Uterine microRNA signature and consequence of their dysregulation in uterine disorders

Nasser Chegini[1]

Division of Reproductive Endocrinology and Infertility, Department of Obstetrics and Gynecology,
University of Florida, Gainesville, FL, 32610

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).

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).

---

[1]Corresponding author: cheginin@ufl.edu
Phone: (352) 273-7566; Fax: (352) 392-6994

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.
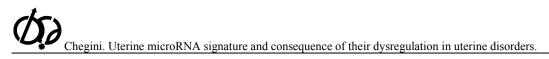
miRNAs and endometrial cell- cycle progression and apoptosis

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

120

Anim. Reprod, v.7, n.3, p.117-128, Jul./Sept. 2010

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 re-programming 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

References

Abal M, Planaguma J, Gil-Moreno A, Monge M, Gonzalez M, Baro T, Garcia A, Castellvi J, Ramon YC, Xercavins J, Alameda F, Reventos J. 2006. Molecular pathology of endometrial carcinoma: transcriptional signature in endometrioid tumors. *Histol Histopathol*, 21:197-204.

Achache H, Revel A. 2006. Endometrial receptivity markers, the journey to successful embryo implantation. *Hum Reprod Update*, 12:731-746.

Adams BD, Furneaux H, White BA. 2007. The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-alpha (ERalpha) and represses ERalpha messenger RNA and protein expression in breast cancer cell lines. *Mol Endocrinol*, 21:1132-1147.

Al-Nakhle H, Burns PA, Cummings M, Hanby AM, Hughes TA, Satheesha S, Shaaban AM, Smith L, Speirs V. 2010. Estrogen receptor Beta 1 expression is regulated by miR-92 in breast cancer. *Cancer Res*, 70:4778-4784.

Altmae S, Martinez-Conejero JA, Salumets A, Simon C, Horcajadas J., Stavreus-Evers A. 2010. Endometrial gene expression analysis at the time of embryo implantation in women with unexplained infertility. *Mol Hum Reprod*, 16:178-187.

Ambros V, Chen X. 2007. The regulation of genes and genomes by small RNAs. *Development*, 134:1635-1641.

Baek D, Villen J, Shin C, Camargo FD, Gygi SP, Bartel DP. 2008. The impact of microRNAs on protein output. *Nature*, 455:64-71.

Bartel DP. 2004. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell*, 116:281-297.

Bartel DP. 2009. MicroRNAs: target recognition and regulatory functions. *Cell*, 136:215-233.

Berezikov E, van Tetering G, Verheul M, van de Belt J, van Laake L, Vos J, Verloop R, van de Wetering M, Guryev V, Takada S, van Zonneveld AJ, Mano H, Plasterk R, Cuppen E. 2006. Many novel mammalian microRNA candidates identified by extensive cloning and RAKE analysis. *Genome Res*, 16:1289-1298.

Bergqvist A, Bruse C, Carlberg M, Carlstrom K. 2001. Interleukin 1beta, interleukin-6, and tumor necrosis factor-alpha in endometriotic tissue and in endometrium. *Fertil Steril*, 75:489-495.

Bhat-Nakshatri P, Wang G, Collins NR, Thomson MJ, Geistlinger TR, Carroll JS, Brown M, Hammond S, Srour EF, Liu Y, Nakshatri H. 2009. Estradiol-regulated microRNAs control estradiol response in breast cancer cells. *Nucleic Acids Res*, 37:4850-4861.

Bircan S, Ensari A, Ozturk S, Erdogan N, Dundar I, Ortac F. 2005. Immunohistochemical analysis of c-myc, c-jun and estrogen receptor in normal, hyperplastic and neoplastic endometrium. *Pathol Oncol Res*, 11:32-39.

Boren T, Xiong Y, Hakam A, Wenham R, Apte S, Wei Z, Kamath S, Chen DT, Dressman H, Lancaster JM. 2008. MicroRNAs and their target messenger RNAs associated with endometrial carcinogenesis. *Gynecol Oncol*, 110:206-215.

Brennecke J, Stark A, Russell RB, Cohen SM. 2005. Principles of microRNA-target recognition. *PLoS Biol*, 3:e85.

Brosens JJ, Gellersen B. 2006. Death or survival--progesterone-dependent cell fate decisions in the human endometrial stroma. *J Mol Endocrinol*, 36, 389-398.

Bukulmez O, Hardy DB, Carr BR, Word RA, Mendelson CR. 2008. Inflammatory status influences aromatase and steroid receptor expression in endometriosis. *Endocrinology*, 149:1190-1204.

