ production (Sun et al., 2012). Increasing miR-155 activity attenuated progesterone's inhibition on IL-6 and interferon γ expression, promoting an inflammatory environment less suitable for placentation. This raises the possibility that miRNA manipulation could be a new strategy for maintenance of progesterone-induced immunosuppressive signals (Sun et al., 2012).

Altered miRNA expression in endometriosis

Eutopic endometrial tissues

Endometriosis causes pelvic pain and infertility in reproductive-age women (Giudice and Kao, 2004). Although the cause of endometriosis is still uncertain, Sampson's theory states that eutopic endometrial tissue is flushed retrogradely through the Fallopian tubes during menstruation, where it attaches and grows (Sampson, 1927). There is increasing evidence that women with endometriosis have a genetic and/or environmental predisposition which enhances ectopic endometrial tissue development, because only 10% of women develop endometriotic disease whereas 90% of women suffer from retrograde menstruation. Inherent abnormalities in eutopic endometrium are likely to contribute to the pathophysiology of endometriosis and may be fundamental to endometriosis-related subfertility due to implantation defects. Endometriosis-associated endometrial changes may also have diagnostic potential as biomarkers to distinguish women with and without disease.

Like proteomic and genomic alterations, differentially regulated miRNA have been identified in eutopic endometrium from women with endometriosis. In a comparison of three control mid-secretory-phase endometrium ($n = 3$) with endometria from four women with severe endometriosis (Burney et al., 2009), six down-regulated endometriosis-associated miRNA were identified from the miR-9 and miR-34 miRNA families. It was suggested that miR-34 participates in maintaining a proliferative endometrial profile and may mediate the delayed proliferative to secretory transition of endometrium observed in women with advanced endometriosis.

A further comparison of eutopic endometrium between women with mild or severe endometriosis was performed using microarrays that incorporated both mRNA and miRNA. An increased expression of miR-21 and DICER was identified in severe endometriosis as were differences in the transcriptome between the two disease stages. This suggests that mild and severe stages of endometriosis are distinct

Table 1 Summary of miRNA profiling studies in human endometrium during the implantation window.

Study	*Samples*	*Detection platform*	*Dysregulated miRNA*	*Predicted target genes*	*Validated target genes*
Qian et al. (2009)	Decidualized versus non-decidualized HESC	Hybridization oligonucleotide miRNA microarray	49 miRNA (16 ↑, 33 ↓): 11 validated by qRT-PCR (miR-27b, miR-30c, miR-143, miR-101, miR-181b, miR-29b, miR-30d, miR-507, miR-23a, miR-222, miR-221: all ↓)	Transcriptional factors, extracellular matrix-related molecules, intracellular signalling molecules, growth factors, cytokines (decidualization)	↓ miR-222 → ↑ CDKN1C/p57kip (cell differentiation)
Estella et al. (2012)	Decidualized versus non-decidualized HESC	Human whole-genome miRNA PCR array	43 miRNA (26 ↑, 17 ↓): ↓ predominantly three miRNA families (miR-181, miR-183, miR-200) and ↑ DICER mRNA	Transcriptional factors, growth factors, interleukins, matrix remodelling enzymes (decidualization)	↑ miR-96 and miR-135b → ↓ FOXO1, HOXA10, IGFPB1 (decidual transformation) DICER knockdown No change in expression of decidual markers (FOXO1, C/EBPB, COX2, SP1)
Altmae et al. (2012a)	Receptive versus pre-receptive endometrium in healthy fertile women (LH + 7, *n* = 4 versus LH + 2, *n* = 5)	Agilent hybridization miRNA microarray	3 miRNA: 2 ↑ (miR-30b, miR-30d), 1 ↓ (miR-494)	CAST, CFTR, FGFR2, LIF, DPYSL2, F11R, MTF1, NPAS2, P4HA2, PPRGC1A, TACC2, RAB40B (implantation) Intersection of the predicted and experimentally validated targets	
Sha et al. (2011)	Receptive versus pre-receptive endometrium in infertile women: natural cycles (LH + 7, *n* = 5 versus LH + 2, *n* = 5) and stimulated IVF cycles (HCG + 7, *n* = 5 versus HCG + 4, *n* = 5)	Next-generation sequencing	20 miRNA: 8 ↑ (miR-30d, miR-30b, miR-30b*, miR-31, miR-21*, miR-193a-5p,,miR-193a3p, miR-203), 12 ↓ (miR-33a, miR-452, miR-125b, miR-455-3p, miR-455-5p, miR-483-5p, miR-143*, miR-100, miR-504, miR-424, miR-424*, miR-503)	Genes involved in cell cycle, cell adhesion and metabolism Intersection of the predicted and experimentally validated targets in independent microarray studies Reciprocal relationship between miR-424 (↓) and osteopontin gene (↑)	

