Published in final edited form as:
Anim Reprod. 2010 ; 7(3): 117–128.

Uterine microRNA signature and consequence of their dysregulation in uterine disorders

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Abstract

MicroRNA (miRNA) has emerged as key post-transcriptional regulator and through this mechanism control many normal developmental and physiological processes. Conversely, aberrant expression of some miRNAs has been correlated with various disorders, more specifically, development and progression of malignancy. Endometrium is a dynamic tissue which undergoes extensive cyclic changes in preparation for embryo implantation during reproductive years, as well as changes that occur following menopause, and establishment of benign and malignant uterine disorders. These processes are highly regulated by ovarian steroids and locally expressed genes in response to steroid hormone receptor-mediated signaling and include genes related to inflammatory reaction, apoptosis, cell-cycle progression, angiogenesis and tissue remodeling. Here we present an overview of our current understanding of uterine miRNA biogenesis and highlights their potential regulatory functions in cellular processes relevant to normal uterine physiological and pathological disorders such as endometriosis, dysfunctional uterine bleeding and endometrial cancer. Understanding the expression, regulation and functional aspects of miRNAs in uterine environment under normal and various disorders may lead to their potential utilization as diagnostic as well as therapeutic tool.

Keywords

microRNA; gene expression; regulation; uterus; endometrium; disorders

Introduction

The endometrium is a dynamic tissue which undergoes highly organized cyclic structural changes in preparation for embryo implantation during the reproductive years. These overlapping and dynamic endometrial morphological and molecular changes are remarkably consistent during each cycle and, in many aspects, resemble the repair processes that occur during wound healing. Wound repair and cyclic endometrial regeneration are both initiated by an inflammatory reaction followed by a rapid cell proliferation, angiogenesis, differentiation (tissue formation), and tissue remodeling. Although wound repair in response to inflammation and/or mechanical injuries is often associated with excess-tissue formation and scarring (Chegini, 2002), cyclic endometrium undergoes near perfect regeneration. These processes are under the control of ovarian steroid actions and are essential for endometrial preparation for embryo implantation. The ovarian steroids actions are regulated by their specific receptors and by steroid metabolizing enzymes. In addition to estrogen and progesterone, evidence suggests that glucocorticoids and androgens may influence the above endometrial cellular activities (for review see (King and Critchley, 2010).

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Estrogen and progesterone receptors (ERs and PRs) each consist of at least two isoforms, ERα and ERβ and PRA and PRB. The endometrial profile and cellular distribution of ERs and PRs have been well documented throughout the normal menstrual cycle, the post-menopausal period and pathological conditions; and knockout mice models implicated their fundamental biological and physiological roles in uterine and other steroid-sensitive tissues (Conneely *et al*., 2003; Zhao *et al*., 2008a; Critchley and Saunders, 2009; Ellmann *et al*., 2009; Deroo and Buensuceso, 2010; King and Critchley, 2010). Upon ligand binding, ERs and PRs undergo conformational changes, bind to specific enhancer DNA elements of their target genes and through recruitment and interactions with co-regulators (co-activators and co-suppressors) modulate their transcription in a cell- and promoter-specific manner (Conneely *et al*., 2003; O'Malley and McKenna, 2008; Zhao *et al*., 2008a; Ellmann *et al*., 2009). Considerable evidence exists regarding the endometrial expression of various autocrine/paracrine factors many of which are direct targets of ER and PR regulatory actions (Girling and Rogers, 2005; Achache and Revel, 2006; Jabbour *et al*., 2006; Makker and Singh, 2006; Du and Taylor, 2007; Horcajadas *et al*., 2007, 2008; Critchley and Saunders, 2009; Altmae *et al*., 2010; King and Critchley, 2010). Transcriptional regulation of these mediators is considered to serve as downstream signal of ER and PR actions in the endometrium and other steroid-sensitive tissues. Any alteration in production of ovarian steroids, exposure to excess exogenous hormones, and changes in ERs and PRs expression, which results in differential regulation of these mediators, seems to be responsible for inappropriate tissue regeneration, embryo implantation failure and other abnormalities, including dysfunctional uterine bleeding, endometriosis and endometrial cancer (Ferenczy, 2003; Abal *et al*., 2006; Harada *et al*., 2007; Prat *et al*., 2007; Horcajadas *et al*., 2008; Critchley and Saunders, 2009; Altmae *et al*., 2010; King and Critchley, 2010).