Burney RO, Hamilton AE, Aghajanova L, Vo KC, Nezhat CN, Lessey BA, Giudice LC. 2009.

MicroRNA expression profiling of eutopic secretory endometrium in women with versus without endometriosis. *Mol Hum Reprod*, 15:625-631.

Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. 2005. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med*, 353:1793-1801.

Calin GA, Croce CM. 2007. Chromosomal rearrangements and microRNAs: a new cancer link with clinical implications. *J Clin Invest*, 117:2059-2066.

Cao WG, Morin M, Sengers V, Metz C, Roger T, Maheux R, Akoum A. 2006. Tumour necrosis factor-alpha up-regulates macrophage migration inhibitory factor expression in endometrial stromal cells via the nuclear transcription factor NF-kappaB. *Hum Reprod*, 21:421-428.

Castellano L, Giamas G, Jacob J, Coombes RC, Lucchesi W, Thiruchelvam P, Barton G, Jiao LR, Wait R, Waxman J, Hannon GJ, Stebbing J. 2009. The estrogen receptor-alpha-induced microRNA signature regulates itself and its transcriptional response. *Proc Natl Acad Sci USA*, 106:15732-15737.

Chakrabarty A, Tranguch S, Daikoku T, Jensen K, Furneaux H, Dey SK. 2007. MicroRNA regulation of cyclooxygenase-2 during embryo implantation. *Proc Natl Acad Sci USA*, 104:15144-15149.

Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, Dang CV, Thomas-Tikhonenko A, Mendell JT. 2008. Widespread microRNA repression by Myc contributes to tumorigenesis. *Nat Genet*, 40:43-50.

Chegini N, Dou Q, Williams RS. 1999. An inverse relation between the expression of tumor necrosis factor alpha (TNF-alpha) and TNF-alpha receptor in human endometrium. A*m J Reprod Immunol*, 42:297-302.

Chegini N, Williams RS. 2000. Cytokine networks in human endometrium from menstruation to implantation. In: Hill J (Ed.). *Cytokines in Human Reproduction*. Ames, IA: J Wiley. pp. 93-132.

Chegini N. 2002. Peritoneal molecular environment, adhesion formation and clinical implication. *Front Biosci*, 7:E91-E115.

Chen D, Farwell MA, Zhang B. 2010. MicroRNA as a new player in the cell cycle. *J Cell Physiol*. doi: 10.1002/jcp.22234.

Chen K, Rajewsky N. 2006. Deep conservation of microRNA-target relationships and 3'UTR motifs in vertebrates, flies, and nematodes. *Cold Spring Harb Symp Quant Biol*, 71:149-156.

Chen Y, Gorski DH. 2008. Regulation of angiogenesis through a microRNA (miR-130a) that down-regulates antiangiogenic homeobox genes GAX and HOXA5. *Blood*, 111:1217-1226.

Cho WC. 2007. OncomiRs: the discovery and progress of microRNAs in cancers. *Mol Cancer*, 6:60.

Chung TK, Cheung TH, Huen NY, Wong KW, Lo KW, Yim SF, Siu NS, Wong YM, Tsang PT, Pang MW, Yu MY, To KF, Mok SC, Wang VW, Li C, Cheung AY, Doran G, Birrer MJ, Smith DI, Wong YF. 2009. Dysregulated microRNAs and their predicted targets associated with endometrioid endometrial adenocarcinoma in Hong Kong women. *Int J Cancer*, 124:1358-1365.

Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S, Dono M, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M, Croce CM. 2005. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci USA*, 102:13944-13949.

Conneely OM, Mulac-Jericevic B, Lydon JP. 2003. Progesterone-dependent regulation of female reproductive activity by two distinct progesterone receptor isoforms. *Steroids*, 68:771-778.

Cork BA, Tuckerman EM, Li TC, Laird SM. 2002. Expression of interleukin (IL)-11 receptor by the human endometrium in vivo and effects of IL-11, IL-6 and LIF on the production of MMP and cytokines by human endometrial cells in vitro. *Mol Hum Reprod*, 8:841-848.

Costinean S, Zanesi N, Pekarsky Y, Tili E, Volinia S, Heerema N, Croce CM. 2006. Pre-B cell proliferation and lymphoblastic leukemia/high-grade lymphoma in E(mu)-miR155 transgenic mice. *Proc Natl Acad Sci USA*, 103:7024-7029.

