Published in final edited form as:

Trends Endocrinol Metab. 2012 May ; 23(5): 223–233. doi:10.1016/j.tem.2012.03.002.

miRNAs and estrogen action

Carolyn M. Klinge

Department of Biochemistry & Molecular Biology, Center for Genetics and Molecular Medicine, University of Louisville School of Medicine, Louisville, KY. 40292

Abstract

MicroRNAs (miRNAs) are short, non-coding RNAs that generally base-pair within the 3' untranslated region of target mRNAs causing translational inhibition and/or mRNA degradation. Estradiol (E₂) and other estrogen receptor (ER) ligands suppress or stimulate miRNA expression in human breast cancer cells, endometrial cells, rat mammary gland, and mouse uterus and post-translationally regulate protein expression. Aberrant miRNA expression is implicated in estrogen-related breast and endometrial cancers and a number of miRNAs downregulate ER α . The role of estrogen-regulated miRNA expression, the target genes of these miRNAs, and the role of miRNAs in health and disease is a "hot" area of research that will yield new insight into molecular mechanisms of estrogen action.

miRNA biogenesis and regulation

The function of the 80–93% of the expressed, non-protein-encoding dark matter RNAs' (excluding rRNA, mRNA, tRNA, and mtRNA) in the human is not yet fully understood [1]. The importance of non-coding RNAs (ncRNAs), which includes short interfering RNAs (siRNAs), microRNAs (miRNAs), and PIWI-interacting RNA (piRNAs), was recently reviewed [2].

miRNA are small (~ 20–22 nt), ncRNAs that regulate mRNA translation or stability [3]. About half of miRNAs are intragenic, and most are found in the 5' introns of host genes [4]. The current miRBase release (18.0) contains 18,226 miRNAs (168 species) and 1,527 human miRNAs (November 2011, <http://www.mirbase.org/>) [5]. Although the number of publications on miRNAs in humans has increased exponentially since the first reports in 2001, there are fewer studies on estrogenic regulation of miRNAs. The first report correlating miRNA expression with estrogen receptor α (ER α) in breast tumors was published in 2005 [6]. The first report on 17 β -estradiol (E₂) regulation of miRNAs, found that E₂ repressed miR-206 which, in turn, repressed ER α protein expression in MCF-7 human breast cancer cells [7]. Here, I will summarize research on E₂ regulation of miRNAs (Tables 1 and 2) and how miRNAs regulate E₂ action through ER, with a focus on human cell line studies.

miRNA biogenesis is diagrammed in Figure 1. RNA polymerase II transcribes ~ 60–100 nt primary-miRNAs (pri-miRNAs) that are 5'-capped and 3'-polyadenylated [8]. Pri-miRNAs

© 2012 Elsevier Ltd. All rights reserved.

Corresponding author: Klinge, C.M. (carolyn.klinge@louisville.edu).

Conflict of interest: none

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

are processed by Drosha, an RNase III endonuclease, that is recruited co-transcriptionally [9]. The Drosha complex associates with at least 20 polypeptides called the 'Drosha microprocessor complex' [10]. The Drosha cofactor DGCR8 (DiGeorge critical region 8), a double-stranded RNA binding domain protein (dsRBD), is required to cleave pri-miRNA into short (60 to 70 nt) imperfect hairpin precursor-miRNAs (pre-miRNAs). DGCR8 stabilizes Drosha and Drosha regulates DGCR8 levels by cleaving *DGCR8* mRNA, forming a regulatory loop [8]. The DEAD-box RNA helicases p68 and p72 are components of the Drosha complex that play redundant roles in the processing of a subset of miRNAs [11]. Pre-miRNAs have a 2 nt 3' overhang recognized by exportin-5 and Ran-GTP for export to the cytoplasm.

In the cytoplasm, pre-miRNAs are processed by the Dicer complex. Dicer is a cytoplasmic RNase III that removes the loop structure from pre-miRNA to generate the mature ~22 nt miRNA duplex with an overhang at the newly formed 3' end [12]. TRBP/PACT, dsRBDs, interact with and stabilize Dicer to enhance pre-miRNA processing [8]. Dicer, TRBP and/or PACT transfer miRNA to Argonaute proteins (Ago1, Ago2, Ago3, and Ago4) in the RNA-induced silencing complex (RISC) [8]. Ago proteins in RISC unwind the miRNA duplexes to form single stranded miRNA-5p and -3p products. The passenger strand is usually, although not always, degraded, and the mature miRNA (guide strand) is incorporated into RISC. Which miRNA is incorporated into RISC varies between tissues and species. Some pre-miRNAs are processed by Ago2 slicer catalytic activity instead of Dicer [13].

The mature miRNA guides the RISC complex to target mRNAs by binding either to the 3' untranslated region (3' UTR) or to the open reading frame (ORF) [8]. Imperfect base pairing complementarity between the mRNA 3'UTR and the miRNA represses translation by interaction of RISC complex with eIF6 to prevent 80S ribosomal assembly or translation [14]. Ago association with mRNA induces binding of eukaryotic 5' m7G cap-dependent initiation machinery, and the RISC/miRNP complex specifically recognizes some feature of the cap-dependent initiation machinery resulting in initiation-targeted translational repression [15]. Thus, miRNA interaction with the 3'UTR of its target mRNA decreases protein, but not necessarily mRNA, levels. Ago2 is the only Ago family member with RNA cleavage activity in the RISC complex [8]. The miRNA-containing ribonucleoprotein particle (miRNP)-silenced mRNA is directed to the P-bodies, where the mRNA is either released upon a cellular signal and/or actively degraded. Studies using miRNA overexpression or knockdown in combination with proteomic profiling concluded that individual miRNAs generally regulate a relatively small number of proteins, at modest levels (<2-fold) [16]. Recent advances in high throughput DNA sequencing directly identify miRNA-mRNA targets. High-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation (HITS-CLIP) identified RNA associated with Ago protein-RNA complexes, in mouse brain [17]. Although there is some evidence for miRNAs increasing translation of select mRNAs in a cell cycle-dependent manner [18], there is no information about E₂-regulation of such an increase.