miRNAs biogenesis and steroid hormone target genes

Whether regulated independently, or through an ovarian steroid-dependent manner, endometrial gene expression is subjected to transcriptional and translational regulation that establishes the framework of its biological functions under normal pathological conditions. Considerable evidence exists regarding the regulatory function of ERs and PRs and other transcription factors in endometrial gene expression. In recent years microRNAs (miRNAs) have also emerged as key posttranscriptional regulators (Bartel, 2004; Ambros and Chen, 2007). As a member of small non-coding RNA family, miRNAs are transcribed from specific genes scattered at multiple locations in all human chromosomes except the Y chromosome (Chen and Rajewsky, 2006; Scherr and Eder, 2007). The initial step in miRNAs biogenesis involves combined actions of Drosha and DiGeorge critical region 8 (DGCR8) which cleave primary miRNA (Pri-miRNA) transcripts into precursor miRNAs (pre-miRNAs) of 60–70 nucleotide (NT) stem-loop structures. Following transport into the cytoplasm with the aid Exportin-5, pre-miRNAs are further processed by Dicer, generating a 20 to 24 NT single strand mature miRNA. The mature miRNAs incorporate into the RNA-induced silencing complex (RISC) and, through complementary binding to the 3′ UTR of specific target genes, post-transcriptionally regulate their expression (Jackson and Standart, 2007; Neilson *et al*., 2007; Pillai *et al*., 2007).

Many putative miRNAs have been identified and/or predicted in the genome of different species, including mammals (Berezikov *et al*., 2006; Yu *et al*., 2007). The specificity of miRNAs interaction with target genes is primarily dictated by the 'seed' region located at residues 2–8 at their 5′ ends. As a result of redundancy within the seed region, predictive algorithms estimate that a third of human genome may contain putative single or multiple binding sites. As such, a single miRNA can potentially target hundreds of genes, or a single gene could be a target of many different miRNAs (Lai, 2002; Brennecke *et al*., 2005; Grimson *et al*., 2007). The biological significance of such diversity implemented by the

action of several miRNAs may be necessary to achieve adequate regression of specific target genes based on the degree of complementary sequence homology (Bartel, 2004; Grimson *et al*., 2007). However, it has been reported that a modest degree of repression occurs in a third of miRNA's predicted target genes, and a fine adjustment of their protein biosynthesis (Baek *et al*., 2008). Functionally, such miRNA-induced post-transcriptional regulation is central to various stages of normal developmental processes and other cellular activities that influence cell-cycle progression, differentiation, apoptosis, inflammatory and immune response, angiogenesis and tissue turnover (Ambros and Chen, 2007; Cho, 2007; Huppi *et al*., 2007; Kuehbacher *et al*., 2007; Linsley *et al*., 2007; Ma and Weinberg, 2007; Ku and McManus, 2008; Lodish *et al*., 2008; Crosby *et al*., 2009; Chen *et al*., 2010; Fabian *et al*., 2010; Farajollahi and Maas, 2010; Iorio *et al*., 2010). Conversely, deregulated expression and activity of Drosha, DGCR8 and Dicer, resulting in alteration of miRNA biogenesis, disrupt many of the above cellular activities, leading to various disorders, more specifically developmental abnormalities, inflammatory and immune reactions, fibrosis, cellular transformation and cancer (Croce, 2008; Han *et al*., 2009; Wang and Olson, 2009; Jiang *et al*., 2010b).