Li et al. (2011)	Endometrium day-6 post oocyte retrieval: 12 infertile women with elevated progesterone on the day of HCG versus 7 women with normal progesterone	Hybridization oligonucleotide miRNA microarray	4 miRNA ↓ (miR-451, miR-424, miR-30b miR-125b), 22 dysregulated mRNA (13 ↑, 9 ↓)	Osteopontin and VEGF both predicted and validated targets (implantation markers)
Revel et al. (2011)	Secretory endometrium; 12 fertile women versus 11 women with repeated implantation failure	PCR-based TaqMan miRNA arrays	13 miRNA: 10 ↑ (miR-23b, miR-145, miR-99a, miR-27b, miR-652, miR-139-5p, miR-195, miR-342-3p, miR-150, miR-374b), 3 ↓ (miR-32, miR-628-5p, miR-874), miR-23b and miR-145 validated by qRT-PCR	Adherens junction, Wnt signalling, cell adhesion molecules (implantation)	↓ H2AFX, sFRP-4, n-cadherin, netrin-4, all targets of miR-145 (cell adhesion)

ESC = endometrial stromal cell; HCG = human chorionic gonadotrophin; qRT-PCR = quantitative reverse-transcription PCR; VEGF = vascular endothelial growth factor.

disorders with significant molecular and signalling pathway differences that are likely to be regulated by miRNA (Aghajanova and Giudice, 2011).

Additional studies have explored individual miRNA in eutopic endometrium from women with endometriosis. Compared with healthy controls, endometrium from women with endometriosis is characterized by over-expression of miR-135a in the proliferative phase and miR-135b in the proliferative and secretory phases (Petracco et al., 2011). These miRNA were predicted and validated to target HOXA10, a key mediator of endometrial receptivity (Taylor et al., 1999), the expression of which was simultaneously repressed in the endometrium of women with endometriosis. Transfection of endometrial stromal cells with miR-135a/b or miR-135a/b inhibitors resulted in an altered expression of HOXA10 mRNA and protein and this may suppress endometrial receptivity in endometriosis.

The transfection of endometrial cells with miR-199a repressed IκB kinase/NFκB signalling and inhibited IL-8 secretion. Therefore, the low expression levels of miR-199a in endometrial stromal cells from women with endometriosis (Dai et al., 2012) would be expected to up-regulate these inflammatory mediators and result in decreased endometrial receptivity and implantation defects. IL-8 is elevated in peritoneal fluid from women with endometriosis and its negative effect on embryo quality could also account for reduced implantation in women with endometriosis (Wu and Ho, 2003).