There are a few examples of miRNAs interacting within the coding sequence (CDS) of target mRNAs. "RIP-ChIP" (AGO protein immunoprecipitation, which pulls down miRNAs and mRNA targets, followed by high density microarray) revealed that members of the miR-103/107 family preferentially bind their target CDS [19]. Individual miRNAs also recognize CDS targets. MiR-181a, repressed by E₂ in MCF-7 cells ([20], see Table 2), binds to 8-mer matched sites within the CDS of zinc finger genes (ZNF family) including *ZNF83*, *ZNF37A*, and *ZNF180* to reduce protein expression [21]. miR-24, also repressed by E₂ ([20], see Table 2), targets the CDS of FAS-associated factor 1 (*FAF1*) and blocks apoptosis in prostate and other cancer cells [22].

ER α , not ER β , interacts with Drosha

ER α interacts with and suppresses Drosha activity in mouse uterine epithelial cells and MCF-7 cells [23] (Figure 1). ER α -Drosha interaction is enhanced by E₂ and by ER α interaction with the p68 and p72 RNA helicases in the Drosha complex [23] (Figure 1). p68 and p72 co-activate ER α and increase its transcriptional activity, by interacting with the N-terminus of ER α , but not ER β [24]. The N-terminus of ER α also interacts directly with an LXXLL motif in the C-terminus of Drosha [23]. Liganded ER α (E₂-ER α) reduces the expression of 39 miRNAs in mouse uterus, by suppressing the Drosha-mediated processing of pri-miRNAs to pre-miRNAs [23] (see Table 2). Similarly, E₂-ER α represses the expression of a small number of mature miRNAs, including miR-125a and miR-145, in MCF-7 cells, without affecting the transcriptional level of the pre-miR-125a or pre-miR-145 [23]. E₂-ER α also inhibits a biochemically purified Drosha complex from processing pri-miR-16 to pre-miR-16, *in vitro*. These data indicate that E₂-ER α suppresses miRNA processing by its direct interaction with Drosha and with p68/p72 [23] (Figure 1). However, ER β does not interact with Drosha or p68 [25].

E₂ and miRNA regulation of Dicer and Ago2

The expression and/or activity of Dicer is regulated by the MAPK pathway: phosphorylation of TRBP enhances the production of growth-promoting miRNAs, *e.g.*, miR-17 and miR-20a, and inhibits let-7 tumor suppressor expression, at least in HeLa cells [26]. E₂ rapidly activates membrane-associated ER α 's activities including activating MAPK in a variety of cell lines [27]; however, no one has evaluated the impact of membrane-initiated ER α regulation of Dicer activity. This possibility is indicated in Figure 1. Dicer is translationally repressed by miR-221/222 and miR-29a [28]. E₂ increased Dicer protein ~ 1.45-fold, with 6 h treatment in MCF-7 cells [29]. *In vivo*, Dicer mRNA correlates positively with ER α mRNA in fallopian epithelial cells from women in the late ovulatory phase and negatively with ER β 2 mRNA in the midsecretory phase [30]. However, the authors did not directly examine if E₂ regulates Dicer expression.

Expression of Ago2, the catalytic component of RISC, is higher in ER α -negative, HER2-positive, than in ER α -positive/HER2 negative (luminal) human breast cancer cell lines and tumors [31]. Despite this observation, E₂ and the ER α -agonist PPT, but not the ER β -agonist DPN, increased Ago2 protein expression in MCF-7 cells [31] (Figure 1). This result would be anticipated to increase miRNA-mediated gene suppression.

miRNA regulation of ER α

Although a number of programs predict miRNA target-gene interaction, such as TargetScan [32] and myMIR [33], specific miRNA-mRNA interaction must be confirmed experimentally by cloning the 3'-UTR of the target mRNA 3' to a luciferase reporter, and then examining if the miRNA of interest reduces luciferase activity, in transiently transfected cells. Ten miRNAs have been identified as *bona fide* ER α regulators (reviewed in [34], unless cited here): miR-22 [35], miR-206; miR-221,222; miR-18a, 18b, 193b, and 302c [36]. In turn, ER α negatively regulates the expression of miR-221 and miR-222 [37] and miR-206 [7] in breast cancer cells (Figure 2). miR-206 is inversely correlated with the expression of ER α , but not ER β , in human breast tumors [38]. E₂ and the ER α -selective agonist PPT decrease miR-206 in MCF-7 cells whereas DPN, an ER β -selective agonist, increases miR-206, pointing to a regulatory loop [7]; however, since ER α expression is higher than ER β expression in MCF-7 and DPN also activates ER α , the significance of these observations remains to be confirmed. Higher miR-206 in ER α -negative MDA-MB-231 cells [7] offers a mechanism, in addition to ER α promoter methylation, for reduced ER α expression in MDA-MB-231 cells. These observations seem to contradict miR-206's

role as a tumor suppressor, but we must remember that miRNAs target multiple genes, and other miR-206 targets include NOTCH3, PAX7 and HSP60, that play roles in cell proliferation and motility [39].

miR-27a indirectly regulates ER α expression by reducing ZBTB10, an Sp repressor, *i.e.*, reducing Sp1, Sp3, and Sp4 [40] (Supplemental Fig. 1). In addition, direct interaction of ER α with Sp1, Sp3, and Sp4 bound to DNA GC-box interactions regulates transcription [41, 42]. A single-nucleotide polymorphism (SNP) has been identified within the terminal loop of pre-miR-27a, which would be expected to block maturation, that reduced risk in familial premenopausal breast cancer [43]. However, no one has examined the correlation of this SNP with ZBTB10, Sp1, 3, or 4 proteins, or ER α function in human breast tumors.