Creighton CJ, Benham AL, Zhu H, Khan MF, Reid JG, Nagaraja AK, Fountain MD, Dziadek O, Han D, Ma L, Kim J, Hawkins SM, Anderson ML, Matzuk MM, Gunaratne PH. 2010. Discovery of novel microRNAs in female reproductive tract using next generation sequencing. *PLoS One*, 5:e9637.

Critchley HO, Saunders PT. 2009. Hormone receptor dynamics in a receptive human endometrium. *Reprod Sci*, 16:191-199.

Croce, CM. 2008. Oncogenes and cancer. *N Engl J Med*, 358:502-511.

Crosby ME, Devlin CM, Glazer PM, Calin GA, Ivan M. 2009. Emerging roles of microRNAs in the molecular responses to hypoxia. *Curr Pharm Des*, 15:3861-3866.

Dalmay T, Edwards DR. 2006. MicroRNAs and the hallmarks of cancer. *Oncogene*, 25:6170-6175.

Deroo BJ, Buen suceso AV. 2010. Minireview: estrogen receptor-beta: mechanistic insights from recent studies. *Mol Endocrinol*. doi:10.1210/me.2009-0288.

Du H, Taylor HS. 2007. Contribution of bone marrow-derived stem cells to endometrium and endometriosis. *Stem Cells*, 25:2082-2086.

El Gazzar M, McCall CE. 2010. MicroRNAs distinguish translational from transcriptional silencing during endotoxin tolerance. *J Biol Chem*, 285:20940-20951.

Ellmann S, Sticht H, Thiel F, Beckmann MW, Strick R, Strissel PL. 2009. Estrogen and progesterone receptors: from molecular structures to clinical targets. *Cell Mol Life Sci*, 66:2405-2426.

124

Anim. Reprod, v.7, n.3, p.117-128, Jul./Sept. 2010

Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, Watts L, Booten SL, Graham M, McKay R, Subramaniam A, Propp S, Lollo BA, Freier S, Bennett CF, Bhanot S, Monia BP. 2006. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab*, 3:87-98.

Esquela-Kerscher A, Trang P, Wiggins JF, Patrawala L, Cheng A, Ford L, Weidhaas JB, Brown D, Bader AG, Slack FJ. 2008. The let-7 microRNA reduces tumor growth in mouse models of lung cancer. *Cell Cycle*, 7: 759-764

Fabian MR, Sonenberg N, Filipowicz W. 2010. Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem*, 79:351-379.

Farajollahi S, Maas S. 2010. Molecular diversity through RNA editing: a balancing act. *Trends Genet,* 26:221-230.

Ferenczy A. 2003. Pathophysiology of endometrial bleeding. *Maturitas*, 45:1-14.

Filigheddu N, Gregnanin I, Porporato PE, Surico D, Perego B, Galli L, Patrignani C, Graziani A, Surico N. 2010. Differential expression of microRNAs between eutopic and ectopic endometrium in ovarian endometriosis. *J Biomed Biotechnol*, 2010:369549.

Fontana L, Pelosi E, Greco P, Racanicchi S, Testa U, Liuzzi F, Croce CM, Brunetti E, Grignani F, Peschle C. 2007. MicroRNAs 17-5p-20a-106a control monocytopoiesis through AML1 targeting and M-CSF receptor upregulation. *Nat Cell Biol*, 9:775-787.

Garofalo M, Quintavalle C, Di Leva G, Zanca C, Romano G, Taccioli C, Liu CG, Croce CM, Condorelli G. 2008. MicroRNA signatures of TRAIL resistance in human non-small cell lung cancer. *Oncogene*, 27:3845-3855.

Girling JE, Rogers PA. 2005. Recent advances in endometrial angiogenesis research. *Angiogenesis*, 8:89-99.

Gleeson NC, Gonsalves R, Bonnar J. 1993. Plasminogen activator inhibitors in endometrial adenocarcinoma. *Cancer*,72:1670-1672.

Gonzalez G, Behringer RR. 2009. Dicer is required for female reproductive tract development and fertility in the mouse. *Mol Reprod Dev*, 76:678-688.

Grimson A, Farh KK, Johnston WK, Garrett-Engele P, Lim LP, Bartel DP. 2007. MicroRNA targeting specificity in mammals: determinants beyond seed pairing. *Mol Cell*, 27:91-105.

Han J, Pedersen JS, Kwon SC, Belair CD, Kim YK, Yeom KH, Yang WY, Haussler D, Blelloch R, Kim VN. 2009. Posttranscriptional crossregulation between Drosha and DGCR8. *Cell*, 136:75-84.

