The role of microRNAs in endometriosis and associated reproductive conditions

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TABLE OF CONTENTS

- Introduction
- Methods
- Discovery and mechanisms of miRNAs
- miRNAs in endometriotic lesion development
- miRNAs and malignant transformation in endometriosis
- miRNAs in endometriosis and subfertility
- Therapeutic and diagnostic potential of miRNAs in endometriosis
- Conclusions and further perspectives

BACKGROUND: microRNAs (miRNAs) are short, single-stranded RNAs that regulate gene expression at the post-transcriptional level. Recent research has shown that miRNAs and their target mRNAs are differentially expressed in endometriosis and other disorders of the female reproductive system. Since miRNAs control a broad spectrum of normal and pathological cellular functions, they may play pivotal roles in the pathogenesis of these disorders.

METHODS: A systematic review was undertaken of the published literature on; (i) the expression and functions of miRNAs in mammalian female reproductive tissues with a focus on endometriosis and the malignancies and fertility disorders related to this disease; and (ii) the potential roles played by validated mRNA targets of endometriosis-associated miRNAs. The current understanding of the biology of miRNAs is overviewed and the potential diagnostic and therapeutic potential of miRNAs in endometriosis is highlighted.

RESULTS: The differential expression of miRNAs in endometriosis, and the putative molecular pathways constituted by their targets, suggests that miRNAs may play an important role in endometriotic lesion development. Models for miRNA regulatory functions in endometriosis are presented, including those associated with hypoxia, inflammation, tissue repair, TGFβ-regulated pathways, cell growth, cell proliferation, apoptosis, extracellular matrix remodelling and angiogenesis. In addition, specific miRNAs which may be associated with malignant progression and subfertility in endometriosis are discussed.

CONCLUSIONS: miRNAs appear to be potent regulators of gene expression in endometriosis and its associated reproductive disorders, raising the prospect of using miRNAs as biomarkers and therapeutic tools in endometriosis.

Key words: endometriosis / microRNA / gene expression / female reproductive tract cancer / female infertility

Introduction

MicroRNAs (miRNAs) are ~21 nucleotide (nt) single stranded non-coding RNAs that bind to target mRNAs, mediating translational repression and/or mRNA degradation (Bartel, 2004). The recent discovery of miRNAs, and a rapidly emerging understanding of their general regulatory function in cellular processes, has provided new insight into the regulation of gene expression (reviewed in
Aberrant miRNA expression is associated with a number of human diseases (reviewed in Singh et al., 2008), including benign gynaecological conditions, gynaecological malignancies and fertility disorders of the human female reproductive tract (reviewed in Carletti and Christenson, 2009).

The miRNA expression profiles of eutopic and ectopic endometrium from women with endometriosis were recently identified by us (Ohlsson Teague et al., 2009), and others (Pan et al., 2007). Furthermore, by performing bioinformatic analyses on miRNA and mRNA microarray data, we identified novel miRNA associated molecular pathways that are likely to play an important role in the pathophysiology of this disorder (Hull et al., 2008; Ohlsson Teague et al., 2009). miRNAs may thus be attractive candidates for novel diagnostic markers and therapeutic interventions in endometriosis, as recently demonstrated in other miRNA regulated diseases (Elmen et al., 2008a; Mitchell et al., 2008).

This review presents the current understanding of miRNA biogenesis, function and regulation. It then examines miRNA expression in female reproductive medicine, specifically delineating the role of miRNAs in endometriotic lesion development. Finally, it outlines how an improved understanding of endometrial miRNAs may translate into novel diagnostic tests and therapies for endometriosis.

**Methods**

An overview of basic miRNA biology was compiled based on seminal articles in this research field. A systematic review of the current literature on miRNAs in the mammalian female reproductive tract was performed, with particular emphasis on the endometrium and endometriosis. The articles cited were selected on the basis of relevance and quality from a list generated using combinations of the following search terms in Entrez Pubmed (http://www.ncbi.nlm.nih.gov/sites/entrez) microRNA and endometrium; endometrial; endometriosis; cervix; cervical; oocyte; ovary; ovaries; oviduct; uterus; uterine; infertility; miscarriage; reproductive tract; endocrine; estrogen; progesterone. An additional Pubmed search was carried out to identify experimentally confirmed miRNA targets of miRNAs associated with endometriosis. Finally, key articles on miRNAs as diagnostic biomarkers and in therapeutic intervention of human disease were reviewed.

**Discovery and mechanisms of miRNAs**

Our present understanding on miRNAs originates from the 1993 discovery of lin-4, a gene found to encode a 22 nt non-coding, hairpin shaped RNA that bound and suppressed the translation of LIN-4 mRNA in nematodes (Lee et al., 1993; Wightman et al., 1993). Seven years later, this novel gene regulatory mechanism was confirmed with the finding that let-7 transcripts have a similar role in post-transcriptional control in nematodes (Reinhart et al., 2000). As more non-coding, short RNAs were recognized, it became apparent that these transcripts and their functions were highly conserved across a wide range of distantly related organisms (Pasquinelli et al., 2000; Li and Mao, 2007). In 2001, three independent research teams identified nearly 100 short hairpin shaped RNAs by cDNA library sequencing (Lagos-Quintana et al., 2001; Lau et al., 2001; Lee and Ambros, 2001). The term microRNA was coined and further research into this field has been exponential.

**miRNA methodology**

As the number of known miRNAs increased, microarray platforms were created that incorporated hundreds of miRNA gene-probes. These high throughput methods, in addition to a range of genomewide and in situ miRNA detection techniques, have been used to identify disease-associated miRNAs (reviewed in Kong et al., 2009). To study the functions of miRNAs, multiple in silico algorithms were developed to predict their miRNA targets. These models generally search for miRNA targets that are homologous to the 5' 'seed sequence' of a miRNA, and in some cases also compensatory binding sites in the miRNA's 3' end (reviewed in Brodersen and Voinnet, 2009). The intersection of two to three different prediction algorithms is currently regarded as the most rigorous method to predict miRNA targets, providing a good balance of sensitivity and specificity and reducing the risk of false positive target predictions (Stark et al., 2005; Sethupathy et al., 2006).

Although these in silico models are now widely used for miRNA target predictions, experimental target validation is still required to confirm the miRNA-mediated repression at protein or miRNA level (reviewed in Kuhn et al., 2008). qRT-PCR and Western blots combined with luciferase reporter assays are the gold standard, the latter utilizing the predicted miRNA binding site of a target mRNA linked to sequences encoding a luminescent reporter. When a miRNA that binds to the miRNA binding site is introduced into a cell line containing the reporter gene, reporter repression is seen, as evidenced by reduced luciferase activity.