Transient overexpression of Let-7a, Let-7b, and Let-7i inhibit ER α expression in MCF-7 cells [44]. Let-7 family members are highly conserved and are considered to be tumor-suppressor miRNAs [45]. It has been reported that 4-hydroxytamoxifen (4-OHT) represses the expression of all eight Let-7 family members in MCF-7 cells and none of the Let-7 family members in endocrine-resistant LY2 breast cancer cells, commensurate with a less differentiated cellular state in LY2 cells [46]. Concordantly, lower *ESR1* mRNA and ER α protein were observed in LY2, compared to endocrine-sensitive MCF-7 cells [46].

To date, only one miRNA has been identified as regulating ER β : miR-92 [47].

Altered miRNA expression in breast cancer

Over 400 studies have been published identifying and examining miRNA in breast cancer with most studies using microarray with confirmation of selected changes by real-time PCR. Aberrant patterns of miRNA expression in human breast cancer have been reviewed, *e.g.*, [48]. miRNAs whose expression is increased in tumor cells, often as a result of chromosomal or molecular genomic aberrations, and which inhibit the translational expression of tumor suppressor genes, are called oncomiRNAs or oncomiRs. One well-characterized oncomiR in breast, and many other cancer types, is miR-21 which suppresses the expression of the tumor suppressors PTEN and PDCD4 [49, 50]. miR-21 expression is increased in breast tumors and antisense to miR-21 (AS-miR-21) suppresses MCF-7 cell growth *in vitro* and in tumor xenografts in mice, by down-regulating the apoptosis regulator BCL-2 [51]. It has also been reported that overexpression of miR-21 in MCF-7 cells increases soft agar colony formation, reflecting increased tumorigenicity of these cells [52]. It was recently demonstrated that miR-21 binds to a seed element in the 3'-UTR of the *PDCD4* gene and reduces PDCD4 protein expression [52].

E₂ regulates miR-21, although whether it stimulates or represses its transcription varies depending on experimental conditions, cell line, and control genes used in miRNA analysis (Tables 1 and 2). Conversely, tumor suppressor miRNAs (tsmiRNAs or tsmiRs) show lower expression in tumors and the reduction in their expression allows translation of mRNAs encoding oncogenes and other genes resulting in increased tumor cell proliferation, invasion, and angiogenesis and an inhibition of apoptosis [53].

Is E₂ synthesis and metabolism regulated by miRNAs?

Estrogens (E₂, estrone, and estriol) regulate development and homeostasis in a wide variety of tissues including the reproductive tract, bone, vasculature, brain, and breast. Although the sequence of events leading to breast tumor formation are not completely understood, lifetime exposure to estrogens is a major risk factor for breast cancer development [54]. Some studies indicated that E₂ is carcinogenic in human breast epithelial cells, perhaps through genotoxic metabolites, *e.g.*, such as 4-OH-E₂ or 4-OH-estrone [55]. In

postmenopausal women, estrogens, primarily estrone (E1) and estriol, are derived from adrenal androgens. Local production of E₂ in breast and other tumors is due to aromatase (*CYP19A*) and 17 β -hydroxysteroid dehydrogenase (17 β -HSD1) activity and may play a role in cancer progression [56]. There are no reports on how androgenic precursors to E₂ or E1 or the ER β ligand 3 β -adiol affect miRNA expression. There is a recent review on miRNAs regulation of P450 drug and steroid metabolism [57]. Pertinent to estrogen action is the observation that miR-27b reduces CYP1B1 [57], a result that would be expected to reduce 4-OH-E₂ or 4-OH-estrone, although this was not examined. Additionally, overexpression of a miRNA precursor library with 172 synthetic miRNA precursors by transient transfection in primary human granulosa cells identified 51 miRNAs that suppressed E₂ release, *e.g.*, miR-15a, miR-24, miR-24, let-7d, let-7g, miR-125a, miR-125b, miR-98, and miR-29a., but none that stimulated E₂ release [58]. The mechanisms for these effects have not been examined.

miRNAs regulating ER activity by repressing coregulator (coactivator/corepressor) expression

ERs regulate gene transcription by recruiting coregulators that modify chromatin structure in a ligand-, gene-, and cell-specific manner [59]. Although miRNAs would be expected to affect estrogen-regulated gene expression by altering coregulator levels, there are only two reports on coregulator regulation by miRNAs directly affecting ER activity. miR-17-5p was found to inhibit translation of the ER coactivator SRC-3/AIB1/NCOA3 [60]. Transfection of CHO-K1 cells with miR-17-5p and ER α inhibited E₂-stimulated ERE-driven luciferase reporter activity by 50%. Transfection of MCF-7 cells (that lack miR-17-5p) with miR-17-5p reduced E₂-induced cyclin D1 transcription and cell proliferation [60]. Overexpression of miR-206 repressed SRC-1/NCOA1 and SRC-3/NCOA3 expression in MCF-7 cells by direct interaction with the 3'-UTRs of each transcript, resulting in inhibition of E₂-induced ERE-luciferase activity and endogenous ER α target genes *PGR* and *CDC6* transcription [61].

There is some evidence that cyclin D1 acts as an ER α coactivator by direct interaction with the receptor C-terminal activation domain AF-2 [62], although ChIP studies fail to confirm direct recruitment of cyclin D1 and ER α to regulate *PGR* expression [63]. Infection of MCF-7 cells with a retroviral vector for the miR-17/20 cluster (including miR-17-5p, 18a, 19a, 20a, 19b, and 92) suppressed cyclin D1 expression and cell proliferation [64]. However, whether the inhibition of cell proliferation was mediated solely by cyclin D1 regulating CDK4 or CDK6 independent of ER α was not examined. It would be interesting to test whether overexpression of the miR-17/20 cluster represses ER α transcriptional activity. Expression of members of the miR-17/20 cluster was higher in LY2 ER- endocrine resistant cells compared to MCF-7 cells and 4-OHT increased the expression of miR-17, 18a, and 19a [46].