Harada T, Taniguchi F, Izawa M, Ohama Y, Takenaka Y, Tagashira Y, Ikeda A, Watanabe A, Iwabe T, Terakawa N. 2007. Apoptosis and endometriosis. *Front Biosci*, 12:3140-3151.

He L, He X, Lowe SW, Hannon GJ. 2007a. microRNAs join the p53 network--another piece in the tumour-suppression puzzle. *Nat Rev Cancer*, 7:819-822.

He X, He L, Hannon GJ. 2007b. The guardian's little helper: microRNAs in the p53 tumor suppressor network. *Cancer Res*, 67:11099-11101.

Heusschen R, van Gink M, Griffioen AW, Thijssen VL. 2010. MicroRNAs in the tumor endothelium: novel controls on the angioregulatory switchboard. *Biochem Biophys Acta*, 1805:87-96.

Hong X, Luense LJ, McGinnis LK, Nothnick WB, Christenson LK. 2008. Dicer1 is essential for female fertility and normal development of the female reproductive system. *Endocrinology*, 149:6207-6212.

Horcajadas JA, Pellicer A, Simon C. 2007. Wide genomic analysis of human endometrial receptivity: new times, new opportunities. *Hum Reprod Update*, 13:77-86.

Horcajadas JA, Minguez P, Dopazo J, Esteban FJ, Dominguez F, Giudice LC, Pellicer A, Simon C. 2008. Controlled ovarian stimulation induces a functional genomic delay of the endometrium with potential clinical implications. *J Clin Endocrinol Metab*, 93:4500-4510.

Hu SJ, Ren G, Liu JL, Zhao ZA, Yu YS, Su RW, Ma XH, Ni H, Lei W, Yang ZM. 2008. MicroRNA expression and regulation in mouse uterus during embryo implantation. *J Biol Chem*, 283:23473-23484.

Huppi K, Volfovsky N, Mackiewicz M, Runfola T, Jones TL, Martin SE, Stephens R, Caplen NJ. 2007. MicroRNAs and genomic instability. *Semin Cancer Biol*, 17:65-73.

Iorio MV, Piovan C, Croce CM. 2010. Interplay between microRNAs and the epigenetic machinery: An intricate network. *Biochim Biophys Acta*. doi:10.1016/j.bbagrm.2010.05.005.

Jabbour HN, Kelly RW, Fraser HM, Critchley HO. 2006. Endocrine regulation of menstruation. *Endocr. Rev*, 27:17-46.

Jackson RJ, Standart N. 2007. How do microRNAs regulate gene expression? *Sci STKE*, (367):re1.

Jiang S, Zhang HW, Lu MH, He XH, Li Y, Gu H, Liu MF, Wang ED. 2010a. MicroRNA-155 functions as an OncomiR in breast cancer by targeting the suppressor of cytokine signaling 1 gene. *Cancer Res*, 70:3119-3127.

Jiang X, Tsitsiou E, Herrick SE, Lindsay MA. 2010b. MicroRNAs and the regulation of fibrosis. *FEBS J*, 277, 2015-2021.

Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovcharenko D, Wilson M, Wang X, Shelton J, Shingara J, Chin L, Brown D, Slack FJ. 2007. The let-7 microRNA represses cell proliferation pathways in human cells. *Cancer Res*, 67:7713-7722.

Jones RK, Searle RF, Bulmer JN. 1998. Apoptosis and bcl-2 expression in normal human endometrium, endometriosis and adenomyosis. *Hum Reprod*, 13:3496-3502.

Jones RL, Findlay JK, Farnworth PG, Robertson DM, Wallace E, Salamonsen LA. 2006. Activin A and inhibin A differentially regulate human uterine matrix metalloproteinases: potential interactions during

decidualization and trophoblast invasion. *Endocrinology*, 147:724-732.

Jovanovic M, Hengartner MO. 2006. miRNAs and apoptosis: RNAs to die for. *Oncogene*, 25:6176-6187.

Kayisli UA, Aksu CA, Berkkanoglu M, Arici A. 2002. Estrogenicity of isoflavones on human endometrial stromal and glandular cells. *J Clin Endocrinol Metab*, 87:5539-5544.