When functionally interpreting miRNA data, it is important to recognize that up-regulation of a specific miRNA represses target mRNA translation, whereas down-regulation relieves the suppressive effect. The combined effects of multiple miRNAs will dictate a particular disease outcome. An indication of miRNA function can be gained from observing the effect on cellular mRNA and protein profiles following miRNA over-expression or inhibition. However, since each miRNA on average may repress hundreds of targets and thus affects numerous downstream molecular pathways, this type of experiment can be difficult to interpret.

Genetically modified mice have been used to identify the impact of miRNAs in mammalian systems. Homozygous Dicer-1 deficient mice have global defects in miRNA synthesis and die during early embryogenesis (Bernstein et al., 2003; Yang et al., 2005). However, mice with partial or conditional Dicer-1 deletions survive to adulthood and have been instrumental in defining the cell or organ specific effects of postnatal miRNA deficiency, such as those involved in female fertility (Hong et al., 2008; Nagaraja et al., 2008; Otsuka et al., 2008).

Currently, over 9000 miRNA sequences have been identified in more than 100 species, as listed on the miRBase sequence database (Griffiths-Jones et al., 2008). Nearly one thousand miRNAs have been cloned in the human genome, and these are expected to target ~30% of the protein encoding genes (Lewis et al., 2005), regulating transcripts from as many as 8000 genes (Bartel, 2004; Griffiths-Jones et al., 2006). However, the lack of high throughput experimental methods for miRNA target confirmation has meant that only a fraction of these human miRNAs are currently functionally characterized.
We are only beginning to understand the impact of miRNAs in pathological and physiological processes.

**miRNA expression and processing**

In this review, we summarize the current models of miRNA biogenesis and post-transcriptional regulatory functions (Fig. 1). Emerging data shows that there are miRNA specific differences in these processes, offering additional complexity to miRNA regulatory functions (reviewed in Winter et al., 2009).

The majority of mammalian miRNA genes are embedded in intronic regions of protein encoding genes (Rodriguez et al., 2004; Kim and Kim, 2007) and are transcribed in the nucleus into primary miRNA (pri-miRNA) transcripts by RNA polymerase II (Lee et al., 2004). Pri-miRNAs have local hairpin shaped structures and may be several hundred kilobases long, in some cases comprising several miRNAs in tandem. Further transcript processing by Drosha, a RNase III endonuclease (Lee et al., 2003) in the Microprocessor complex (Denli et al., 2004; Gregory et al., 2004), releases ~70 nt long hairpin pre-miRNAs.

Pre-miRNAs are exported to the cytoplasm by Exportin-5 (Yi et al., 2003; Lund et al., 2004), cleaved into 21–24 nt long miRNA–miRNA* (sense-antisense* strand) duplexes by the RNase III-like enzyme Dicer (Grishok et al., 2001; Hutvagner et al., 2001; Ketting et al., 2001) and unwound to single stranded transcripts by helicases. Although both strands can generate functional miRNAs, the strand with the weakest base pairing in the 5’ end generally forms the mature miRNA, while the antisense miRNA* strand is degraded (Khourova et al., 2003; Schwarz et al., 2003). The mature single-stranded miRNAs may then guide the RNA-induced silencing effector complex (RISC) to complementary miRNAs sequences. The conserved seed sequence of the miRNA provides target specificity by base-pairing to target mRNAs, whereas Argonaute proteins (AGO1-4) and other RISC ribonucleoproteins execute the various forms of miRNA directed RNA processing, as outlined below (reviewed in Filipowicz et al., 2008).

**Models of miRNA mediated gene repression**

Mammalian miRNAs bind by imperfect base-pairing to multiple sites in 3’ untranslated regions, and sometimes protein encoding open reading frames of target mRNAs. The degree of miRNA–mRNA sequence homology dictates how miRNA target repression is executed (Fig. 1). Partial complementarity generally leads to mRNA decay and/or translational inhibition, whereas highly complementary target sites (which are mainly confined to plants), result in endonucleolytic mRNA cleavage and degradation (reviewed in Brodersen and Voinnet, 2009). Additional factors influencing miRNA target repression include the assembly of RISC accessory proteins, since only AGO2 has endonucleolytic activity, and the number of miRNA target sites within a mRNA, as the magnitude of miRNA directed translational repression is amplified by target site multiplicity (Saetrom et al., 2007).

miRNA repressed mRNAs accumulate in cytoplasmic structures called P-bodies where they are degraded or stored (reviewed in Filipowicz et al., 2008). During cellular stress, miRNA repression appears to be reversible and there is evidence miRNAs can be
released from P-bodies and become translationally active (Bhattachar-yya et al., 2006). There are also examples of miRNAs that have a stimulatory effect on target mRNA translation during cell cycle arrest, but these appear to be rare (Vasudevan et al., 2007).

**Cellular functions of miRNAs**

A single miRNA can repress hundreds of transcript targets (Lewis et al., 2005; Lim et al., 2005; Baek et al., 2008; Selbach et al., 2008), and each mRNA may also be targeted by multiple miRNAs (Enright et al., 2003; Grun et al., 2005; Krek et al., 2005; Stark et al., 2005). miRNAs generally act in concert to fine tune target protein expression. However, some miRNAs are able to mediate a complete switch of biological function by altering specific proteins, such as let-7 and lin-4 regulation of early development in nematodes (Lee et al., 1993; Wightman et al., 1993; Reinhart et al., 2000).

Some miRNAs with common mRNA targets are co-expressed from a single miRNA gene cluster, generating an amplified repressive effect on cellular functions. Members of miRNA families have similar or identical sequences, and can thus repress identical target miRNAs. Furthermore, miRNAs and their mRNA targets can form regulatory loops, through which reciprocal regulation provides additional control of gene expression, as exemplified in Fig. 2 (Fazi et al., 2005; Taganov et al., 2006; Sylvestre et al., 2007; Bracken et al., 2008; Burk et al., 2008). A picture thus emerges of a complex network of thousands of interacting miRNA-target mRNA associations.

Cui et al. reported that miRNAs typically target (i) positive regulatory motifs (three or four proteins positively regulating each other); (ii) highly connected scaffolds and most downstream network components such as signalling transcription factors and (iii) genes whose promoter regions include a large number of putative transcription factor binding sites (Cui et al., 2006). A combination of transcriptional and miRNA regulation is therefore likely to occur, particularly in scenarios where precise control of transcript activity is required. miRNAs may therefore, like transcription factors, be regarded as potential master regulators of cellular processes (reviewed in Hobert, 2008).

The findings in some miRNA target repression experiments have been contradictory, probably due to the diverse cellular systems in which they were performed. Certain miRNAs are expressed in a cell lineage specific manner, whereas others are widely expressed but nevertheless show distinct functions in specific cell types. There are several determinants of cell lineage restricted phenotypes, including the expression of target mRNAs, as well as miRNA modulators (Kedde et al., 2007; Huang et al., 2009a).

For this reason, the functional activity of a miRNA in one cell line may not necessarily be applicable to all cell lineages.