Expression and potential regulatory function of miRNAs in the endometrium

Since their discovery over a decade ago, our understanding of miRNA expression and regulatory function has increased exponentially (Ambros and Chen, 2007; Cho, 2007; Huppi *et al*., 2007; Ma and Weinberg, 2007; Lodish *et al*., 2008; Crosby *et al*., 2009; Chen *et al*., 2010; Fabian *et al*., 2010; Farajollahi and Maas, 2010; Iorio *et al*., 2010). In reproductive tract tissues, expression profiling has also identified a large number of miRNAs in the ovarian, fallopian tube and uterine tissues in both normal and diseased states(Chakrabarty *et al*., 2007; Boren *et al*., 2008; Hong *et al*., 2008; Hu *et al*., 2008; Luo and Chegini, 2008; Merritt *et al*., 2008; Nagaraja *et al*., 2008; Pan and Chegini, 2008; Burney *et al*., 2009; Chung *et al*., 2009; Gonzalez and Behringer, 2009; Kobel *et al*., 2009; Qian *et al*., 2009; Creighton *et al*., 2010; Filigheddu *et al*., 2010; Kuokkanen *et al*., 2010; Myatt *et al*., 2010). The menstrual-dependent expression of some miRNAs imply their possible regulation by ovarian steroids, while their aberrant expression in endometriosis and endometrial cancer associates these miRNAs with endometrial disorders(Boren *et al*., 2008; Pan and Chegini, 2008; Burney *et al*., 2009; Qian *et al*., 2009; Creighton *et al*., 2010; Filigheddu *et al*., 2010). Expression profiling of endometrial epithelial cells isolated from mid-luteal phase, or isolated endometrial cells treated with ovarian steroids provided support for potential regulatory functions of ovarian steroids on miRNA expression (Pan and Chegini, 2008; Maillot *et al*., 2009; Kuokkanen *et al*., 2010). Functional regulatory interactions between ovarian steroids, their receptors and miRNAs expression have also been reported in other steroids-sensitive cells(Chakrabarty *et al*., 2007; Kondo *et al*., 2008; Zhao *et al*., 2008b; Bhat-Nakshatri *et al*., 2009; Maillot *et al*., 2009; Pandey and Picard, 2009; Wickramasinghe *et al*., 2009; Yamagata *et al*., 2009; Al-Nakhle *et al*., 2010; Li *et al*., 2010; Loven *et al*., 2010; Zhao *et al*., 2010). Although the molecular mechanisms to explain the regulatory action of ovarian steroids on miRNA expression remain unclear, recent reports suggested that inhibition of miRNA maturation at the level of processing of pri-miRNAs into pre-miRNAs and estrogen-dependent association with Drosha complex may account for ERα mediated actions on target gene expression (Castellano *et al*., 2009; Yamagata *et al*., 2009).

miRNAs such as miR-21, miR-18a, miR-181a, miR-206, miR-133 and miR-142-5p; predicted to target a large number of genes, including transforming growth factor beta (TGF-β), TGF-β receptor, ERs, and PRs (Adams *et al*., 2007; Maillot *et al*., 2009; Kuokkanen *et al*., 2010; Pan *et al*., 2010) are expressed in the endometrium. The product of these genes plays a critical role in various endometrial activities. Functional analysis in other

cells has also provided support for regulatory influence of these miRNAs on the expression of genes involved in cell-cycle progression, differentiation, apoptosis, inflammatory and immune response and angiogenesis (Ambros and Chen, 2007; Cho, 2007; Huppi *et al*., 2007; Ma and Weinberg, 2007; Lodish *et al*., 2008; Crosby *et al*., 2009; Chen *et al*., 2010; Fabian *et al*., 2010; Farajollahi and Maas, 2010; Iorio *et al*., 2010). These cellular processes are integrated parts of the cyclic endometrial regeneration and pathogenesis of a number of disorders, including endometriosis and endometrial cancer. Although cell- and tissue-specific biological function of many miRNAs, including in the endometrium, remains to be established, conditional inactivation of Dicer has provided evidence for the pivotal function of miRNAs in ovarian as well as oviductal and uterine mesenchymal cellular development, female sterility and multiple reproductive defects (Hong *et al*., 2008; Hu *et al*., 2008; Nagaraja *et al*., 2008; Gonzalez and Behringer, 2009). Similar tubal morphological abnormalities seen as a result of Dicer conditional-inactivation are often observed in fallopian tubes of women with endometriosis (Chaudhri *et al.*, 2010). Fallopian tubes of women with endometriosis display an altered expression of miRNAs and mRNAs; personal communication). Although, alterations in peritoneal environment, endometrial receptivity, ovarian reserve, and oocyte quality often attend endometriosis-associated infertility, the contribution of an overt tubal disease may influence early embryonic development and be reflected in an altered expression of a number of miRNAs. Through expression profiling of a large number of miRNAs and mRNAs in fallopian tubes of women with and without endometriosis, we identified not only the expression of DGCR8, Dicer and Exportin-5, but also the expression of the same set of miRNAs that were altered in the oviduct of mice with Dicer conditional-inactivation in the fallopian tubes of women with endometriosis (Chaudhri *et al*., 2010).