There is another report on miRNA regulation of two known ER coregulators: SRC-1/NCOA1 and RIP140/NRIP1 [65], but this study did not directly examine how these miRNAs affected ER-mediated activities. miR-22 inhibited SRC-1/NCOA1 expression by directly binding to the 3'UTR of NCOA1 in Huh7 human hepatocarcinoma cells [65]. miR-140-3p interacts directly with a seed element in the 3'UTR and represses the translation of RIP140/NRIP1 in Huh 7 cells [65].

There are 3 reports on miRNA regulation of corepressors. SMRT/NCOR2 suppresses basal ER α activity [66], although SMRT also acts as a gene-specific ER coactivator in MCF-7 cells [67]. miR-10a and -10b repress SMRT/NCOR2 expression by direct interaction with the 3'-UTR of NCOR2, in neuroblastoma cell lines [68]. However, the ability of miR-10a

and -10b to regulate ER activity is unknown. It has been reported that miR-10a and -10b expression is higher in LY2 endocrine-resistant breast cancer cells, compared to MCF-7 cells [46]. miR-184 interacts with the 3'-UTR of SMRT/NCOR2 and represses translation in transfected HeLa cells [69]. miR-184 is less abundant in breast tumors of patients who develop metastasis [70], but no one has examined how miR-184 affects ER activity in breast cancer cells. MTA1 (metastatic tumor antigen 1) suppresses ER α activity and was repressed by miR-661, but ER α function was not examined [71]. miR-615-3p repressed LCoR expression in THP-1 cells and normal splenic macrophages [72], but its ability to regulate ER activity has not been tested. Overall, further study of miRNA regulation coregulators and downstream effects on ER transcriptional activity is needed.

Estrogen regulation of miRNA expression

Because of its role in breast cancer, much of what we know about estrogenic regulation of miRNA expression comes from studies of how E₂ treatment of breast cancer cell lines affects mature miRNA expression. Although it is likely that estrogens regulate miRNAs by both genomic (transcriptional) and 'non-genomic' mechanisms of action, *e.g.*, plasma membrane ER α or GPR30- associated signaling cascades, investigators are only beginning to examine these pathways. A PubMed search for estrogen AND miRNA revealed a total of 195 papers and the pace research has increased since the initial reports in 2001. Rodent studies identified E₂-mediated changes in miRNA expression in traditional estrogen target tissues, including mouse uterus and rat mammary gland (Tables 1 and 2). The effect of E₂ on miRNA expression in male zebrafish (*Danio rerio*), ACI rats, and mouse splenocytes and was previously reviewed [34]. New information on E₂ regulated miRNAs in embryonic chicken gonads [73] and mouse uterus is presented in Tables 1 and 2.

There are, to my knowledge, only 19 studies in which miRNA regulation by E₂ has been directly examined in human cell lines (see Tables 1 and 2). Notably, 14 of the studies of E₂ regulated changes in miRNA expression were performed in MCF-7 cells [7, 20, 29, 34, 37, 74–82]. As noted by us and others, Tables 1 and 2 reflect the lack of consistency of E₂-regulated changes in miRNA expression even within the best-studied MCF-7 cell line, a result that may be attributed to different times and treatment conditions, *e.g.*, the method used to 'serum starve' the cells in order to see a response; E₂ concentration; differences in MCF-7 cells between labs; the control gene used for miRNA normalization; and the assay method used to measure mature miRNA expression.

One study compared the E₂-time course of mRNA expression profiling with global mapping of genomic ER α binding sites in MCF-7 and ZR-75-1 breast cancer cells [76]. That study identified miRNA binding sites within the UTRs of all E₂-regulated transcripts in MCF-7 cells and predicted miRNAs that would be expected to pair with at least 15% of genes within gene clusters that were regulated at particular times of E₂ treatment (1–32 h) [76]. The authors reported that E₂ increased miR-760 and miR-424 in a time-dependent manner and decreased miR-618, -570, and -107 [76]. They noted that hormone deprivation of MCF-7 reduced miR-101, -30e, and 340 and increased miR-103, -125b, -222, 30d, and 513a-5p, but appropriately concluded that dextran-coated charcoal stripping of serum eliminates many hormones from the serum, so they could not conclude that estrogens regulate these miRNAs [76]. This study reminds us to be cautious in interpreting E₂-induced changes in miRNA expression.

In contrast to reports of E₂-regulated miRNAs in MCF-7 cells, no miRNAs were significantly regulated by 10 nM E₂ with a 24 h treatment of T47D breast cancer cells, a result that the authors attributed to variations between treatments and the false positives generated by both microarray (LCSciences) and TaqMan Low Density Array technology

(Applied Biosystems) [83]. One explanation that the authors offered for previous reports showing E₂-ER α regulation of miRNA expression is through rapid upregulation of cMYC which, in turn regulates miRNA expression [83]. This possibility is included in Supplemental Fig. 2.

Direct regulation of let-7g by E₂-ER α was recently reported. E₂ suppressed the expression of let-7g, but not pri-let-7g, in a time-dependent, Fulvestrant-sensitive, and MEK/MAPK-dependent manner in MCF-7 cells [82]. The precise mechanism for the lack of effect of E₂ on pri-let-7g while decreasing mature let-7g was not examined other than to say that the effect of E₂ “may be achieved through a posttranscriptional mechanism” [82]. The reduction in mature let-7g could result from the ER α -Drosha complex interactions described above [23], although this was not examined.

There are only 3 studies directly examining tamoxifen or 4-OHT regulation of miRNA expression, were performed in MCF-7 cells [46, 77, 84]. Tables 1 and 2 summarize these data and indicate the need for further examination of the effect of these and other ER ligands on miRNA expression, particular as to effects on pri-miRNA expression.