**miRNAs in endometriotic lesion development**

Endometriosis is defined by the presence of endometrial glands and stroma at sites outside the uterus. Approximately 6–10% of
reproductive aged women suffer from painful periods, chronic pelvic pain and subfertility as a result of endometriosis (reviewed in Giudice and Kao, 2004), at considerable cost to society (Gao et al., 2006a).

Although the aetiology of endometriosis is still controversial, in 1927, Sampson proposed his widely accepted theory, that endometriosis arises from the implantation of endometrial fragments in menstrual fluid that have passed retrogradely through the fallopian tubes into the pelvic cavity (Sampson, 1927).

Animal models of endometriosis and microarray methods have identified numerous individual endometriosis-associated transcripts that encode the proteins involved in broad signalling pathways. These pathways mediate inflammation, tissue remodelling, apoptosis, cellular proliferation, angiogenesis and wound healing in endometriotic tissues (Arimoto et al., 2003; Matsuzaki et al., 2006a; Eyster et al., 2007; Flores et al., 2007; Hever et al., 2007; Borghese et al., 2008; Hull et al., 2008). These findings support a model of endometriotic lesion development whereby displaced endometrial tissue in retrograde menstrual fluid progresses through a process of attachment, tissue degradation, acute inflammation, macrophage infiltration, tissue remodelling, neovascularisation and fibrosis, in order to become established at an ectopic site (Flores et al., 2007; Hull et al., 2008).

Differentially regulated miRNAs in endometriosis

Many mRNA transcripts are differentially regulated in endometriotic lesions when compared with eutopic tissues (Arimoto et al., 2003; Eyster et al., 2007; Hever et al., 2007; Hull et al., 2008; Borghese et al., 2008). However, an *in silico* analysis has revealed a mismatch between the expression levels of transcriptomes and proteins associated with the endometriotic disease process (Wren et al., 2007). This suggests that gene expression is not solely regulated at the level of transcription, and that post-transcriptional regulatory mechanisms may operate in endometriosis. miRNA regulation may provide at least some of this additional level of gene regulation, since two studies have identified differentially expressed miRNAs in endometriotic tissues (Pan et al., 2007; Ohlsson Teague et al., 2009).

Pan et al. identified differential expression of 48 miRNAs in a microarray analysis of (i) four eutopic endometrial tissues from endometriosis-free volunteers, (ii) four paired eutopic and (iii) ectopic endometrial tissues from women with endometriosis, as well as (iv) four additional unpaired ectopic endometrial tissues (Pan et al., 2007). No direct comparisons were undertaken between eutopic and ectopic endometrial tissues from women with endometriosis, nor of diseased versus control eutopic tissues and all tissue samples were taken at early to mid secretory phase of the menstrual cycle from women with ASRM stage III endometriosis. The majority of the differentially expressed miRNAs displayed reduced expression in eutopic and ectopic tissue from women with endometriosis when compared with control endometrium. qRT-PCR was used to validate the microarray results, confirming dysregulation of two miRNAs (miR-21 and miR-26a) in eutopic versus ectopic tissues.

We have recently identified 22 differentially expressed miRNAs in a microarray analysis of seven paired eutopic versus ectopic peritoneal endometrial tissues from women with rAFS stage II–IV endometriosis (Ohlsson Teague et al., 2009). qRT-PCR confirmed the expression of six miRNAs using two ubiquitously expressed endogenous miRNA controls. The samples were taken in both the proliferative (*n* = 3) and secretory (*n* = 4) phase of the menstrual cycle, therefore the differentially expressed miRNAs were associated with endometriosis across the menstrual cycle. The intersection of two target prediction algorithms was used to identify miRNAs that may be regulated by endometriosis-associated miRNAs, and a subset of these predicted miRNA targets were previously verified as differentially regulated in endometriotic lesions (Hever et al., 2007; Hull et al., 2008). This subset provided a database for an *in silico* functional analysis which identified several novel miRNA regulated molecular networks associated with endometriosis (Ohlsson Teague et al., 2009).

Eight miRNAs were differently expressed in both the above studies; however, the direction of dysregulation was not concordant between the studies for any of these miRNAs. This could be explained by differences in study design, methods of analysis, measures of stringency, qRT-PCR endogenous controls and menstrual cycle phase at the time of biopsy. Despite the disparity of these studies, there is evidence that endometriotic tissues have altered miRNA profiles when compared with eutopic endometrium and that specific miRNAs are likely to have a function in the pathophysiology of endometriosis.

Models for miRNA regulation in endometriotic lesion development

We utilized Gene Ontology and Ingenuity Pathway Analysis (IPA) databases to generate hypotheses for miRNA regulated processes involved in endometriotic lesion development (Ohlsson Teague et al., 2009). The study was based on mRNA and miRNA microarray analyses of eutopic and ectopic endometrium, where there are differences in cellular composition. Although numerous and compelling, the putative mechanisms linking miRNAs and endometriosis in these *in silico* analyses remain speculative until experimentally confirmed in endometrial cell lineages. To our knowledge, no such validation studies have yet been performed. The models for miRNA regulation in endometriosis presented here (summarized in Tables I and II; Figs. 2 and 3) are restricted to the functions of experimentally confirmed miRNA targets of endometriosis-associated miRNAs in other diseases, including in epithelial, fibroblast and myoblast cell lineages. Nevertheless, these cells constitute endometriotic lesions and participate in pathological processes, such as cell proliferation, migration and angiogenesis, that are common to endometriosis, cancer and many other diseases. The control of cellular events these miRNAs exert in other diseases may thus also occur in endometriosis.

Hypoxic injury

Several researchers have identified markers of hypoxia in ectopic endometrial lesions from women with endometriosis and in animal models of this disorder. We suggest that the broad effects of hypoxia on the up-regulation of hypoxia inducible transcription factors (HIFs) are modified by miRNAs in endometriosis.

CREB binding protein (CREBBP) is a co-activator of HIF1α, a hypoxia induced and pro-angiogenic transcription factor (Kaluz et al., 2008). CREBBP/HIF1α activities are likely to be increased in ectopic endometriotic lesions due to loss of transcript suppression by two down-regulated miRNAs, miR-20a and miR-200b (Ohlsson Teague et al., 2009).
et al., 2009). Reduced repression of HIF1α mRNA translation by miR-20a (Taguchi et al., 2008) is consistent with the elevated HIF1α mRNA levels seen in endometriotic lesions (Wu et al., 2007; Becker et al., 2008). Similarly, since CREBBP is predicted to be targeted by miR-200b, reduced miR-200b suppression may contribute to the up-regulation of CREBBP mRNAs in endometriosis (Hull et al., 2008). CREBBP was also central in one of our miRNA regulated IPA networks associated with angiogenesis in endometriosis (Ohlsson Teague et al., 2009). The result of reduced miRNA translational suppression of CREBBP, HIF1α and other hypoxic markers may be increased angiogenesis, an improvement in oxygen delivery to ischaemic tissues and potentially, survival of injured endometrial tissues at an ectopic site.