Ectopic endometrial tissues

Proteins, transcripts and miRNA that are specific to endometriotic lesion establishment can be identified when eutopic and ectopic endometrial tissues are compared. As outlined in recent reviews (Hawkins et al., 2011a; Ohlsson Teague et al., 2010), four studies have determined the miRNAome of ectopic endometrial tissues (ovarian or peritoneal) with paired or unpaired eutopic endometrium from women with endometriosis or eutopic endometrium from healthy controls, using microarrays or next-generation sequencing (**Table 2**) (Filigheddu et al., 2010; Hawkins et al., 2011b; Ohlsson Teague et al., 2009; Pan et al., 2007). There was considerable heterogeneity between the studies in terms of design, control group selection, analysis methods and the location of ectopic endometrial tissues. Each study identified a unique set of differentially expressed miRNA, with at least 22% of the dysregulated miRNA in every experiment being present in gene lists from other papers (**Figure 2**). Twenty-two endometriosis-associated miRNA were identified in the analysis by Ohlsson Teague et al. (2009), of which 60% were concordant with Filigheddu et al. (2010), 36% with Pan et al. (2007) and 23% with Hawkins et al. (2011b; **Figure 2**). Predicted targets of miRNA differentially expressed in endometriosis revealed biologically important pathways and molecular networks known to be associated with endometriosis including angiogenesis, tissue remodelling and TGFβ signalling pathways. Postulation regarding the role of miRNA in endometriotic disease was able to be undertaken (Hawkins et al., 2011a; Ohlsson Teague et al., 2010). It is important to note that the evidence supporting these hypotheses is largely indirect, based on in-silico computational analyses.

Table 2 Global miRNA profiling studies in endometriosis.

Study	*Sample tested*	*Cycle phase*	*Results*
Burney et al. (2009)	Eutopic in endometriosis (n = 4) versus eutopic controls (n = 3)	Early secretory	6 miRNA
Ohlsson Teague et al. (2009)	Eutopic versus ectopic (n = 8) in endometriosis	Proliferative and secretory	22 miRNA
Filigheddu et al. (2010)	Eutopic versus ectopic in endometriosis (n = 13)	Early proliferative	50 miRNA
Hawkins et al. (2011b)	Eutopic in controls (n = 11) versus ectopic in endometriosis (n = 10)	Proliferative and secretory	22 miRNA
Pan et al. (2007)	Paired ectopic–eutopic patients (n = 4), ectopic patients (n = 4), eutopic controls (n = 4)	Early to mid-secretory	50 miRNA
Abe et al. (2013)	Endometriotic endometrial stromal cell cultures (n = 8) versus normal endometrial stromal cell cultures (n = 8)	Mid to late proliferative	12 miRNA

One study has recently explored the miRNA microarray profile of eight primary endometriotic cyst stromal cell cultures in comparison to eight primary normal endometrial stromal cell cultures. Four up-regulated and eight down-regulated miRNA were identified in endometriotic cyst stromal cell cultures, with three (miR-99b-5p, -99a-3p and -424) being previously identified in the microarray dataset of Ohlsson Teague et al. (2009). Subsequent luciferase assay experiments determined that down-regulated miR-96b directly repressed its target mRNA, c-myc and B cell lymphoma/leukaemia 2 (BCL2) in the same established cell cultures. Furthermore, induced over-expression of miR-96b in ECSCs resulted in increased apoptosis and suppression of proliferation in functional assays. The low miR-96b concentrations in endometriomas would be predicted to promote proliferation and suppress apoptosis, both of which are likely to promote endometriotic tissue growth (Abe et al., 2013).

It is difficult to assess the effect of cell culture conditions on miRNA expression profiles from isolated stromal cells. Gene expression profiling studies of laser-captured tissue compartments from endometriotic tissues may clarify this in the future although the stromal tissue compartment is still likely to contain a heterogeneous mix of several cell types.

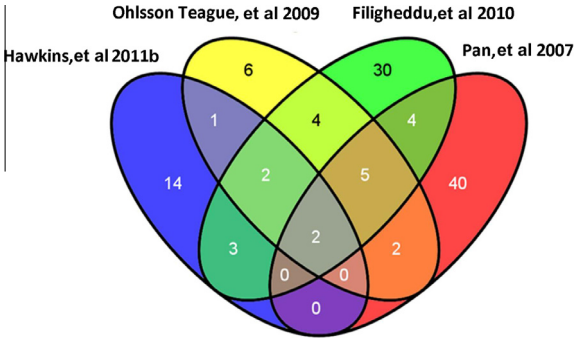

Figure 2 Concordance between published miRNA profiling studies in endometriosis. The Venn diagram represents comparison between the miRNA studies and demonstrates the number of overlapping miRNA in the datasets.