Khan KN, Masuzaki H, Fujishita A, Kitajima M, Hiraki K, Sekine I, Matsuyama T, Ishimaru T. 2005. Interleukin-6- and tumour necrosis factor alpha-mediated expression of hepatocyte growth factor by stromal cells and its involvement in the growth of endometriosis. *Hum Reprod*, 20:2715-2723.

Kim HK, Lee YS, Sivaprasad U, Malhotra A, Dutta A. 2006. Muscle-specific microRNA miR-206 promotes muscle differentiation. *J Cell Biol*, 174:677-687.

King AE, Critchley HO. 2010. Oestrogen and progesterone regulation of inflammatory processes in the human endometrium. *J Steroid Biochem Mol Biol*, 120:116-126.

Kobel M, Gilks CB, Huntsman DG. 2009. Dicer and Drosha in ovarian cancer. *N Engl J Med*, 360:1150-1151.

Kondo N, Toyama T, Sugiura H, Fujii Y, Yamashita H. 2008. miR-206 Expression is down-regulated in estrogen receptor alpha-positive human breast cancer. *Cancer Res*, 68:5004-5008.

Krichevsky AM, Gabriely G. 2009. miR-21: a small multi-faceted RNA. *J Cell Mol Med*, 13:39-53.

Ku G, McManus MT. 2008. Behind the scenes of a small RNA gene-silencing pathway. *Hum Gene Ther*, 19:17-26

Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S. 2007. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. *Circ Res*, 101:59-68.

Kuehbacher A, Urbich C, Dimmeler S. 2008. Targeting microRNA expression to regulate angiogenesis. *Trends Pharmacol Sci*, 29:12-15.

Kuokkanen S, Chen B, Ojalvo L, Benard L, Santoro N, Pollard JW. 2010. Genomic profiling of microRNAs and messenger RNAs reveals hormonal regulation in microRNA expression in human endometrium. *Biol Reprod*, 82:791-801.

Lai EC. 2002. Micro RNAs are complementary to 3' UTR sequence motifs that mediate negative post-transcriptional regulation. *Nat Genet*, 30:363-364.

Le Sage C, Nagel R, Egan DA, Schrier M, Mesman E, Mangiola A, Anile C, Maira G, Mercatelli N, Ciafre SA, Farace MG, Agami R. 2007. Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. *EMBO J*, 26:3699-3708.

Lee DY, Deng Z, Wang CH, Yang BB. 2007. MicroRNA-378 promotes cell survival, tumor growth, and angiogenesis by targeting SuFu and Fus-1 expression. *Proc Natl Acad Sci USA*, 104:20350-20355.

Lee YS, Dutta A. 2007. The tumor suppressor

microRNA let-7 represses the HMGA2 oncogene. *Genes Dev*, 21:1025-1030.

Lewis BP, Shih IH, Jones-Rhoades MW, Bartel DP, Burge CB. 2003. Prediction of mammalian microRNA targets. *Cell*, 115:787-798.

Lewis BP, Burge CB, Bartel DP. 2005. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*, 120:15-20.

Li QJ, Chau J, Ebert PJ, Sylvester G, Min H, Liu G, Braich R, Manoharan M, Soutschek J, Skare P, Klein LO, Davis MM, Chen CZ. 2007a. miR-181a is an intrinsic modulator of T cell sensitivity and selection. *Cell*, 129:147-161.

Li R, Luo X, Pan Q, Zineh I, Archer DF, Williams RS, Chegini N. 2006. Doxycycline alters the expression of inflammatory and immune-related cytokines and chemokines in human endometrial cells: implication in irregular uterine bleeding. *Hum Reprod*, 21:2555-2563.

Li R, Luo X, Archer DF, Chegini N. 2007b. Doxycycline alters the expression of matrix metalloproteases in the endometrial cells exposed to ovarian steroids and pro-inflammatory cytokine. *J Reprod Immunol*, 73:118-129.

Li X, Mertens-Talcott SU, Zhang S, Kim K, Ball J, Safe S. 2010. MicroRNA-27a indirectly regulates estrogen receptor alpha expression and hormone responsiveness in MCF-7 breast cancer cells. *Endocrinology*, 151:2462-2473.

Linsley PS, Schelter J, Burchard J, Kibukawa M, Martin MM, Bartz SR, Johnson JM, Cummins JM, Raymond CK, Dai H, Chau N, Cleary M, Jackson AL, Carleton M, Lim L. 2007. Transcripts targeted by the microRNA-16 family cooperatively regulate cell cycle progression. *Mol Cell Biol*, 27:2240-2252.