miR-15b and miR-16 comprise a down-regulated miRNA cluster in endometriosis (Pan et al., 2007) which was repressed during hypoxic conditions in a human epitheloid carcinoma cell line (Hua et al., 2006). Hypoxic repression of miR-15b/miR-16 activities resulted in increased transcription of two of its confirmed targets, the pro-angiogenic factors vascular endothelial growth factor (VEGF)-A (Hua et al., 2006) and cyclooxygenase-2 (COX-2) (Shanmugam et al., 2008). In endometriosis, increased expression of VEGF-A (Hull et al., 2008) and COX-2 (Ota et al., 2001) may thus be a response to hypoxic injury, as well as being strongly implicated in the growth and survival of endometriotic deposits (Matsuzaki et al., 2004; Nap et al., 2004; Ozawa et al., 2006; Eyster et al., 2007; Laschke et al., 2007; Becker et al., 2008; Hull et al., 2008), as discussed below.

**Inflammation**

In endometriosis, pro-inflammatory conditions, such as elevated levels of interleukin-1 beta and tumour necrosis factor alpha (TNF-α) (Mori et al., 1991; Keenan et al., 1995), activate nuclear factor κB (NFκB) (Paik et al., 2002; Cao et al., 2005, 2006) and HIF1α signalling (Jung et al., 2003) leading to, among other effects, enhanced COX-2 transcription (Tamura et al., 2002a, b; Wu et al., 2002). COX-2 is a rate limiting enzyme in the pro-inflammatory prostaglandin pathway that also demonstrates pro-angiogenic activity (Smith et al., 1996). Additionally, COX-2 participates in a positive feed forward loop that enhances aromatase activity and local estradiol production in endometriotic lesions, thereby promoting a proliferative local hormonal environment (Bulun et al., 2000).

COX-2 translation is known to be suppressed by miR-199a* and miR-16 (Chakrabarty et al., 2007; Shanmugam et al., 2008) and both of these miRNAs were down-regulated in endometriosis (Pan et al., 2007). Moreover, miR-199a targets IκB kinase-β (IκKB), which is a co-factor required for NFκB activation (Chen et al., 2008). This signalling network was also evident in a miRNA regulated IPA network associated with endometriosis (Ohlsson Teague et al., 2009). Therefore, it is possible that reduced expression of miR-199a and miR-16 may work synergistically to promote an inflammatory environment by up-regulating COX-2 protein levels, thereby promoting prostaglandin production, neoangiogenesis and estradiol mediated cellular proliferation in endometriotic tissues. The importance of COX-2 activity in endometriosis was highlighted by the finding by several (Matsuzaki et al., 2004; Ozawa et al., 2006; Laschke et al., 2007).
but not all (Hull et al., 2005) research groups, that COX-2 inhibitors reduce ectopic endometrial lesion development in animal models of endometriosis. It seems reasonable to propose that a similar therapeutic effect could be achieved through therapeutic enhancement of miR-199a or miR-16 activities.

**Tissue repair and TGFβ-regulated pathways**

TGFβ activity is up-regulated in the peritoneal fluid of women with endometriosis (Oosterlynck et al., 1994) and several studies have identified this growth factor as a central component in molecular signalling networks associated with endometriosis (Animoto et al., 2003; Komiyama et al., 2007; Hull et al., 2008; Kyama et al., 2008a,b). We have recently found that human endometriotic lesion development is restricted in TGFβ1 knockout mice on a SCID background (unpublished data). We hypothesize that TGFβ mediates a switch from an initially ischaemic and inflammatory environment that causes tissue damage and necrosis, to a ‘healing’ milieu that promotes cellular proliferation and tissue remodelling during endometriotic lesion development (Hull et al., 2008).

There is evidence that TGFβ activity is subject to miRNA control in endometriosis. TGFβ1 and -2 mRNA translation is repressed by miR-21 (Lu et al., 2009) and miR-141 (Burk et al., 2008), respectively. These miRNAs are down-regulated in endometriosis (Pan et al., 2007; Ohlsson Teague et al., 2009) and relief of miR-21/141 suppression could account for the enhanced TGFβ activity in endometriosis. Additionally, a negative feedback mechanism exists whereby TGFβ signalling induces TGFβ-induced factor (TGFIF) transcription, which in turn suppresses TGFβ activity (Chen et al., 2003). This regulation may be deficient in ectopic endometrial tissues, as TGFIF mRNA transcripts were down-regulated (Hull et al., 2008) and TGFβ signalling pathways augmented, miR-1 and miR-194 are both up-regulated in endometriotic tissues (Ohlsson Teague et al., 2009) and predicted to suppress TGIF translation. We propose that dysregulation of miR-1, miR-21, miR-141 and miR-194 may synergistically enhance TGFβ signalling in endometriotic lesions by increasing TGFβ expression and restraining TGIF’s suppressive activity. TGFβ also interacts with the retinoid receptor RXRα (Bartholini et al., 2006), and a TGFβ2–TGIF–RXR axis appeared in two of our endometriosis associated miRNA regulated IPA networks (Ohlsson Teague et al., 2009). Thus, miR-1/194 mediated TGIF repression may have effects on retinoic acid signalling, promoting tissue remodelling, cell proliferation and invasiveness in endometriosis.

**Cell growth, proliferation and apoptosis**

Apoptotic resistance is mediated by intracellular proteins such as B-cell CLL/lymphoma 2 (BCL2), leading to enhanced survival of stressed endometrial cells in endometriosis (Meresman et al., 2000). miR-15b/16 targets BCL2 (Cimmino et al., 2005), and the reduced expression of these miRNAs may possibly contribute to increased activities of this anti-apoptotic protein in endometriosis.

Cell proliferation is promoted by the cell cycle regulator insulin receptor substrate-1 (IRS1). Two highly up-regulated miRNAs in endometriosis, miR-126 and miR-145 (Ohlsson Teague et al., 2009) target this mitogenic protein (Shi et al., 2007a; Zhang et al., 2008a) and may inhibit endometrial cell proliferation. miR-145 is co-expressed with miR-143 in an up-regulated miRNA cluster (Ohlsson Teague et al., 2009) and miR-143 may additionally suppress cell proliferation via repression of the growth promoting proteins mitogen-activated protein kinase 7 (MAPK7) (Esau et al., 2004; Akao et al., 2007) and KRAS (Chen et al., 2009).