Global genome binding studies to identify E₂-regulated miRNAs

GRO-seq (global nuclear run-on and sequencing) was used to identify the position and orientation of all engaged RNA polymerases (RNA pol I, II, and III) across the genome of MCF-7 cells treated with 100 nM E₂ for 10, 60, or 160 min [75]. This study reported that ER α enhancer transcripts were predominately upregulated by E₂, whereas the intergenic transcripts (which would include miRNAs and Long Intergenic Noncoding (linc) RNAs) were predominantly downregulated. The authors identified 322 expressed miRNA-containing transcripts and 119 were regulated by E₂ at least at one of the time points examined. Of those 119 pri-miRNA E₂-regulated transcripts, 47 showed more than a 3-fold up- or down-regulation. Further, using RefSeq, 2,700 putative mRNA targets for these miRNAs were identified and the authors reported that MCF-7 cells express a larger fraction of the 2,700 target mRNAs than expected, suggesting an “integrated regulatory program” [75]. Although the authors stated that miR-181a, miR-181b, and miR-21 were E₂-regulated, the published data showed only that miR-181a and -181b were downregulated and no confirmatory real time PCR studies were performed.

Less is known about ER β regulation of miRNA expression. One cause of this deficiency is that no one has examined miRNA regulation in a cell or tissue with endogenous selective ER β expression. E₂-induced ER β binding sites were identified in MCF-7 cells engineered to express comparable levels of ER α and ER β 1 using ChIP-seq after 45 min of 10 nM E₂ treatment [85]. Fifty-two E₂-induced ER β binding sites were identified in proximity to miRNA-encoding loci [85]. This list included miR-21, but not miR-206 that was reported to be upregulated by the ER β selective ligand DPN in MCF-7 cells [7]. Recently this group performed microarray miRNA expression profiling in the ER β 1-stable MCF-7 cell lines [25]. Seventy-three miRNAs were differentially expressed in ER β ⁺/ER β ⁻ cells with 44 increased and 29 decreased. Eight miRNAs: miR-1285, 30a, 450b-3p, 548d-3p, 616, 663b, and 708 were also differentially expressed in ER β ⁺ *versus* ER β ⁻ breast tumors. E₂ treatment of ER β ⁺ cells suppressed pri-, pre-, and mature miR-30a* and increased miR-23b, 23b*, 27b, 27b*, 24, and 24-1* [25]. Interestingly, ER β increased pre-miR-23b, 27b, and 24-1 synthesis from pri-RNA transcripts without increasing pri-RNA syntheses, indicating that ER β stimulates pri-RNA processing, an effect opposite of ER α 's suppression of Drosha activity [23]. Further research on endogenous ER β regulation of miRNA expression is needed.

E₂ or fulvestrant - regulated miRNA and their *bona fide* targets

Supplemental Table 1 lists E₂ or fulvestrant (ER antagonist, ICI 182,780)-regulated mRNAs and their *bona fide* (experimentally proven) targets. The major caveat is that the relationship between E₂ or fulvestrant regulation of the miRNA and the concordant effect of E₂ or fulvestrant on the mRNA gene target has rarely been examined, thus providing direction for future research.

miRNAs related to endocrine resistance in breast cancer

Approximately 40% of breast cancer patients are initially responsive to tamoxifen and other endocrine therapies relapse [86]. The mechanisms for acquired endocrine resistance, despite continued ER α expression, are complex [86]. To date, the role of microRNAs in endocrine-resistance has been examined by relatively few investigators. miRNA-221/222 are overexpressed in tamoxifen-, fulvestrant-, and tumor necrosis factor (TNF)- resistant MCF-7 cell line derivatives [81, 87, 88] and in ER α negative tumors [89]. This makes sense because miR-221/222 target ER α (Figure 2). Indeed, silencing of miR-221/222 in ER α -negative, endocrine-resistant MDA-MB-468 breast cancer cells increased ER α and sensitized the cells to tamoxifen-induced apoptosis [89]. Other targets of miR-221/222 in breast cancer were recently reviewed [90].

Overexpression of an oncogenic isoform of HER2 (HER2 Δ 16) causes tamoxifen-resistance in MCF-7 cells by reducing miR-15a and miR-16 which normally suppress BCL-2 [91]. miR-342 is downregulated in HER2 Δ 16-MCF-7 cells, in a HER2-/ tamoxifen resistant MCF-7 variant (TAMR1), in LCC2 ER α +/- tamoxifen-resistant breast cancer cells, and in a panel of tamoxifen refractory breast tumors [78]. Overexpression of miR-342 in HER2 Δ 16-MCF-7 cells sensitizes the cells to tamoxifen-induced apoptosis and reduces expression of BMP-7, GEMIN4, and SEMAD3, although further studies are required to determine the significance of these observations [78]. miR-301 is overexpressed in lymph node negative breast tumors and is associated with tumor recurrence [92]. Transfection of high miR-301-expressing MCF-7 cells with antagomir-31 increases the tamoxifen-sensitivity [92]. Microarrays identified 97 miRNAs differentially expressed in MCF-7 endocrine-sensitive *versus* resistant LY2 breast cancer cells [46]. Opposite expression of miRs-10a, 21, 22, 29a, 93, 125b, 181, 200a, 200b, 200c, 205, and 222 in MCF-7 *versus* LY2 cells was confirmed by quantitative real-time PCR [46]. miR-200 family members suppress expression of the transcription factor ZEB1 that initiates epithelial to mesenchymal transition (EMT), by repressing E-cadherin transcription [93, 94]. LY2 cells express ZEB1 and have reduced E-cadherin compared to ZEB1 negative, E-cadherin-expressing MCF-7 cells [46]. Other targets of miR-200 family members in cancer was recently reviewed [90].

A screen of miRNAs involved in estrogen-resistance in MCF-7 cells identified upregulation of miR-101 as promoting estrogen-independent growth of MCF-7 cells, without affecting ER α levels or activity [95]. The mechanism involves downregulation of MAGI-2, a scaffold protein required for PTEN activity by miR-101, and consequent increased Akt activity [95].