In-vitro methods are required to verify miRNA regulation of a predicted target mRNA. In cell lines, quantitative PCR combined with Western blotting can quantify the concentrations of a mRNA target and the protein it encodes in the absence and presence of the miRNA of interest. This can provide indirect evidence that a miRNA represses the protein production of a particular target. The luciferase assay provides direct evidence of miRNA regulation of a specific mRNA target and is the gold standard for target verification. In this assay, the predicted miRNA-binding site of a target transcript is linked to the luciferase gene, which encodes a luminescent protein. When a miRNA that targets the mRNA-binding site is introduced into a cell line containing this reporter construct, luciferase activity is repressed and chemiluminescence is measurably reduced. Only a small number of miRNA–mRNA interactions have been experimentally confirmed in endometriosis and there is a need to develop high-throughput methodologies to enhance the efficiency of validating miRNA target transcripts.

Endometriotic tissue is highly complex and to date all miRNA expression profiling studies, apart from the study by Abe et al. (2013), have been performed in whole tissues. There is a complex interaction between the ectopic endometrium and its host site of attachment at the cellular and molecular levels. When human endometrium is xenografted into immunocompromised mice, species-specific immunohistochemical staining demonstrates that the glandular epithelium retains human characteristics, whereas species derivation in the stroma is heterogeneous, with macrophage, myofibroblast and endotheial cell populations being predominantly host derived (Hull et al., 2008). Using in-situ hybridization, this study group has shown that miRNA which are dysregulated in endometriosis can be confined to the epithelial or stromal compartment of endometrial tissues (**Figure 3**). It is critical to determine the cellular origin of endometriosis-associated miRNA in order to select appropriate cell lines that will demonstrate a representative functional effect that is relevant to the endometriotic disease process.

Early functional studies in endometriosis which have undertaken gain- or loss-of-function manipulations in eutopic and ectopic endometrial cell lineages have provided

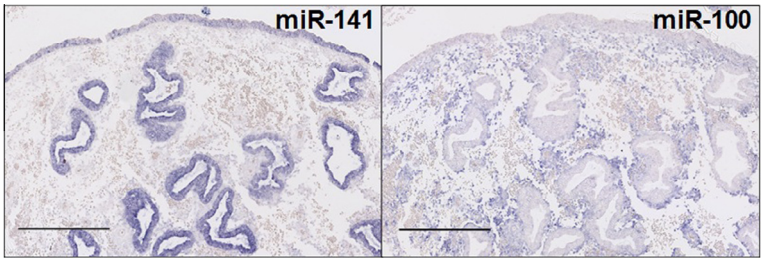

Figure 3 Cellular localization of miR-141 and miR-100 in eutopic endometrial tissue. In-situ hybridization reveals that miR-141 localizes to the glandular and luminal epithelium, whereas miR-100 localizes to stromal cells in eutopic endometrial tissues. Bars = 300 μm. Courtesy of Dr. Jonathan McGuane.

experimental confirmation of some miRNA—mRNA relationships in endometriosis (**Table 3**). For example, the extracellular matrix molecule collagen type VII A1 (COL7A1), transcription factor AP-2γ (TFAP2C) and the transmembranous signalling protein uroplakin 1B are predicted targets of miR-29c, which was found to be up-regulated in endometrioma when compared with independent eutopic endometrial tissue samples from women without endometriosis (Hawkins et al., 2011b). These targets were down-regulated in endometrioma tissues, which is consistent with their regulation by miR-29c. A miR-29c mimic suppressed their expression, whereas an inhibitor of miR-29c enhanced expression of COL7A and TFAP2C when transfected into human endometrial stromal cell cultures from eight control women.