The up-regulation of cell cycle repressors represents another mechanism of miRNA action in endometriosis. The low levels of miR-20a (Ohlsson Teague et al., 2009), miR-221 and miR-222 (Pan et al., 2007) seen in endometriotic tissues may ease post-transcriptional suppression of their up-regulated mRNA targets, the cell cycle repressors cyclin-dependent kinase inhibitor 1A (CDKN1A/p21) (Dufournet et al., 2006; Hever et al., 2007; Inomata et al., 2009), CDKN1B (p27) and CDKN1C (p57) (Matsuzaki et al., 2001; Galardi et al., 2007; Visone et al., 2007; Formari et al., 2008; Hull et al., 2008; Medina et al., 2008; Inomata et al., 2009). In summary, high miR-15b/16, miR-143, miR-145 and low miR-20a, miR-221 and miR-222 expression are consistent with repressed cell proliferation and enhanced cell survival in endometriosis.

**Extracellular matrix remodelling**

Endometriotic lesion development has been associated with an aberrant expression of extracellular matrix proteins (Kleemann et al., 2007). Collagens COL1A1 (Hever et al., 2007; Hull et al., 2008), COL1A2 (Hull et al., 2008), COL3A1 (Hever et al., 2007), COL5A1 (Eyster et al., 2007) and secreted protein, acidic, cysteine-rich, osteonectin (SPARC) (Hull et al., 2008) mRNAs were up-regulated in human ectopic endometrial lesions and encode extracellular matrix proteins involved in cell migration and cancer metastasis. We found by IPA analysis that miRNA regulated networks converging on JUN/FOS, IFNγ and progesterone incorporated COL1A1, COL1A2, COL3A1, COL5A1 and SPARC, which are all experimentally proven targets of miR-29c (Sengupta et al., 2008). As miR-29c is up-regulated in endometriotic tissue (Ohlsson Teague et al., 2009) it may be that control of extracellular matrix production is integral to endometriotic lesion development. Precise organization of the extracellular matrix scaffold may be crucial for the correct placement of developing glands and stroma in remodelling tissues and miRNAs may contribute by fine tuning this process.

**Angiogenesis**

The importance of angiogenesis in endometriotic lesion development is demonstrated by the poor development of endometriosis-like lesions in animal models exposed to anti-angiogenic agents (Hull et al., 2003; Nap et al., 2004; Becker et al., 2008). We found that miRNAs may be implicated in this process, since several angiogenesis related transcripts are confirmed targets of endometriosis-associated miRNAs.

In endothelial cells, miR-126 enhances VEGF and fibroblast growth factor (FGF) signalling via repression of inhibitors of these pathways, leading to neoangiogenesis and the development of a mature vasculature (Fish et al., 2008; Guo et al., 2008; Kuhnert et al., 2008; Wang et al., 2008). Moreover, a recent in vivo study showed that silencing of miR-126 impaired ischemia-induced angiogenesis in mice (van Solingen et al., 2008). miR-126 is embedded in the EGF-like-domain, multiple 7 (EGFL7) gene, and both these transcripts were highly up-regulated in ectopic versus eutopic endometrium (Hull et al., 2008; Ohlsson Teague et al., 2009) which is indicative of co-transcription. EGFL7 enhances the effect of miR-126 by inducing endothelial cell migration (Schmidt et al., 2007) during neovascularisation. The angiogenic functions of miR-126 are summarized in Fig. 4.
Perspectives on differential miRNA expression in endometriosis

The evidence supporting our hypotheses regarding the mode of action of miRNAs in endometriotic disease is largely indirect. To confirm the role of miRNA expression in endometriosis it will be necessary to characterize the miRNA expression profiles and to validate mRNA targets in endometrial and ectopic endometrial cell lineages.

Many endometriosis-associated miRNAs are also implicated in other pathological conditions such as reproductive malignancies and fertility disorders, as summarized in Supplemental Table SI. Although as yet there is no direct evidence that miRNAs mediate the effect of endometriosis on these conditions, this possibility will be discussed in the following sections.

miRNAs and malignant transformation in endometriosis

Cancer risk in endometriosis

Historically, endometriosis has been comparatively similar to malignant reproductive disease in terms of familial predisposition, ability to spread to distant organs, identified genetic alterations in tissues and existence of an altered immunobiological, angiogenic and hormonal environment (Nezhat et al., 2008). Several large population based studies have demonstrated that women diagnosed with endometriosis have a statistically significant increased risk of developing several types of cancer (Brinton et al., 1997; Olson et al., 2002; Melin et al., 2006). Ovarian cancer was the cancer most strongly associated with endometriosis, with a standard incidence ratio of between 1.3 and 2.2 (Brinton et al., 1997; Ness et al., 2000; Borgfeldt and Andolf, 2004; Brinton et al., 2004; Melin et al., 2006).

Van Gorp and colleagues estimated that ovarian cancer prevalence was 0.9% for women with endometriosis, and that 35.9% of clear cell cancers and 19% of endometriod carcinomas were associated with concomitant pelvic endometriosis (Van Gorp et al., 2004). Common genetic alterations were also seen in ovarian cancers adjacent to endometriotic tissues (Sato et al., 2000; Wells, 2004). These studies suggest that malignant change can occur in endometriotic tissues in a small number of women.

miRNA abnormalities in cancer

miRNA genes are frequently dysregulated in cancer and many miRNA genes are located within genomically unstable sites and cancer-associated genomic regions (Calin et al., 2004; Calin and Croce, 2006; Zhang et al., 2006). Additionally, abnormal DNA copy numbers, mutations, epigenetic alterations of miRNA expression (such as hypermethylation) and chromosomal translocation can silence miRNA expression with oncogenic consequences (Calin and Croce, 2007).

miRNA dysregulation may promote tumourigenesis in two ways. Firstly, miRNAs that target tumour suppressing activities are often up-regulated in cancer. These so-called oncomiRs act to remove tumour repressor constraints on cellular growth and proliferation, promoting cancer development. Alternatively, down-regulation of tumour suppressor miRNAs permits unsuppressed oncogene
<table>
<thead>
<tr>
<th>miRNA (hsa)</th>
<th>Experimentally confirmed miRNA repression of target mRNA</th>
<th>Target mRNA</th>
<th>Cell line used for luciferase assay</th>
<th>References</th>
<th>miRNA target in endometriosis</th>
<th>Proposed miRNA function</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-1i</td>
<td>TMSB4X, HAND2, ADAR, CAND1, KIF2A, POGK, HCN2, HCN4</td>
<td>COS1</td>
<td>HeLa (human cervical epithelial adenocarcinoma)</td>
<td>Zhao et al. (2005)</td>
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<td>LMO2</td>
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Up-regulated (↑) and down-regulated (↓) expression of validated miRNA targets in endometriosis. Extracellular matrix (ECM); i Ohlsson Teague et al. (2009); ii* down-regulated in (Pan et al., 2007).
<table>
<thead>
<tr>
<th>miRNA (hsa-)</th>
<th>Experimentally confirmed miRNA repression of target mRNA</th>
<th>Cell line used for luciferase assay</th>
<th>References</th>
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<th>Target mRNA regulation</th>
<th>Proposed miRNA function</th>
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<td>miR-15b</td>
<td>BCL2 MEG-01 (human megakaryocytes), HeLa (human cervical epithelial adenocarcinoma)</td>
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Up-regulated (↑) and down-regulated (↓) expression of validated miRNA targets in endometriosis. Epithelial-Mesenchymal Transition (EMT). i Ohlsson Teague et al. (2009); ii Pan et al. (2007).
translation and activity, enhancing malignant events. Some miRNAs and their targets possess both oncogenic and tumour suppressive functions, demonstrating the high level of complexity of miRNA mediated regulation in malignant disease (Fabbri et al., 2008).