miRNAs and endometrial inflammatory response

Inflammatory response and processes that regulate it are not only central to normal cyclic endometrial activities, but also the outcome of pathogenesis of endometrial disorders, including dysfunctional bleeding, endometriosis and endometrial cancer. The onset of menstruation is associated with local endometrial inflammatory response and its regulated progression is necessary for endometrial tissue regeneration. While deficiency in inflammatory responses often results in impairment of the wound repair process, excess production of inflammatory mediators is a leading cause of disorder, including dysfunctional endometrial bleeding, endometriosis and endometrial cancer. The means and the mechanisms that control the expression of inflammatory mediators and terminate their activities are critical for developing better treatment strategies for various endometrial disorders. A number of studies have associated the expression and regulation of a number miRNAs, including miR-17-5p, miR-20a, miR-106a, miR-125b, miR-146 and miR-155, with mediating the inflammatory response (Costinean *et al*., 2006; O'Connell *et al*., 2007; Rodriguez *et al*., 2007; Tili *et al*., 2007; Meng *et al*., 2007b, 2008). However, it remains to be established whether inflammation causes the expression of specific miRNAs or, unregulated expression of miRNAs, resulting in activation of inflammation-associated genes which account for inflammatory response. Experimental evidence indicated that agents such as endotoxin, tumor necrosis factor alpha (TNF-α), and increased NF-κB activity, known mediators of inflammatory response, altered the expression of a number of miRNAs (Tili *et al*., 2007; El Gazzar and McCall, 2010; Jiang *et al*., 2010a). Endometrium expresses these miRNAs and several pro-inflammatory cytokines, including increased TNF-α and NF-κB activity, during menstruation (Chegini *et al*., 1999; Bergqvist *et al*., 2001; Cork *et al*., 2002; Kayisli *et al*., 2002; Khan *et al*., 2005; Cao *et al*., 2006), raising the possibility that these mediators may function in similar manners in regulating the endometrial miRNA expression and function. Such regulatory interaction is specifically relevant to the endometrium during

menses and dysfunctional uterine bleeding, both characterized by an increased expression of pro-inflammatory mediators.

The expression of miR-125b and miR-155 and their regulation by TNF-α has also been found to be cell lineage-dependent with differential expression of miR-125b in immune-related cells, limiting the level and duration of immune response before it becomes detrimental (Tili *et al*., 2007). Additionally, miR-125b in the breast cancer cell line, MCF7, has been found to regulate cell homeostasis and proliferation. Since miR-125b is expressed and differentially regulated in the endometrium, it may serve in the above capacity in regulating endometrial immune response, cell homeostasis and proliferation during embryo implantation which is an immune privileged environment. Induction of miR-146 as a result of an NF-κB-dependent mechanism has also been shown to inhibit the expression of TNF-α receptor-associated factor 6 (TRAF6) and interleukin-1 receptor associated kinase 1 (IRAK1) which act downstream from Toll-like receptor (TLR) and cytokine signaling (Taganov *et al*., 2007). Because miR-146a expression was triggered by cell surface and not by the intracellular TLR, which mainly sense viral nucleic acids, miR-146a is considered to function in response to bacterial rather than viral infection (Neilson *et al*., 2007; O'Connell *et al*., 2007; Taganov *et al*., 2007). In this respect, endometrial expression of selective number miRNAs such as, miR-125, miR-146a and miR-155, may function, not only in regulating inflammatory- and immune-mediated responses, but also events triggered by intrauterine bacterial infection resulting in endometriosis.