Lodish HF, Zhou B, Liu G, Chen CZ. 2008. Micromanagement of the immune system by microRNAs. *Nat Rev Immunol*, 8:120-130.

Loven J, Zinin N, Wahlstrom T, Muller I, Brodin P, Fredlund E, Ribacke U, Pivarcsi A, Pahlman S, Henriksson M. 2010. MYCN-regulated microRNAs repress estrogen receptor-alpha (ESR1) expression and neuronal differentiation in human neuroblastoma. *Proc Natl Acad Sci USA*, 107:1553-1558.

Lu Y, Thomson JM, Wong HY, Hammond SM, Hogan BL. 2007. Transgenic over-expression of the microRNA miR-17-92 cluster promotes proliferation and inhibits differentiation of lung epithelial progenitor cells. *Dev Biol*, 310:442-453.

Luo X, Chegini N. 2008. The expression and potential regulatory function of microRNAs in the pathogenesis of leiomyoma. *Semin Reprod Med*, 26:500-514.

Ma L, Weinberg RA. 2007. MicroRNAs in malignant progression. *Cell Cycle*, 7:570-572.

Maillot G, Lacroix-Triki M, Pierredon S, Gratadou L, Schmidt S, Benes V, Roche H, Dalenc F, Auboeuf D, Millevoi S, Vagner S. 2009. Widespread estrogen-dependent repression of micrornas involved in breast

tumor cell growth. *Cancer Res*, 69:8332-8340.

Makker A, Singh MM. 2006. Endometrial receptivity: clinical assessment in relation to fertility, infertility, and antifertility. *Med Res Rev*, 26:699-746.

Matsubara H, Takeuchi T, Nishikawa E, Yanagisawa K, Hayashita Y, Ebi H, Yamada H, Suzuki M, Nagino M, Nimura Y, Osada H, Takahashi T. 2007. Apoptosis induction by antisense oligonucleotides against miR-17-5p and miR-20a in lung cancers overexpressing miR-17-92. *Oncogene*, 26:6099-6105.

McAvoy S, Ganapathiraju SC, Ducharme-Smith AL, Pritchett JR, Kosari F, Perez DS, Zhu Y, James CD, Smith DI. 2007. Non-random inactivation of large common fragile site genes in different cancers. Cytogenet. *Genome Res*, 118:260-269.

Meng F, Henson R, Lang M, Wehbe H, Maheshwari S, Mendell JT, Jiang J, Schmittgen TD, Patel T. 2006. Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. *Gastroenterology*, 130:2113-2129.

Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. 2007a. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. *Gastroenterology*, 133:647-658.

Meng F, Henson R, Wehbe-Janek H, Smith H, Ueno Y, Patel T. 2007b. The MicroRNA let-7a modulates interleukin-6-dependent STAT-3 survival signaling in malignant human cholangiocytes. *J Biol Chem*, 282:8256-8264.

Meng F, Wehbe-Janek H, Henson R, Smith H, Patel T. 2008. Epigenetic regulation of microRNA-370 by interleukin-6 in malignant human cholangiocytes. *Oncogene* 27:378-386.

Merritt WM, Lin YG, Han LY, Kamat AA, Spannuth WA, Schmandt R, Urbauer D, Pennacchio LA, Cheng JF, Nick AM, Deavers MT, Mourad-Zeidan A, Wang H, Mueller P, Lenburg ME, Gray JW, Mok S, Birrer MJ, Lopez-Berestein G, Coleman RL, Bar-Eli M, Sood AK. 2008. Dicer, Drosha, and outcomes in patients with ovarian cancer. *N Engl J Med*, 359:2641-2650.

Mertens-Talcott SU, Chintharlapalli S, Li X, Safe S. 2007. The oncogenic microRNA-27a targets genes that regulate specificity protein transcription factors and the G2-M checkpoint in MDA-MB-231 breast cancer cells. *Cancer Res*, 67:11001-11011.

Murphy D, Dancis B, Brown JR. 2008. The evolution of core proteins involved in microRNA biogenesis. *BMC Evol Biol*, 8:92.

Myatt SS, Wang J, Monteiro LJ, Christian M, Ho KK, Fusi L, Dina RE, Brosens JJ, Ghaem-Maghami S, Lam EW. 2010. Definition of microRNAs that repress expression of the tumor suppressor gene FOXO1 in endometrial cancer. *Cancer Res*, 70:367-377.