miRNAs in epithelial–mesenchymal transition

Epithelial–mesenchymal transition (EMT) is a reversible biological process induced by growth factors where stationary epithelial cells obtain a migratory, mesenchymal phenotype (reviewed in Thiery and Sleeman, 2006). It has been suggested that many ovarian cancers develop during wound healing at the time of ovulation, when EMT induced migratory ovarian surface epithelium becomes trapped in inclusion cysts in the ovarian stroma (reviewed in Auer-sperg et al., 2001). Neoplastic progression may occur in these cysts leading to ovarian cancer and altered miRNA expression is implicated in this process (Zhang et al., 2008b).

Epithelial/mesenchymal phenotypic plasticity is regulated by a set of regulatory feedback loops involving TGFβ signalling, members of the miR-200 family, and transcription factors zinc finger E-box binding transcription factor 1 (ZEB1) and ZEB2. When TGFβ signalling is increased, this system is shifted towards low miR-200 expression and high ZEB1/ZEB2 mediated E-cadherin suppression, promoting a mesenchymal phenotype, summarized in Fig. 5 (reviewed in Gregory et al., 2008). Metastatic spread of tumour cells is also promoted by miRNA regulated EMT, giving rise to an invasive cellular phenotype that enables tumour cells to detach and spread through the blood system to secondary sites (Bracken et al., 2009). It has thus been suggested that forced miR-200 expression in aggressive tumours may prevent metastasis in a therapeutic setting.

In addition to wound healing, EMT also occurs during fibrosis and tissue remodelling, processes strongly associated with endometriosis (Sillem et al., 1998; Hudelist et al., 2005; Hull et al., 2008). The low miR-200 expression (Ohlsson Teague et al., 2009) and increased TGFβ activity (Oosterlynck et al., 1994; Arimoto et al., 2003; Komiyama et al., 2007; Hull et al., 2008) in endometriosis may promote EMT and a migratory mesenchymal phenotype in endometriotic tissues (Gaetje et al., 1995; Demir et al., 2004).

Oncogene and tumour suppressor regulation by miRNAs

miR-125a and miR-125b are tumour suppressor miRNAs that repress ERBB2 (HER2) and ERBB3 (HER3), oncogenic components of the

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**Figure 5** miR-200 regulates epithelial–mesenchymal transition. 

(A) Members of the miR-200 family (miR-141, miR-200a, miR-200b, miR-200c and miR-429) are expressed in two miRNA clusters from chromosome (Chr) 1 and 12. (B) Mature miR-200 promotes E-cadherin expression and an epithelial cell phenotype via post-transcriptional repression of zinc finger E-box binding transcription factor 1 (ZEB1), ZEB2 and TGFβ. During epithelial–mesenchymal transition (EMT), augmented TGFβ signalling induces ZEB1 and ZEB2 expression, leading to transcriptional repression of E-cadherin and a mesenchymal, invasive cell phenotype. This is maintained via ZEB1/ZEB2 repression of miR-200 transcription in a double-negative regulatory feedback loop. This process is reversible, since enforced miR-200 expression initiates mesenchymal–epithelial transition (MET), reinstalls E-cadherin expression and an epithelial phenotype.
epidermal growth factor (EGF) receptor family (Scott et al., 2007). Members of the miR-125 family were up-regulated in the endometriotic lesions (Ohlsson Teague et al., 2009), which would repress ERBB signalling as well as control cell migration and invasion (Fig. 2) (Nasu et al., 1995; Hull et al., 2008). In ovarian cancers, the loss of miR-125a/b activity (Iorio et al., 2007; Nam et al., 2008; Yang et al., 2008) may account for elevated ERBB2 levels seen in ovarian endometrioid adenocarcinomas arising from endometriosis, when compared with endometriosis-independent ovarian cancers (Prefumo et al., 2003). Therapeutic reinstatement of miR-125a/b expression could therefore have preventive effects on tumour progression and metastasis in these cancers. An IPA network comprised of targets of differentially regulated miRNAs in endometrial cancer (Boren et al., 2008) contained members of the ERBB2 signalling pathway, providing further evidence of a role for miR-125a/b in endometriosis associated malignancy.

Progression from endometriosis to endometrioid cancer was dependent on activation of the KRAS oncogene and deletion of the PTEN tumour suppressor gene in a genetic mouse model (Dinulescu et al., 2005). Moreover, conditional deletion of endometrial PTEN, particularly when combined with loss of p53, resulted in aggressive endometrioid cancer lesion development and mortality in a mouse cancer model (Daikoku et al., 2008). PTEN is a confirmed target of miR-21 (Meng et al., 2007), miR-26a (Huse et al., 2009) and miR-214 (Yang et al., 2008), and its activity is likely to be suppressed in ovarian and endometrioid cancers as high miR-21 and miR-214 levels were present. In endometriosis, down-regulation of miR-21, miR-26a and miR-214 transcript levels (Pan et al., 2007) may enhance tumour suppressor activities. It appears that PTEN inactivation is an early event in malignant transformation in endometriosis, and that this potentially is a combined effect of miRNA regulation and mutation of the PTEN gene (Sato et al., 2000).

Cell proliferation is controlled by a complex miRNA regulatory network, involving members of the miRNA cluster miR-17–92, miR-26a, the regulator of gene expression enhancer of zeste homolog 2 (EZH2), oncogenes KRAS and MYC, as well as EZF transcription factors, summarized in Fig. 2 (reviewed in Bueno et al., 2008; Sander et al., 2009). Aguda et al. (2008) suggested that the miR-17–92 miRNA cluster has a critical role in regulating the steady state levels of the components of these regulatory feedback loops, carrying out both oncogenic and tumour suppressive properties. Transcripts of miR-20a, a member of the miR-17–92 cluster (Ohlsson Teague et al., 2009), miR-26a (Pan et al., 2007), MYC (Meola et al., 2009), as well as EZF1 and EZF3 (Hull et al., 2008) were all down-regulated in ectopic versus eutopic endometrial lesions. On the basis of these findings, we propose that the collective dysregulation of miR-16, miR-20a, miR-21, miR-26a, miR-34c, miR-125a/125b, miR-126, miR-143, miR-145, miR-221 and miR-222 strictly controls the cell cycle and suppresses malignant transformation in endometriosis (Fig. 2).