The onset on menstruation, dysfunctional uterine bleeding, endometriosis, ectopic endometrium and endometrial cancer progression are also associated with an increased production of proteolytic enzymes, such as matrix metalloproteinases (MMPs; (Jabbour *et al*., 2006; Li *et al*., 2006, 2007b). Several miRNAs including miR-20, miR-21, miR-23a, miR-222 are predicted to target the expression of MMPs and their physiological inhibitor, tissue inhibitor of MMPs (TIMPs; (Dalmay and Edwards, 2006). Resolution of endometrial inflammatory response, control of proteolytic enzymes, and decrease in the rate of apoptosis and increase in cell proliferation are also necessary for reparative mechanisms to take place. Several miRNAs, including overexpression of miR-17-5p, miR-20a and miR-106a have been reported to inhibit differentiation and maturation of monocytes, the major source of inflammatory mediators and proteolytic enzymes (Fontana *et al*., 2007). In addition, increased miR-181a expression in mature T cells was found to regulate the expression of anti-apoptotic proteins, such as B-cell lymphoma 2 (BCL2) and the cell surface regulator CD69 (Li *et al*., 2007a; Neilson *et al*., 2007). The endometrium expresses miR-181a and a recent report demonstrated PRA as a direct target of this miRNA in breast cancer cell line (Maillot *et al*., 2009). Although the biological significance of these miRNAs in the endometrium is yet to be established, their potential functional regulation of genes with pro- and anti-inflammatory and anti-apoptotic activities, as well as steroid receptors, may serve in promoting transition from an inflammatory phase into a reparative stage as well as progression into disease states, when irregularly expressed.

miRNAs and endometrial cell- cycle progression and apoptosis

Apoptosis and cell cycle progression are highly regulated and play central roles in endometrial regeneration and the outcome of disorders such endometriosis and endometrial cancer (Brosens and Gellersen, 2006; Harada *et al*., 2007). Interestingly, many miRNAs have been identified or predicted to functionally regulate several apoptotic and cell cycle-related genes, including miR-10a, miR-21, miR-28, miR-196a, miR-96, miR-145, miR-150, miR-155, and miR-188 (Jovanovic and Hengartner, 2006; Meng *et al*., 2006; Matsubara *et al*., 2007; Mertens-Talcott *et al*., 2007; Si *et al*., 2007). TNF-α and, TNF-related apoptosis-inducing ligand (TRAIL, Apo-2L) which activates caspase-dependent apoptotic machinery,

has been associated with the altered expression of several of these miRNAs. In addition, TRAIL-resistant cells displayed different miRNA profile with increased expression of miR-221 and miR-222 by targeting c-Kit and p27 (kip1) expression (Ovcharenko *et al*., 2007; Garofalo *et al*., 2008). TRAIL-induce apoptosis is also triggered by miR-155 (O'Connell *et al*., 2007; Tili *et al*., 2007). Many cell cycle- and apoptotic-associated genes and their inhibitors (Watanabe *et al*., 1997; Jones *et al*., 1998; Chegini and Williams, 2000; Selam *et al*., 2001; Brennecke *et al*., 2005; Jabbour *et al*., 2006; Harada *et al*., 2007;) and miRNAs that target their expression are expressed in endometrium, ectopic endometrium and endometrial cancer. More specifically, the expression of E2F transcription factors, PDCD4, PTEN, and Bcl2, which are known cell cycle regulator genes, is the direct target of several miRNAs in various cell types, including endometrial cancer cell line and isolated myometrial cells (He *et al*., 2007a, b; Matsubara *et al*., 2007; Meng *et al*., 2007a; O'Connell *et al*., 2007; Raver-Shapira *et al*., 2007; Si *et al*., 2007; Sylvestre *et al*., 2007; Welch *et al*., 2007; Woods *et al*., 2007; Myatt *et al*., 2010; Pan *et al*., 2010). The manner in which ovarian steroids regulate the uterine expression of specific miRNAs that functionally target pro- and anti-apoptotic as well as cell cycle-associated genes, may be critical not only during the menstrual cycle, but also during establishment and progression of disorders affecting this tissue.