Concluding Remarks

E₂ and 4-OHT stimulate and repress miRNA expression in a cell and tissue-specific manner through ER α and possibly ER β . Little is known about how other ER ligands affect miRNA expression or how miRNAs may affect the synthesis or metabolism of E₂ and other estrogens. Because ER α interacts with components of the miRNA biogenesis pathway, more research is needed to distinguish E₂ regulation at the level of pri-miRNA transcription *versus* miRNA processing and whether transcriptional or membrane-initiated signaling (or both) regulate miRNA expression. Additionally, E₂-activated ER activity may be regulated

by miRNAs that target coregulators. Identification and characterization of estrogen-regulated miRNAs may provide new biomarkers and therapeutic targets in estrogen-associated diseases including breast and endometrial cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Special thanks to Dr. Barbara J. Clark for her astute reading and comments to improve this review.

References Cited

1. Kapranov P, et al. The majority of total nuclear-encoded non-ribosomal RNA in a human cell is 'dark matter' un-annotated RNA. *BMC Biol.* 2010; 8:149. [PubMed: 21176148]
2. Wery M, et al. Noncoding RNAs in gene regulation. *Wiley Interdiscip Rev Syst Biol Med.* 2011
3. Zeng Y. Principles of micro-RNA production and maturation. *Oncogene.* 2006; 25:6156–6162. [PubMed: 17028594]
4. Hinske L, et al. A potential role for intragenic miRNAs on their hosts' interactome. *BMC Genomics.* 2010; 11:533. [PubMed: 20920310]
5. Kozomara A, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res.* 2011; 39:D152–D157. [PubMed: 21037258]
6. Iorio MV, et al. MicroRNA Gene Expression Deregulation in Human Breast Cancer. *Cancer Res.* 2005; 65:7065–7070. [PubMed: 16103053]
7. Adams BD, et al. The Micro-Ribonucleic Acid (miRNA) miR-206 Targets the Human Estrogen Receptor- α (ER α) and Represses ER α Messenger RNA and Protein Expression in Breast Cancer Cell Lines. *Mol Endocrinol.* 2007; 21:1132–1147. [PubMed: 17312270]
8. Davis-Dusenbery BN, Hata A. Mechanisms of control of microRNA biogenesis. *J Biochem (Tokyo).* 2010; 148:381–392. [PubMed: 20833630]
9. Suzuki HI, Miyazono K. Emerging complexity of microRNA generation cascades. *J Biochem (Tokyo).* 2011; 149:15–25. [PubMed: 20876186]
10. Gregory RI, et al. The Microprocessor complex mediates the genesis of microRNAs. *Nature.* 2004; 432:235–240. [PubMed: 15531877]
11. Fukuda T, et al. DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. *Nat Cell Biol.* 2007; 9:604–611. [PubMed: 17435748]
12. Katahira J, Yoneda Y. Nucleocytoplasmic Transport of MicroRNAs and Related Small RNAs. *Traffic.* 2011; 12:1468–1474. [PubMed: 21518166]
13. Cifuentes D, et al. A Novel miRNA Processing Pathway Independent of Dicer Requires Argonaute2 Catalytic Activity. *Science.* 2010; 328:1694–1698. [PubMed: 20448148]
14. Lowery AJ, et al. MicroRNAs as Prognostic Indicators and Therapeutic Targets: Potential Effect on Breast Cancer Management. *Clin Cancer Res.* 2008; 14:360–365. [PubMed: 18223209]
15. Djuranovic S, et al. A parsimonious model for gene regulation by miRNAs. *Science.* 2011; 331:550–553. [PubMed: 21292970]
16. Selbach M, et al. Widespread changes in protein synthesis induced by microRNAs. *Nature.* 2008; 455:58–63. [PubMed: 18668040]
17. Chi S, et al. Argonaute HITS-CLIP decodes microRNA-mRNA interaction maps. *Nature.* 2009; 460:479–486. [PubMed: 19536157]
18. Vasudevan S, et al. Switching from Repression to Activation: MicroRNAs Can Up-Regulate Translation. *Science.* 2007; 318:1931–1934. [PubMed: 18048652]
19. Nelson PT, et al. Specific sequence determinants of miR-15/107 microRNA gene group targets. *Nucleic Acids Res.* 2011; 39:8163–8172. [PubMed: 21724616]