Nagaraja AK, Andreu-Vieyra C, Franco HL, Ma L,

Chen R, Han DY, Zhu H, Agno JE, Gunaratne PH, DeMayo FJ, Matzuk MM. 2008. Deletion of Dicer in somatic cells of the female reproductive tract causes sterility. *Mol. Endocrinol*, 22:2336-2352.

Neilson JR, Zheng GX, Burge CB, Sharp PA. 2007. Dynamic regulation of miRNA expression in ordered stages of cellular development. *Genes Dev*, 21:578-589.

O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. 2007. MicroRNA-155 is induced during the macrophage inflammatory response. *Proc Natl Acad Sci USA*, 104:1604-1609.

O'Malley BW, McKenna NJ. 2008. Editorial: Coactivators and corepressors: what's in a name? *Mol Endocrinol*, 22:2213-2214.

Ovcharenko D, Kelnar K, Johnson C, Leng N, Brown D. 2007. Genome-scale microRNA and small interfering RNA screens identify small RNA modulators of TRAIL-induced apoptosis pathway. *Cancer Res*, 67:10782-10788.

Pan Q, Chegini N. 2008. MicroRNA signature and regulatory functions in the endometrium during normal and disease states. *Semin Reprod Med*, 26:479-493.

Pan Q, Luo X, Chegini N. 2010. microRNA 21: response to hormonal therapies and regulatory function in leiomyoma, transformed leiomyoma and leiomyosarcoma cells. *Mol Hum Reprod*, 16:215-227.

Pandey DP, Picard D. 2009. miR-22 inhibits estrogen signaling by directly targeting the estrogen receptor alpha mRNA. *Mol Cell Biol*, 29:3783-3790.

Perron MP, Provost P. 2009. Protein components of the microRNA pathway and human diseases. *Methods Mol Biol*, 487:369-385.

Pillai RS, Bhattacharyya SN, Filipowicz W. 2007. Repression of protein synthesis by miRNAs: how many mechanisms? *Trends Cell Biol*, 17:118-126.

Poliseno L, Tuccoli A, Mariani L, Evangelista M, Citti L, Woods K, Mercatanti A, Hammond S, Rainaldi G. 2006. MicroRNAs modulate the angiogenic properties of HUVECs. *Blood*, 108:3068-3071.

Prat J, Gallardo A, Cuatrecasas M, Catasus L. 2007. Endometrial carcinoma: pathology and genetics. *Pathology*, 39:72-87.

Qian K, Hu L, Chen H, Li H, Liu N, Li Y, Ai J, Zhu G, Tang Z, Zhang H. 2009. Hsa-miR-222 is involved in differentiation of endometrial stromal cells in vitro. *Endocrinology*, 150:4734-4743.

Raver-Shapira N, Marciano E, Meiri E, Spector Y, Rosenfeld N, Moskovits N, Bentwich Z, Oren M. 2007. Transcriptional activation of miR-34a contributes to p53-mediated apoptosis. *Mol Cell*, 26:731-743.

Rhoton-Vlasak A, Chegini N, Hardt N, Williams RS. 2005. Histological characteristics and altered expression of interleukins (IL) IL-13 and IL-15 in endometria of levonorgestrel users with different uterine bleeding patterns. *Fertil Steril*, 83:659-665.

Rinaldi A, Poretti G, Kwee I, Zucca E, Catapano CV, Tibiletti MG, Bertoni F. 2007. Concomitant MYC

and microRNA cluster miR-17-92 (C13orf25) amplification in human mantle cell lymphoma. *Leuk Lymphoma*, 48:410-412.

Rodriguez A, Vigorito E, Clare S, Warren MV, Couttet P, Soond DR, van Dongen S, Grocock RJ, Das PP, Miska EA, Vetrie D, Okkenhaug K, Enright AJ, Dougan G, Turner M, Bradley A. 2007. Requirement of bic/microRNA-155 for normal immune function. *Science*, 316:608-611.

Scherr M, Eder M. 2007. Gene silencing by small regulatory RNAs in mammalian cells. *Cell Cycle*, 6:444-449.

Selam B, Kayisli UA, Mulayim N, Arici A. 2001. Regulation of Fas ligand expression by estradiol and progesterone in human endometrium. *Biol Reprod*, 65:979-985.

Shi H, Li P, Liang W, Chen J, Gao Y. 2010. Mechanisms of microRNA-mediated regulation of angiogenesis. *Front Biosci (Elite Ed)*, 2:1304-1319.

Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY, 2007. miR-21-mediated tumor growth. *Oncogene*, 26:2799-2803.