Hawkins et al. (2011b) demonstrated some of the difficulties in undertaking functional miRNA studies in endometrial tissues when they attempted to demonstrate that miR-29c had a direct regulatory effect on COL7A1 and TFAP2C expression using the luciferase assay. Human endometrial stromal fibroblasts were resistant to transfection of the luciferase construct and mimics or inhibitors. An alternate cell line, HEK293T, which does not constitutively express miR-29c and is unrelated to the endometrium, was required to demonstrate the direct targeting effect of miR-29c on COL7A1 and TFAP2C and this finding was extrapolated to endometrial tissues

Lin et al. (2012) utilized luciferase assays to explore direct targeting of dual-specificity phosphatase 2 (DUSP2) by miR-20a after this group had measured miR-20a as up-regulated in endometriotic tissues, although they did not state their quantitative PCR normalization methods. The 3′-untranslated region of DUSP2 was cloned into a luciferase reporter system and transfected with pre-miR-20a into normal endometrial stromal cells. Pre-miR-20 suppressed luciferase activity and DUSP2 protein concentrations whereas an anti-miR20a hairpin inhibitor induced the opposite effect. miR-20a-mediated down-regulation of DUSP2 was shown to enhance the activity of several angiogenic genes (Lin et al., 2012) and may promote the increased vascularity associated with endometriosis (Hull et al., 2003).

Table 3 Functional studies of the selected miRNA in endometriosis.

Study	*miRNA tested*	*Sample tested*	*Conclusion*
Abe et al. (2013)	*miR-96b*	Endometriotic cyst cell cultures versus normal endometrial stromal cell cultures (8 patients, 8 controls)	miR-96b inhibits proliferation and induces apoptosis via c-myc and bcl-2 suppression in endometriosis
	miR-199a	Endometrioma versus eutopic endometrium (12 patients, 12 controls)	Increase in cell invasion, activation of NFκB pathway, elevation of IL-8
Lin et al. (2012)	*miR-20a*	Ectopic versus eutopic endometrium (37 patients, 17 controls)	Up-regulation of angiogenic genes and FGF-9, stimulation of proliferation
Petracco et al. (2011)	*miR-135a/b*	Eutopic endometrium (32 patients, 50 controls)	Down-regulation of HOXA10 that regulates endometrial receptivity
Ramon et al. (2011)	*miR-125a, miR-222, miR-17-5p*	Endometrioma versus eutopic endometrium (58 patients, 38 controls)	Lower expression of VEGF-A; higher expression of the angiogenic inhibitor TSP-1
Hawkins et al. (2011b)	*miR-29c*	Endometrioma versus eutopic endometrium (8 patients, 11 controls)	miR-29c induces down-regulation of COL7A1, TFAP2C and UPK1B

Green indicates down-regulated miRNA; red indicates up-regulated miRNA.COL7A1 = collagen type VII A1; FGF = fetal growth factor; HOXA10 = homeobox A10; IL = interleukin; NFκB = nuclear factor kappa B; TFAP2C = transcription factor AP-2γ; UPK1B = uroplakin 1B; TSP = Thrombospondin; VEGF = vascular endothelial growth factor

In contrast and consistent with the microarray findings of Ohlsson Teague et al. (2009) and Filigheddu et al. (2010), miR-20a was identified as being down-regulated in endometrioma ($n = 58$) when compared with eutopic endometrium from women with endometriosis ($n = 58$) and from controls ($n = 38$) when quantitative PCR assays were performed normalizing to RNU6B. Of the three up-regulated (miR-21, -125 and -222) and three down-regulated miRNA (miR-15b, -17-5p and -20a) identified, a decrease in the pro-angiogenic miR-17-5p was shown to correlate inversely with elevated thrombospondin protein concentrations (an angiogenic factor) in endometrioma tissues. Conversely elevations in miR-125a and miR-222 were associated with a decrease in VEGF-A protein concentrations in endometriomas. Ramon et al. (2011) concluded that miRNA could regulate angiogenic factors in a way that enhanced angiogenic activity in endometriosis.