20. Maillot G, et al. Widespread Estrogen-Dependent Repression of microRNAs Involved in Breast Tumor Cell Growth. *Cancer Res.* 2009; 69:8332–8340. [PubMed: 19826037]
21. Huang S, et al. MicroRNA-181a modulates gene expression of zinc finger family members by directly targeting their coding regions. *Nucleic Acids Res.* 2010; 38:7211–7218. [PubMed: 20591824]
22. Qin W, et al. miR-24 Regulates Apoptosis by Targeting the Open Reading Frame (ORF) Region of FAF1 in Cancer Cells. *PLoS ONE.* 2010; 5:e9429. [PubMed: 20195546]
23. Yamagata K, et al. Maturation of MicroRNA Is Hormonally Regulated by a Nuclear Receptor. *Mol Cell.* 2009; 36:340–347. [PubMed: 19854141]
24. Watanabe M, et al. A subfamily of RNA-binding DEAD-box proteins acts as an estrogen receptor alpha coactivator through the N-terminal activation domain (AF-1) with an RNA coactivator, SRA. *EMBO J.* 2001; 20:1341–1352. [PubMed: 11250900]
25. Paris O, et al. Direct regulation of microRNA biogenesis and expression by estrogen receptor beta in hormone-responsive breast cancer. *Oncogene.* 2012
26. Paroo Z, et al. Phosphorylation of the Human MicroRNA-Generating Complex Mediates MAPK/Erk Signaling. *Cell.* 2009; 139:112–122. [PubMed: 19804757]
27. Levin ER. Minireview: Extranuclear Steroid Receptors: Roles in Modulation of Cell Functions. *Mol Endocrinol.* 2011; 25:377–384. [PubMed: 20861220]
28. Cochrane D, et al. MicroRNAs Link Estrogen Receptor Alpha Status and Dicer Levels in Breast Cancer. *Hormones and Cancer.* 2010; 1:306–319. [PubMed: 21761362]
29. Bhat-Nakshatri P, et al. Estradiol-regulated microRNAs control estradiol response in breast cancer cells. *Nucl Acids Res.* 2009; 37:4850–4861. [PubMed: 19528081]
30. Shao R, et al. Distinct Expression Pattern of Dicer1 Correlates with Ovarian-Derived Steroid Hormone Receptor Expression in Human Fallopian Tubes during Ovulation and the Midsecretory Phase. *J Clin Endocrinol Metab.* 2011; 96:E869–E877. [PubMed: 21346072]
31. Adams BD, et al. Argonaute-2 Expression is Regulated by EGFR/MAPK Signaling and Correlates with a Transformed Phenotype in Breast Cancer Cells. *Endocrinology.* 2008 en.2008–0984.
32. Lewis BP, et al. Conserved Seed Pairing, Often Flanked by Adenosines, Indicates that Thousands of Human Genes are MicroRNA Targets. *Cell.* 2005; 120:15–20. [PubMed: 15652477]
33. Corrada D, et al. myMIR: a genome-wide microRNA targets identification and annotation tool. *Briefings in Bioinformatics.* 2011; 12:588–600. [PubMed: 22021901]
34. Klinge CM. Estrogen Regulation of MicroRNA Expression. *Curr Genomics.* 2009; 10:169–183. [PubMed: 19881910]
35. Pandey DP, Picard D. miR-22 Inhibits Estrogen Signaling by Directly Targeting the Estrogen Receptor {alpha} mRNA. *Mol Cell Biol.* 2009; 29:3783–3790. [PubMed: 19414598]
36. Leivonen SK, et al. Protein lysate microarray analysis to identify microRNAs regulating estrogen receptor signaling in breast cancer cell lines. *Oncogene.* 2009; 28:3926–3936. [PubMed: 19684618]
37. Di Leva G, et al. MicroRNA Cluster 221–222 and Estrogen Receptor {alpha} Interactions in Breast Cancer. *J Natl Cancer Inst.* 2010; 102:706–721. [PubMed: 20388878]
38. Kondo N, et al. miR-206 Expression Is Down-regulated in Estrogen Receptor {alpha}-Positive Human Breast Cancer. *Cancer Res.* 2008; 68:5004–5008. [PubMed: 18593897]
39. Chen X, et al. Expression of the tumor suppressor miR-206 is associated with cellular proliferative inhibition and impairs invasion in ER α -positive endometrioid adenocarcinoma. *Cancer Lett.* 2012; 314:41–53. [PubMed: 21983130]
40. Li X, et al. MicroRNA-27a Indirectly Regulates Estrogen Receptor {alpha} Expression and Hormone Responsiveness in MCF-7 Breast Cancer Cells. *Endocrinology.* 2010; 151:2462–2473. [PubMed: 20382698]
41. Safe S, Kim K. Non-classical genomic estrogen receptor (ER)/specificity protein and ER/activating protein-1 signaling pathways. *J Mol Endocrinol.* 2008; 41:263–275. [PubMed: 18772268]
42. Wu F, et al. Role of SP transcription factors in hormone-dependent modulation of genes in MCF-7 breast cancer cells: microarray and RNA interference studies. *J Mol Endocrinol.* 2009; 42:19–33. [PubMed: 18952783]