Singh M, Zaino RJ, Filiaci VJ, Leslie KK. 2007. Relationship of estrogen and progesterone receptors to clinical outcome in metastatic endometrial carcinoma: a Gynecologic Oncology Group Study. *Gynecol Oncol*, 106:325-333.

Siomi H, Siomi MC. 2010. Posttranscriptional regulation of microRNA biogenesis in animals. *Mol Cell*, 38:323-332.

Smith OP, Critchley HO. 2005. Progestogen only contraception and endometrial break through bleeding. *Angiogenesis*, 8:117-126.

Smith SK. 2001. Angiogenesis and reproduction. *BJOG*, 108:777-783.

Suarez Y, Fernandez-Hernando C, Pober JS, Sessa WC. 2007. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. *Circ Res*, 100:1164-1173.

Suarez Y, Sessa, WC. 2009. MicroRNAs as novel regulators of angiogenesis. *Circ Res*, 104:442-454.

Sylvestre Y, De Guire V, Querido E, Mukhopadhyay UK, Bourdeau V, Major F, Ferbeyre G, Chartrand P. 2007. An E2F/miR-20a autoregulatory feedback loop. *J Biol Chem*, 282:2135-2143.

Taganov KD, Boldin MP, Baltimore D. 2007. MicroRNAs and immunity: tiny players in a big field. *Immunity*. 26:133-137.

Tili E, Michaille JJ, Cimino A, Costinean S, Dumitru CD, Adair B, Fabbri M, Alder H, Liu CG, Calin GA, Croce CM. 2007. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. *J Immunol*, 179:5082-5089.

Visone R, Croce CM. 2009. MiRNAs and cancer. *Am J Pathol*, 174:1131-1138.

Wang S, Olson, EN. 2009. AngiomiRs--key regulators of angiogenesis. *Curr Opin Genet Dev*, 19:205-211.

Watanabe, H Kanzaki H, Narukawa S, Inoue T, Katsuragawa H, Kaneko Y, Mori T. 1997. Bcl-2 and Fas expression in eutopic and ectopic human endometrium during the menstrual cycle in relation to endometrial cell apoptosis. *Am J Obstet Gynecol*, 176:360-368.

Welch C, Chen Y, Stallings RL. 2007. MicroRNA-34a functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cells. *Oncogene*, 26:5017-5022.

Wickramasinghe NS, Manavalan TT, Dougherty SM, Riggs KA, Li Y, Klinge CM. 2009. Estradiol downregulates miR-21 expression and increases miR-21 target gene expression in MCF-7 breast cancer cells. *Nucleic Acids Res*, 37:2584-2595.

Woods K, Thomson JM, Hammond SM. 2007. Direct regulation of an oncogenic micro-RNA cluster by E2F transcription factors. *J Biol Chem*, 282:2130-2134.

Wu F, Yang Z, Li G. 2009. Role of specific microRNAs for endothelial function and angiogenesis. *Biochem Biophys Res Commun*, 386:549-553.

Yamagata K, Fujiyama S, Ito S, Ueda T, Murata T, Naitou M, Takeyama K, Minami Y, O'Malley BW, Kato S. 2009. Maturation of microRNA is hormonally regulated by a nuclear receptor. *Mol Cell*, 36:340-347.

Yang WJ, Yang DD, Na S, Sandusky GE, Zhang Q, Zhao G. 2005. Dicer is required for embryonic angiogenesis during mouse development. *J Biol Chem*, 280:9330-9335.

Yu Z, Jian Z, Shen SH, Purisima E, Wang E. 2007. Global analysis of microRNA target gene expression reveals that miRNA targets are lower expressed in mature mouse and Drosophila tissues than in the embryos. *Nucleic Acids Res*, 35:152-164.

Zhao C, Dahlman-Wright K, Gustafsson JA. 2008a. Estrogen receptor beta: an overview and update. *Nucl Recept Signal*, 6:e003.

Zhao JJ, Lin J, Yang H, Kong W, He L, Ma X, Coppola D, Cheng JQ. 2008b. MicroRNA-221/222 negatively regulates estrogen receptor alpha and is associated with tamoxifen resistance in breast cancer. *J Biol Chem*, 283:31079-31086.

Zhao Y, Deng C, Wang J, Xiao J, Gatalica Z, Recker RR, Xiao GG. 2010. Let-7 family miRNAs regulate estrogen receptor alpha signaling in estrogen receptor positive breast cancer. *Breast Cancer Res Treat*. doi: 10.1007/s10549-010-0972-2.