The miRNA regulation of other molecular pathways in endometriosis has been explored. Dai et al. (2012) found lower concentrations of miR-199a in 12 endometriotic tissues when compared with paired eutopic endometrium from women with endometriosis and 12 eutopic endometrial samples from disease-free women, when reverse-transcription PCR was performed and the data was normalized to U6. The 3′-untranslated region of the IκB kinase B (IκκB) promoter was cloned into the luciferase reporter construct and co-transfected with miR-199a or a noncoding mutant sequence into primary endometrial stromal cell cultures. The reduction in luciferase activity confirmed the ability of miR-199a to directly down-regulate IκκB, and this was substantiated by a reduction of IκκB protein. Il-8 production and cell migration and invasion was suppressed in the miR-199a-transfected cell cultures, suggesting that low miR-199a concentrations in endometriotic tissues may promote disease progression and cell migration and invasion via the NFκB pathway.

HOXA10 is a marker of endometrial receptivity that is reduced in the eutopic endometrium from women with endometriosis. Sex steroid regulation of HOXA10 appears to be intact in endometriosis and Petracco et al. (2011) explored the hypothesis that epigenetic regulation by miR-135a and -135b altered HOXA10 expression in the eutopic endometrium from women with endometriosis. Both miR-135a and-135b were up-regulated in the proliferative phase and miR-135b concentrations were increased in the secretory phase. A concomitant decrease in HOXA10 mRNA suggested direct regulation of this transcription factor by miR-135a/b, which was subsequently confirmed by luciferase assay in endometrial stromal cells. Transfection of endometrial stromal cells with miR-135a/b or their inhibitors repressed and enhanced HOXA10 mRNA and protein concentrations respectively. It was concluded that increased miR-135a/b repressive activity may result in reduced HOXA10 mRNA and protein concentrations in eutopic endometrium from women with endometriosis thus impairing implantation events.

Although, miRNA can be predicted to have an effect on target mRNA translation and this effect can be experimentally validated *in vitro*, there are several additional factors that determine the functional effect of a miRNA *in vivo*. Each miRNA can regulate up to a hundred transcripts and a target transcript can be regulated by a multitude of miRNA so models of miRNA activity may be overly simple. Some miRNA clusters with common mRNA targets are co-transcribed, amplifying their repressive effect on translation. Furthermore, there are families of miRNA that have extremely similar target profiles adding to the complexity of *in-vivo* miRNA function. This complexity of regulation is difficult to emulate *in vitro* and manipulable animal models to test miRNA activity will need to be developed for endometriosis. Mouse models genetically deficient in specific miRNA have been created (Blüml et al., 2011; Li et al., 2010). Furthermore, miRNA mimics and antagonists have been introduced into rodent and primate models in the context of other diseases (Elmen et al., 2008a,b). These models are starting to be utilized in endometriosis research and are likely to lead to advances in understanding the role of miRNA in endometriosis in the future.

In-silico models for miRNA activity in endometriotic lesions have been formulated that indicate that miRNA regulation is likely to control several processes in ectopic endometrial development including attachment, inflammation, tissue remodelling, angiogenesis and fibrosis (Ohlsson Teague et al., 2010). Further work is required that experimentally validates the functional activity of specific miRNA and their potential to alter the pathophysiology of endometriosis. It may be possible to alter miRNA activity in endometriotic lesions to achieve a therapeutic benefit for women with endometriosis with the use of synthetic or antagonistic miRNA. Translation to clinical practice is likely to require the development of targeted delivery systems to avoid unwanted on-target but off-disease side effects. Physiological miRNA transport mechanisms present in the human circulation could represent a delivery system for inhibitors and enhancers of miRNA activity.

Circulating miRNA and endometrial function

miRNA have been detected in all types of body fluids (Weber et al., 2010) including the cell-free compartment of peripheral blood. Various diseases have been associated with signature serum or plasma miRNA concentrations; however, the physiological role of these miRNA remains unclear. Initial evidence that circulating miRNA were passively released from damaged cells (Wang et al., 2009) has been challenged by observations of active secretion of miRNA from tissues or cells. It is now thought that circulating miRNA can influence gene transcription at distant target sites (Valadi et al., 2007; Vickers et al., 2011). A model has been formulated regarding the biogenesis and function of circulating miRNA (**Figure 1**); however, the exact mechanisms by which miRNA are released from donor cells and taken up by recipient cells are the subject of ongoing investigation.