In summary, our findings suggest that the miRNA profile in endometriosis may regulate transcripts involved in the cell cycle and cancer, and this is consistent with microarray gene expression studies by others (Borghese et al., 2008; Zafrakas et al., 2008). Although these findings could be a result of comparing highly regenerative eutopic endometrium with less actively proliferating pockets of endometriotic tissue, it may be that strong miRNA mediated cell cycle control in the surviving tissue explants is a mechanism that results in the maintenance of a benign phenotype in endometriotic tissues.

### miRNAs in endometriosis and subfertility

Many studies have reported an association between endometriosis and subfertility (Mahutte and Arici, 2002; Gupta et al., 2008) however, the mechanisms underlying this association are controversial. Poor oocyte and embryo development, decreased fertilization, an abnormal peritoneal environment and blunted endometrial receptivity may all contribute to reduced conception in women with endometriosis (Gupta et al., 2008). Although there is strong evidence that miRNAs are integral in these fertility events, no studies have directly explored the possibility that miRNAs mediate endometriosis-associated suppression of fertility. The role of miRNAs in oocyte and embryo function and in endometrial receptivity will be presented here, as this will inform future studies that explore the role of miRNAs in endometriosis-associated subfertility.

**Oocyte and embryonic development is regulated by maternal miRNAs**

Female mice with global miRNA deficiency as a consequence of a germ line specific deletion of Dicer1 are sterile from several causes, including defects in oocyte function (Murchison et al., 2007; Tang et al., 2007). Oocytes from these mice have disorganized meiotic spindles and elevated abundance of maternal mRNA transcripts that are normally degraded during meiotic maturation. Many of these maternally-derived oocyte transcripts are predicted targets of oocyte-specific miRNAs. These experiments show that DICER1 controls oocyte and early zygotic development by enhancing miRNA repression of maternally-inherited cytoplasmic mRNAs. The sequential breakdown of maternally-derived cytoplasmic mRNAs in the oocyte appears to be vital to gamete production.

Women with endometriosis have a longer follicular phase (Cahill et al., 1997), a slower follicular growth rate (Doody et al., 1988) and reduced dominant follicle size (Tummon et al., 1988) compared with women with unexplained infertility. Therefore, it is possible that in endometriosis, the sequence of oocyte maturation events is disrupted by altered miRNA degradation of maternally-derived miRNAs.

### miRNAs in implantation

A large number of miRNAs are up-regulated in the receptive uterus and appear to play a significant role in implantation (Shingara et al., 2005; Chakrabarty et al., 2007). For example, miR-199a targets COX-2 (Chakrabarty et al., 2007) and leukemia inhibitory factor (LIF) (Oskowitz et al., 2008), which are markers of uterine receptivity. In murine endometrium, miR-199a was expressed with COX-2 in a spatiotemporal manner during implantation and this coincided with reduced COX-2 protein levels (Chakrabarty et al., 2007). Similarly, miR-21 and miR-26a were up-regulated at murine implantation sites whereas RECK, a confirmed target of miR-21, was down-regulated (Hu et al., 2008). miR-21 and miR-26a were both highly expressed...
in human normal endometrium, and down-regulated in eutopic versus
eutopic endometrium from women with endometriosis (Pan et al.,
2007). It appears that miRNA regulation of COX-2 and RECK is a
mechanism that ensures precise control of implantation events, and
this regulation may be altered in endometriosis (Ota et al., 2001; Hever
et al., 2007; Hull et al., 2008).

Women with endometriosis have reduced implantation rates
(Barnhart et al., 2002; Trinder and Cahill, 2002) and researchers have
developed the relative influence of poor embryo quality and altered uterine receptivity in this effect (Mahutte and Arici 2002;
Gupta et al., 2008). Progesterone resistance, a hallmark for endome-
triosis, can be studied in Fkbp52−/− mice deficient in immunophilin, a
cochaperone progesterone receptor. The profound implantation failure in these mice may be rescued by progesterone supplementation
(Tranguch et al., 2007). Implantation phase, eutopic endometrium from
baboons with endometriosis demonstrated a Fkbp52-associated reduced response to progesterone, when compared with endome-
trium from the same animals prior to disease induction (Jackson et al., 2007). Furthermore, an attenuated response to progesterone
was revealed in a human mRNA microarray study comparing implant-
ation phase eutopic endometrium from women with and without endometriosis (Burney et al., 2007). In donor oocyte programmes,
women with endometriosis who received oocytes from disease-free
women had similar rates of implantation compared with those
without endometriosis (Pellicer et al., 2001; Katsoff et al., 2006).
Although this finding suggests that implantation is minimally effected
by endometriosis, endometriosis-affected recipients of donor oocytes receive estrogen and progesterone supplements in an artificial
cycle, which may improve their uterine receptivity.

Ovarian steroids regulate miRNA expression in vitro (Pan et al.,
2007; Toloubeydokhti et al., 2008) and in vivo (Dai et al., 2008) and
alter the expression of miRNAs predicted to target transcripts
involved in COX-2 and RECK signalling in human endometrial stromal cell lines (Pan et al., 2007; Toloubeydokhti et al., 2008). Hu
et al. (2008) concluded that miR-21 may fine tune the uterine func-
tions of estrogen and progesterone during implantation in mammals.
It is possible that an underlying steroid dysregulation in eutopic endo-
metrium of women with endometriosis alters miRNA-regulated
COX-2 and RECK (Hever et al., 2007; Hull et al., 2008) activities, and thereby implantation in natural cycles, and that this effect is
attenuated in steroid-supplemented recipients with endometriosis under-
going donor oocyte conception programmes.

Therapeutic and diagnostic potential of miRNAs in
endometriosis

Women with endometriosis commonly experience a diagnostic delay
of 6–12 years (Dmowski et al., 1997) and this delay is associated with
increased severity of disease (Matsuzaki et al., 2006b). Laparoscopic visualization of endometriotic lesions remains the ‘gold standard’
in diagnostic tests for endometriosis, however, only 70–75% of visually
diagnosed lesions are confirmed histologically (Spaczynski and
Duleba, 2003). The identification of a non-invasive biomarker for
endometriosis would reduce surgical risk, potentially permit non-
invasive monitoring of endometriotic disease progression and facilitate

early treatment of endometriosis. Early diagnosis of endometriosis has
been associated with improved pain levels and physical and psycho-
logical functioning (Gao et al., 2006b).

miRNAs as biomarkers

miRNAs can be readily detected in tissues from routine pathology
archives, as they are less subject to RNase degradation compared
with other RNAs (Hoefig et al., 2008). In a study of various human
cancers, tissue miRNA expression profiles identified both physiological and pathological conditions more accurately than mRNA abundance
signatures (Lu et al., 2005). Malignancies have highly specific miRNA
profiles that reflect their developmental lineages and differentiation
(Rosenfeld et al., 2008) and potential miRNA biomarkers that
display high sensitivity and specificity have been identified for a
variety of tumour types (Iorio et al., 2007). It is thus clear that cellular
processes are regulated by miRNAs in a cell-lineage and disease-
dependent manner.