miRNAs and endometrial angiogenesis

Angiogenesis is a self-limiting and strictly regulated event involving the degradation of vascular basement membrane and interstitial matrix, migration and proliferation of endothelial cells, and tubulogenesis and formation of capillary loops. These processes are regulated by many angiogenic and anti-angiogenic mediators and their optimal and timely production is critical to the outcome of angiogenesis, including in the endometrium (Smith, 2001; Girling and Rogers, 2005). Altered production of many of these angiogenic factors has been directly associated with dysfunctional uterine bleeding, endometriosis and endometrial cancer. As with other cellular activities, the regulatory function of miRNA in angiogenesis has been illustrated in a number of in vitro and in vivo models (Yang *et al*., 2005; Suarez and Sessa, 2009; Wang and Olson, 2009; Wu *et al*., 2009; Heusschen *et al*., 2010; Shi *et al*., 2010). Alteration or silencing of Dicer expression in mice embryos, as well as gain- or loss- of function of miR-221/222 in human endothelial cells resulted in defective angiogenesis during embryogenesis and altered expression of vascular endothelial growth factor (VEGF), and its receptors, fms-related tyrosine kinase 1 (FLT1) and kinase insert domain receptor (KDR), as well as tyrosine kinase with immunoglobulin and epidermal growth factor homology domains 1 (TIE1), endothelial nitric oxide synthase and IL-8 (Yang *et al*., 2005; Poliseno *et al*., 2006; Suarez *et al*., 2007). Silencing of Drosha and Dicer was also associated with reduced capillary sprouting and tube forming activity through a mechanism involving decreased migration of endothelial cells; inhibition of let-7f and miR-27b expression reduced sprout formation, suggesting their possible regulatory function in angiogenesis by targeting anti-angiogenic genes (Kuehbacher *et al*., 2007, 2008;). Furthermore, miR-130a regulates the expression of growth arrest homeobox transcription factor and homeobox A5 (Chen and Gorski, 2008), while miR-378 enhanced the survival of vascular endothelial cells (Lee *et al*., 2007) which promote angiogenesis. Although the expression of several of these miRNAs has been identified in human endometrium, ectopic endometrium and endometrial cancers, as well as in pre-implantation mouse uterus (Chakrabarty *et al*., 2007), their regulatory functions on endometrial angiogenic and anti-angiogenic genes expression remains to be investigated. However, the existing evidence obtained in other systems strongly supports the role of miRNA in regulating the expression of genes involved in various aspects of angiogenesis.

miRNAs and endometrial embryo implantation

Embryo implantation is a complex process, and the underlying mechanisms involving this process and the establishment of maternal tolerance to the embryo remains incompletely understood. As stated earlier ovarian steroids and steroid-mediated expression many autocrine/paracrine mediators are essential for endometrial receptivity and embryo implantation. To illustrate the importance of miRNA in embryo implantation, conditional inactivation of Dicer and expression profiling during peri-and pre-implantation periods in mice implicated the potential role of some miRNAs, including miR-125, miR-155 and miR-99 in this process (Chakrabarty *et al*., 2007; Hong *et al*., 2008; Hu *et al*., 2008; Nagaraja *et al*., 2008; Gonzalez and Behringer, 2009). Although miRNAs are predicted to target the expression of several hundred genes, miR-99, possibly by targeting COX2, and miR-155 which was found to be differentially expressed during peri-implantation, and up-regulated at day 4 pregnancy (receptive period) uteri, may have a specific regulatory function in processes leading to embryo implantation (Chakrabarty *et al*., 2007). Ovarian steroids, as well as a number of growth factors, cytokines and chemokines regulate the expression of COX2. miR-155 also targets the expression of TNF-α, a key pro-inflammatory cytokine which regulate COX2 expression (Tili *et al*., 2007; Lodish *et al*., 2008). As such a feed-back regulatory mechanism between miRNAs and their target genes in the endometrium may be critical during the receptive period as compared to other stages of menstrual cycle. Many of the miRNAs expressed in this peri- and pre-implantation mouse model are also expressed in human endometrium during the mid-luteal phase, including miR-19a, miR-18, miR-20a, miR-21, miR-26, miR-199b, miR-101, miR-17-3p, and miR-181b (Kuokkanen *et al*., 2010). The miR-26 family has been predicted to target the expression of LIF, a key cytokine with direct role in embryo implantation (Lewis *et al*., 2003, 2005), while miR-21, miR-18 and miR-181 target the expression of TGF-β receptors, ER and PR, respectively (Maillot *et al*., 2009; Pan *et al*., 2010). These observations suggest that differential regulation of subsets of miRNAs may be required in order to stabilize the endometrial expression of specific genes critical for embryo implantation. However, detailed studies are needed to identify the specific endometrial genes targeted by these miRNAs and their associations with embryo implantation.