Global miRNA profiling in whole blood has been performed in healthy reproductive-age women and age-related differences in miRNA plasma concentrations have been demonstrated, although no attempt was made to determine if cycle phase confounded the results (Sredni et al., 2011). Similarly, none of the many studies that have explored circulating miRNA concentrations in a variety of pathological conditions have accounted for the effect of menstrual cycle phase on miRNA profiling in women of reproductive age.

This information is of particular importance in biomarker research, where the stage of the menstrual cycle in which blood is drawn and the use of hormonal medications might have a negative impact on the performance of a miRNA-based test.

This study group recently profiled plasma miRNA concentrations in three different phases of the menstrual cycle in eight healthy women and in eight women with endometriosis and did not demonstrate significant menstrual-phase variations in plasma miRNA expression for any of 674 screened miRNA (Nisenblat et al., 2012). The data indicate that the miRNA profile in reproductive-age women is disease specific rather than cycle dependent. This implies that plasma miRNA-based diagnostic tests in reproductive-age women are likely to be accurate at any time in the menstrual cycle, although it is possible that small cycle-related changes could be revealed in a larger study. This finding does not contradict previous reports of cycle-dependent miRNA patterns in endometrial cells and tissues, which have higher local concentrations of oestradiol and progesterone than the systemic circulation. Also, the study did not detect cycle-phase differences that could identify the window of implantation and, therefore, did not detect plasma miRNA with potential as uterine receptivity markers. A better study design to detect biomarkers for uterine receptivity would be a comparison of plasma miRNA profiles from fertile women with those from women with unexplained recurrent implantation failure.

Women with endometriosis have a range of systemic health problems, including an increased risk of certain types of cancer (Nezhat et al., 2008; Somigliana et al., 2006; Swiersz, 2002; Van Gorp et al., 2004). A review of 11 case–control and cohort studies demonstrated a positive relationship between endometriosis and ovarian cancer, with all association entities (odd ratio, relative risk and standardized incidence ratio) between 1.3 and 3.0 and all confidence intervals above 1 with one exception (Somigliana et al., 2006). A positive association was also identified between endometriosis and breast cancer (association entity 0.8–4.3 in 11 studies), melanoma (association entity 0.7–3.9 in nine studies) and non-Hodgkin's lymphoma (association entity 1–1.8 in four studies).

There is also an association between a history of endometriosis and auto-immune, endocrine and atopic disorders (Clauw and Chrousos, 1997; Sinaii et al., 2002), susceptibility to infection (Gemmill et al., 2010) and cardiovascular disease (Mu et al., 2012; Santoro et al., 2012). In a cross-sectional survey of 3680 women with endometriosis who responded to a survey of women with surgically defined endometriosis from the American Endometriosis Society, the prevalence odds ratio was 20.7 (95% CI 14.3–29.9, $P < 0.0001$) for systemic lupus erythematosus, 7.1 (95% CI 4.4–11.3, $P < 0.0001$) for multiple sclerosis, 1.5 (95% CI 1.2–1.9, $P = 0.001$) and for rheumatoid arthritis and 23.9 (95% CI 15.5–36.5, $P \leq 0.0001$) for Sjögren's syndrome, when compared with the estimated prevalence in the general American female population (Sinaii et al., 2002). In the same study, women with endometriosis had a prevalence odds ratio of 1.8 (95% CI 1.6–2.1, $P \leq 0.0001$) for fibromyalgia and 180.5 (95% CI 147.2–242.0, $P \leq 0.0001$) for chronic fatigue syndrome. Nisenblat et al. (2012) identified plasma miRNA signatures that are dysregulated in women with endometriosis and postulated that they could contribute to the aberrant systemic epiphenomena associated with endometriosis.