Women with endometriosis have characteristic differences in
eutopic endometrial transcript and protein profiles when compared
with women without endometriosis. Several researchers have tried
to develop a semi-invasive test for endometriosis by analysing
eutopic endometrial biopsies obtained at an outpatient clinic visit. It
seems reasonable to postulate that the eutopic endometrial miRNA
profile can be used to distinguish eutopic endometrium from
women with and without endometriosis in a simple, reliable way
with good sensitivity and specificity.

A strong correlation was demonstrated between the miRNA pro-
files of serum and cancer tissue in patients with ovarian cancer
(Taylor and Gercel-Taylor, 2008; Resnick et al., 2009), suggesting
that miRNAs may be secreted from tissues into the bloodstream.
This hypothesis was proven in a mouse model of human prostate
cancer, where human-specific miRNAs originating from transplanted
human prostate cells were readily detected in serum of xenografted
mice, but not in controls (Mitchell et al., 2008). If endometriosis-
associated miRNAs can be identified in serum, a non-invasive blood
test could be developed to diagnose this chronic condition.

miRNAs as therapeutic targets in
endometriosis

Medical therapies such as GnRH analogues, progestrone
and Danazol are equally effective and better than placebo in treating endo-
metriosis pain (Kennedy et al., 2005). However, these treatments have
side effects such as bone loss and hirsutism that limit their use.
Additionally these medications act to suppress ovulation and are not
suitable for patients who wish to conceive (Hughes et al., 2007).
In 2008, an international consensus group identified the development of
non-hormonal medical treatments to prevent or treat endometrio-
sis as a research priority (Rogers et al., 2009). There are several strat-
egies by which miRNA technologies might be utilized in novel,
non-hormonal therapeutic approaches for endometriosis.

Antagonism of miRNAs

AntimiRs and antagomiRs are single-stranded oligonucleotides that are
designed to specifically base-pair to mature complementary miRNAs,
preventing their suppression of target miRNAs, as exemplified in Fig. 4
(reviewed in Esau and Monia, 2007; Esau 2008; Stenvang et al., 2008).
Chemical modification of the original 2′-O-methyl miRNA antisense oligonucleotides (Hutvagner et al., 2004) has improved cellular uptake, thermodynamic stability, toxicity and solubility profiles.

Cholesterol-conjugated antagonomiRs, 2′-O-methoxymethyl-modified (2′MOE) oligonucleotides, and locked nucleic acid (LNA)-antimiRs have been used to successfully treat miR-122 associated disease in mice (Krutzenfeldt et al., 2005; Esau et al., 2006; Krutzenfeldt et al., 2007; Elmen et al., 2008b) and in non-human primate models (Elmen et al., 2008a). In these studies, antagonism of miR-122, a liver-specific miRNA, led to up-regulation of several transcripts that act to inhibit cholesterol synthesis, and caused a long lasting dose-dependent lowering of plasma cholesterol. Other in vivo studies showed that antagonism of miR-29 and miR-133 improved outcomes in cases of cardiac fibrosis and hypertrophy (Care et al., 2007; van Rooij et al., 2008). As the first human clinical trials of LNA-antimiRs and 2′MOEs are in progress, anti-miRNA targeting therapeutics may become available for the treatment of miRNA-associated human disease in the near future.

**miRNA decoys**

An alternative approach to prevent miRNA target binding is to introduce a vector expressing a series of target sequences complementary to the miRNA seed region into diseased cells. These synthetic decoy miRNA targets compete with endogenous miRNAs for miRNA regulation and miRNA suppression of the targeted endogenous miRNA is relieved, enhancing its functional activity (Ebert et al., 2007). This approach is a powerful way of inhibiting the function of an entire miRNA family, as these recognize the same binding site in a target.

Unlike antagonomiRs and antimiRs, miRNA decoys can be stably integrated into the genome, thus limiting their use in humans. However, they are useful as a tool to generate miRNA depleted cell lines or transgenic mice and they can be used to simultaneously study the complex functions of multiple miRNAs in vivo. miRNA decoys have been successfully used in mouse models and may become an important supplement to genetic knockouts (Gentner et al., 2009).

**miRNA mimics**

Another potential therapeutic approach is to increase the degree of miRNA regulation and suppress transcription of miRNAs that promote disease activity. miRNA mimics are synthetic double stranded RNA duplexes, similar to endogenous miRNA–miRNA* duplexes, unwind to allow the guiding miRNA mimic single strand to be loaded into the RISC and execute post-transcriptional regulation of target miRNAs. This technology has mainly been used to study miRNA functions in vitro and miRNA mimics have repressed the Bcr-Abl oncogene, altering the leukemogenic potency of hematopoietic cells in mice (McLaughlin et al., 2007).

**Challenges in miRNA based therapeutics**

There are several obstacles in translating advances in miRNA research into medical practice, including a paucity of appropriate animal models, difficulties delivering miRNAs to specific cells in vivo and the need to optimize the design of miRNA-based therapeutic agents. The possibility of unpredictable and unacceptable side effects due to either ‘off-target’ actions of a miRNA-based therapeutic agent, or insertional mutagenesis of a viral vector used for therapeutic delivery, must be excluded before miRNA based therapies can be trialed in humans. Currently, many techniques developed in the fields of antisense technology and gene therapy are being adapted to reduce the risk and enhance the development of new miRNA therapeutics.

As miRNAs can regulate multiple, functionally-related mRNAs, entire molecular pathways can hypothetically be controlled by miRNA-based therapeutics, and this may prove beneficial in disease treatment. However, before a new miRNA based treatment is adopted, these positive effects have to be carefully weighed against the adverse effects of altering the function of a single miRNA. With improved delivery and vector design, the use of these miRNA-based technologies may provide new strategic options for the treatment of miRNA-related diseases in humans.

**Conclusions and further perspectives**

The recent discovery of differential expression of miRNAs in endometriotic tissue has generated a new bioinformatic approach to reveal functional information from miRNA and miRNA tissue profiling in this disorder. It is only beginning to become apparent that miRNAs may regulate cellular events that are critical to the development of endometriosis, its maintenance as a benign condition, as well as its suppression of fertility. With further studies on the role of miRNA expression in endometriotic tissue, miRNA research is emerging as an exciting field that holds promise for improvements in diagnosis and treatment for endometriosis.

In the near future, our understanding of female reproductive pathol-

ogy is likely to expand rapidly alongside our growing knowledge of non-coding RNAs such as miRNAs. We hope that this knowledge can be translated into genuine benefit for women with this debilitating disease.

**Supplementary data**

Supplementary data are available at http://humupd.oxfordjournals.org/.

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microRNA expression in endometriosis


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