miRNAs and endometrial disorders

In addition to Dicer, altered expression of DGCR8 and XPO-5 has been associated with deficiency in post-transcriptional and processing of miRNAs during tumorigenesis (Murphy *et al*., 2008; Bartel, 2009; Perron and Provost, 2009; Visone and Croce, 2009; Iorio *et al*., 2010; *et al*., 2010b; Siomi and Siomi, 2010). Aberrant expression of several miRNAs has been associated with a number of disorders, more specifically cancers (Ambros and Chen, 2007; Cho, 2007; Huppi *et al*., 2007; Ma and Weinberg, 2007; Lodish *et al*., 2008; Crosby *et al*., 2009; Chen *et al*., 2010; Fabian *et al*., 2010; Farajollahi and Maas, 2010; Iorio *et al*., 2010). Among hundreds of genes predicted as target of these miRNAs are a number of oncogenes and tumor suppressor genes (Chang *et al*., 2008; Croce, 2008). In addition, miRNA genes are frequently located at fragile sites and regions of loss of heterozygosity or common breakpoint regions (Calin and Croce, 2007; Croce, 2008). Common fragile sites are large, genomically unstable regions, which are hot-spots for deletions and other alterations, especially in cancer cells, including endometrial cancer cells (McAvoy *et al*., 2007). In addition to oncogenes and tumor suppressor genes, the expression of pro-inflammatory, angiogenic, cell cycle-related and adhesion molecules, which regulate various cellular activities critical to tumorigenesis, are targeted by many miRNAs. The expression of many of these oncogenes and tumor-suppressor genes, pro-inflammatory, angiogenic, cell-cycle related genes are expressed and associated with establishment and progression of endometrial cancer (Abal *et al*., 2006) and in pathogenesis of endometriosis and

dysfunctional uterine bleeding. Although altered expression of a subsets of miRNAs has been identified in ectopic endometrium and endometrial cancer as compared to normal endometrium, and their regulatory function on specific genes expression awaits detailed investigation. However, the results suggest that ectopic endometrial tissues and endometrial cancer are programmed differently with respect to their gene expression regulation. Women with endometrial cancer, endometriosis and, more specifically, contraceptive users often experience dysfunctional endometrial bleeding, characterized by an increased inflammatory reaction and increased production of angiogenic mediators as well as excess production of proteolytic enzymes (Rhoton-Vlasak *et al*., 2005; Smith and Critchley, 2005; Jabbour *et al*., 2006; Jones *et al*., 2006). A detailed study involving miRNA expression profile during dysfunctional bleeding would allow for better understating of the disorder and its similarities to and differences from normal menstruation.

Among the miRNA profiled, let7 family, which clusters with miR-99/miR-100 and miR-125 family, has been considered to serve as tumor suppressor (Johnson *et al*., 2007; Lee and Dutta, 2007; Tili *et al*., 2007; Esquela-Kerscher *et al*., 2008). Additionally, the expression of miR-15a and miR-16-1 inversely correlated with Bcl-2 expression (Cimmino *et al*., 2005). miR-15a and miR-16-1 cluster are located at 13q14.3, a region often rearranged in a number of disorders, and germline mutation in miR-16-1 precursor has been found to be associated with a low level of miR-16-1 expression in chronic lymphocytic leukemia (Calin *et al*., 2005). miR-15a and miR-16-1 as well as miR-17-92 which is a target of c-myc, are expressed in endometriosis and endometrial cancer tissues and are regulated by ovarian steroids (Bircan *et al*., 2005; Lu *et al*., 2007; Rinaldi *et al*., 2007). It has been suggested that miR-17-92 cluster in cooperation with increased c-myc expression results in accelerated tumor development (Lu *et al*., 2007; Rinaldi *et al*., 2007). The transcription factor E2F1 which is associated with cell cycle progression is a target of the miR-17-92 cluster and miR-21 (Woods *et al*., 2007), while increased expression of miR-221 and miR-222 promotes cell growth by inhibiting p27 (le Sage *et al*., 2007). miR-27a suppresses cdc2/cyclin B inhibitor Myt-1 in MDA-MB-231 cells, promoting cell proliferation (Mertens-Talcott *et al*., 2007). Experimental evidence also indicates that overexpression of miR-206 promotes cellular differentiation in C2C12 myoblasts (Kim *et al*., 2006) and targets the expression of ERα in ER-positive breast cancer cells (Adams *et al*., 2007). Altered expression of ERα and ERβ has been associated with several uterine disorders, including endometrial cancer and endometriosis (Gleeson *et al*., 1993; Singh *et al*., 2007; Bukulmez *et al*., 2008), and miR-206 as well as miR-18 and miR-181 may potentially regulate their expression in these and other steroid target tissues (Adams *et al*., 2007; Pan and Chegini, 2008; Maillot *et al*., 2009; Al-Nakhle *et al*., 2010). Although evidence suggests that ovarian steroids either directly and/or indirectly may regulate the expression of miRNAs, resulting in reprogramming of their target genes expression, detailed studies are needed to ascertain the molecular mechanism that account for such regulation.