miRNA that differentiate women with and without endometriotic disease have potential to be biomarkers that could underpin the development of a noninvasive diagnostic test for endometriosis. Wang et al. (2012a) identified 145 serum miRNA with a differential expression >2-fold and 61 with >10-fold difference, when a multiplex reverse-transcription PCR analysis was performed using two pooled samples from 10 endometriotic and 10 disease-free women (Wang et al., 2012a); four miRNA, miR-199a, miR-122, miR-145* and miR-542-3p, predicted endometriosis with 93.2% sensitivity and 96% specificity in a large retrospective cohort of 60 endometriosis and 25 disease-free women (Wang et al., 2012a). Similarly, Jia et al. (2012) performed a similar miRNA TaqMan microarray analysis with three plasma samples from women with endometriosis and three from healthy women; 27 down-regulated miRNA were identified, but none overlapped with Wang's diagnostic set of 4 miRNA. In a cohort of 23 women with endometriosis and 23 endometriosis free controls, miR-15b-5p, miR-17-5p, miR-20a, miR-21, miR-22 and miR-26a displayed an area under the receiver operating curve of between 0.79–0.87, much higher than the area under the curve of 0.5 which is expected by chance alone. Similar to Wang et al. (2012a), the development and validation of the diagnostic value of these biomarkers was in the same cohort rather than an independent test cohort, which is not recommended by the QUADAS guidelines for diagnostic test development (Whiting et al., 2011). Nevertheless, both studies demonstrate the potential for plasma miRNA as biomarkers of endometriotic disease.

The current study group utilized TaqMan arrays to identify differentially regulated miRNA in the plasma of eight women with endometriosis and eight healthy controls at three different cycle stages (47 total samples). Twelve miRNA were differentially expressed with P value < 0.01 although only three remained significant after correction for multiple testing (Nisenblat et al., 2012). miR-23a was the only miRNA identified in any other study (Jia et al., 2012). The inconsistency between studies could be due to different laboratory-based methodologies or alternate approaches to data normalization, which can lead to large variations in microarray outcomes using the same dataset. Nisenblat et al. (2012) was unable to replicate the findings of Wang et al. (2012a) and Jia et al. (2012), which raises the possibility of inherent differences in the sample population, either due to geographical divergence or from confounding factors such as disease location, disease severity stage or smoking.

Further effort will be needed to determine whether circulating miRNA are reliable biomarkers for endometriosis, and it will be particularly important to optimize experimental methods and workflow. Ultimately, large independent cohorts are required to firstly develop a robust noninvasive diagnostic test, then assess the sensitivity, specificity and diagnostic accuracy of the test in an independent population in accordance with the QUADAS guidelines for diagnostic tests (Whiting et al., 2011). Confounding factors such as age, disease stage and smoking will also need to be assessed in subgroup analyses. International collaborations will

ultimately be required to determine if the test is able to be applied to global populations.

Conclusion

Recurrent implantation failure, recurrent miscarriage and endometriosis are conditions that are often diagnosed after years of heartbreaking reproductive illness which currently have limited therapeutic modalities. Large-scale miRNA profiling studies have provided the first evidence that miRNA contribute to these endometrial disorders, as well as the physiological menstrual cycle changes in the endometrium. In-silico analyses have been able to predict biological processes that these miRNA and their targets may influence, and some functional studies have experimentally validated miRNA/target mRNA interactions in endometrial cells. A recent exciting advance has been the identification of serum and plasma endometriosis-associated miRNA, raising the possibility that these small molecules may mediate a systemic interaction with non-endometrial cells at distant sites and could have potential as components of noninvasive diagnostic tests.

As knowledge of the function of miRNA in endometrial disease increases, miRNA-based therapies for disorders of implantation, placentation and endometriosis are likely to become more realistic. At this stage, however, much more work needs to be done to establish true biological role of miRNA in endometrial disease, with a view to translating this knowledge into clinically effective outcomes.

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Declaration: The authors report no financial or commercial conflicts of interest.

Received 29 January 2013; refereed 24 July 2013; accepted 30 July 2013.