Conclusion and future direction

In recent years, an unprecedented advancement has been made into the understanding of the uterine molecular environment under normal and diseased conditions. More specifically, expression profiling of thousand of genes allowed identification of new genomic pathways with potential regulatory functions in development and homeostasis of uterine normal biological and physiological activities, as well as uterine disorders. Through these studies it has become more clear that the products of several of these genes, either alone, or through interactive mechanisms, function as regulators of inflammatory and immune responses, cell cycle progression, differentiation, apoptosis, and tissue remodeling. These events play key roles in normal development as well as establishment and progression of uterine disorders. Clearly, identifying the precise regulation of these genes at transcriptional and translational

levels, the influence of epigenetic mechanism and genomic re-arrangement, is central to understanding of their functions in normal endometrial integrity and pathological outcomes.

Expression profiling, cloning strategies and next generation sequencing have identified a large number of miRNAs, and functional analysis has implicated miRNAs as major component of post-transcriptional regulatory mechanism. Various experimental models have provided valuable information regarding the biological relevance of miRNAs in numerous cells and tissues under normal and diseased conditions. The expression profiling and, to a limited extent, functional analysis, also supports the biological relevance of miRNAs in normal endometrial developmental processes as well as disorders such as endometriosis and endometrial cancer. Moreover, evidence suggests that uterine miRNAs expression either directly or indirectly is regulated by ovarian steroids; however the manners by which ovarian steroids mediate their actions in unclear. This is of particular importance because ovarian steroids are central to uterine normal physiological function and the outcome of uterine pathogenesis and tissue and serum profiling of miRNAs appears to be more superior to mRNA expression profiling to differentiate normal from diseased conditions. If the expression pattern of a number of miRNAs differentiates the normal endometrium from diseased conditions, the result may have a significant impact on prognostics and on diagnostic approaches as tools for assessing response to various treatment strategies. Evidence generated in other cell and in vivo systems supports the experimental utilization of miRNA modulation for therapeutic and medical management of disorders such as lowering of plasma cholesterol (miR-122), cancer therapy (miR-21), cardiac hypertrophy (miR-21) and cardiac arrhythmia (miR-1; Esau *et al*., 2006; Krichevsky and Gabriely, 2009; Jiang *et al*., 2010b).

Moreover, genetic variations, single nucleotide polymorphisms and chromosomal rearrangements, which can impact the interaction between miRNAs and/or their target genes, have been associated with various aspects of cellular development, and serve as underlying cause of many human diseases and disorders, including reproductive tract tissues. Additionally, many new small RNA families and novel miRNAs are being discovered, further underscoring the biological regulatory importance of these RNAs and their interactions with their target genes. As such major challenges lie ahead in deciphering many functional aspects of miRNAs in uterine and other reproductive tract tissues. Since the biological relevance of a vast majority of the genes profiled in uterine and other reproductive tissues remains unknown, and the discovery of small RNAs, including miRNAs, added another layer of complexity, individual and combined collaborative efforts are required to enhance our understanding of their interactions and regulatory functions at cellular and tissue levels.

Acknowledgments

I thank Drs. Luo and Bukulmez, and fellows working with me on various projects to make this article possible. I also apologize to those whose work could not be discussed owing to space constraints. This work is supported in part by grants HD37432 and HD58664 from NIH.

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