43. Yang R, et al. A genetic variant in the pre-miR-27a oncogene is associated with a reduced familial breast cancer risk. *Breast Cancer Res Treat.* 2010; 121:693–702. [PubMed: 19921425]
44. Zhao Y, et al. Let-7 family miRNAs regulate estrogen receptor alpha signaling in estrogen receptor positive breast cancer. *Breast Cancer Res Treat.* 2011; 127:69–80. [PubMed: 20535543]
45. Boyerinas B, et al. The role of let-7 in cell differentiation and cancer. *Endocr Relat Cancer.* 2010; 17:F19–36. [PubMed: 19779035]
46. Manavalan TT, et al. Differential expression of microRNA expression in tamoxifen-sensitive MCF-7 versus tamoxifen-resistant LY2 human breast cancer cells. *Cancer Lett.* 2011; 313:26–43. [PubMed: 21955614]
47. Al-Nakhle H, et al. Estrogen Receptor {beta}1 Expression Is Regulated by miR-92 in Breast Cancer. *Cancer Res.* 2010; 70:4778–4784. [PubMed: 20484043]
48. Corcoran C, et al. Intracellular and Extracellular MicroRNAs in Breast Cancer. *Clin Chem.* 2011; 57:18–32. [PubMed: 21059829]
49. Qi L, et al. Expression of miR-21 and its targets (PTEN, PDCD4, TM1) in flat epithelial atypia of the breast in relation to ductal carcinoma in situ and invasive carcinoma. *BMC Cancer.* 2009; 9:163. [PubMed: 19473551]
50. Frankel LB, et al. Programmed Cell Death 4 (PDCD4) Is an Important Functional Target of the MicroRNA miR-21 in Breast Cancer Cells. *J Biol Chem.* 2008; 283:1026–1033. [PubMed: 17991735]
51. Si ML, et al. miR-21-mediated tumor growth. *Oncogene.* 2007; 26:2799–2803. [PubMed: 17072344]
52. Lu Z, et al. MicroRNA-21 Promotes Cell Transformation by Targeting the Programmed Cell Death 4 Gene. *Oncogene.* 2008; 27:4373–4379. [PubMed: 18372920]
53. Iorio MV, et al. Breast cancer and microRNAs: therapeutic impact. *The Breast.* 2011; 20(Supplement 3):S63–S70. [PubMed: 22015296]
54. Henderson BE, Feigelson HS. Hormonal carcinogenesis. *Carcinogenesis.* 2000; 21:427–433. [PubMed: 10688862]
55. Liehr JG. Genotoxicity of the steroidal oestrogens oestrone and oestradiol: possible mechanism of uterine and mammary cancer development. *Hum Reprod Update.* 2001; 7:273–281. [PubMed: 11392373]
56. Sasano H, et al. In situ estrogen production and its regulation in human breast carcinoma: From endocrinology to intracrinology. *Pathol Int.* 2009; 59:777–789. [PubMed: 19883428]
57. Nakajima M, Yokoi T. MicroRNAs from biology to future pharmacotherapy: Regulation of cytochrome P450s and nuclear receptors. *Pharmacol Ther.* 2011; 131:330–337. [PubMed: 21565218]
58. Sirotkin AV, et al. Identification of MicroRNAs controlling human ovarian cell steroidogenesis via a genome-scale screen. *J Cell Physiol.* 2009; 219:415–420. [PubMed: 19194990]
59. Lydon JP, O'Malley BW. Minireview: Steroid Receptor Coactivator-3: A Multifarious Coregulator in Mammary Gland Metastasis. *Endocrinology.* 2011; 152:19–25. [PubMed: 21047941]
60. Hossain A, et al. Mir-17-5p Regulates Breast Cancer Cell Proliferation by Inhibiting Translation of AIB1 mRNA. *Mol Cell Biol.* 2006; 26:8191–8201. [PubMed: 16940181]
61. Adams BD, et al. The Role of miR-206 in the Epidermal Growth Factor (EGF) Induced Repression of Estrogen Receptor-{alpha} (ER{alpha}) Signaling and a Luminal Phenotype in MCF-7 Breast Cancer Cells. *Mol Endocrinol.* 2009; 23:1215–1230. [PubMed: 19423651]
62. Neuman E, et al. Cyclin D1 stimulation of estrogen receptor transcriptional activity independent of cdk4. *Mol Cell Biol.* 1997; 17:5338–5347. [PubMed: 9271411]
63. Yang C, et al. Cyclin D1 Enhances the Response to Estrogen and Progesterone by Regulating Progesterone Receptor Expression. *Mol Cell Biol.* 2010; 30:3111–3125. [PubMed: 20404095]
64. Yu Z, et al. A cyclin D1/microRNA 17/20 regulatory feedback loop in control of breast cancer cell proliferation. *J Cell Biol.* 2008; 182:509–517. [PubMed: 18695042]
65. Takata A, et al. MicroRNA-22 and microRNA-140 suppress NF-κB activity by regulating the expression of NF-κB coactivators. *Biochem Biophys Res Commun.* 2011; 411:826–831. [PubMed: 21798241]

66. Smith CL, et al. Coactivator and corepressor regulation of the agonist/antagonist activity of the mixed antiestrogen, 4-hydroxytamoxifen. *Mol Endocrinol.* 1997; 11:657–666. [PubMed: 9171229]
67. Peterson TJ, et al. The Silencing Mediator of Retinoic Acid and Thyroid Hormone Receptor (SMRT) Corepressor Is Required for Full Estrogen Receptor {alpha} Transcriptional Activity. *Mol Cell Biol.* 2007; 27:5933–5948. [PubMed: 17591692]
68. Foley NH, et al. MicroRNAs 10a and 10b are potent inducers of neuroblastoma cell differentiation through targeting of nuclear receptor corepressor 2. *Cell Death Differ.* 2011; 18:1089–1098. [PubMed: 21212796]
69. Wu J, et al. MicroRNA-184 downregulates nuclear receptor corepressor 2 in mouse spermatogenesis. *BMC Dev Biol.* 2011; 11:64. [PubMed: 22017809]
70. Farazi TA, et al. MicroRNA Sequence and Expression Analysis in Breast Tumors by Deep Sequencing. *Cancer Res.* 2011; 71:4443–4453. [PubMed: 21586611]
71. Bui-Nguyen TM, et al. Stimulation of Inducible Nitric Oxide by Hepatitis B Virus Transactivator Protein HBx Requires MTA1 Coregulator. *J Biol Chem.* 2010; 285:6980–6986. [PubMed: 20022949]
72. Jiang A, et al. miR-615-3p promotes the phagocytic capacity of splenic macrophages by targeting ligand-dependent nuclear receptor corepressor in cirrhosis-related portal hypertension. *Exp Biol Med.* 2011; 236:672–680.
73. Bannister SC, et al. Manipulation of Estrogen Synthesis Alters MIR202* Expression in Embryonic Chicken Gonads. *Biol Reprod.* 2011; 85:22–30. [PubMed: 21389341]
74. Castellano L, et al. The estrogen receptor-alpha induced microRNA signature regulates itself and its transcriptional response. *Proc Natl Acad Sci USA.* 2009; 106:15732–15737. [PubMed: 19706389]
75. Hah N, et al. A Rapid, Extensive, and Transient Transcriptional Response to Estrogen Signaling in Breast Cancer Cells. *Cell.* 2011; 145:622–634. [PubMed: 21549415]
76. Cicatiello L, et al. Estrogen Receptor {alpha} Controls a Gene Network in Luminal-Like Breast Cancer Cells Comprising Multiple Transcription Factors and MicroRNAs. *Am J Pathol.* 2010; 176:2113–2130. [PubMed: 20348243]
77. Wickramasinghe N, et al. Estradiol downregulates miR-21 expression and increases miR-21 target gene expression in MCF-7 breast cancer cells. *Nucleic Acids Res.* 2009; 37:2584–2595. [PubMed: 19264808]
78. Cittelly DM, et al. Downregulation of miR-342 is associated with tamoxifen resistant breast tumors. *Mol Cancer.* 2010; 9:317. [PubMed: 21172025]
79. Masri S, et al. The role of microRNA-128a in regulating TGFbeta signaling in letrozole-resistant breast cancer cells. *Breast Cancer Res Treat.* 2010; 124:89–99. [PubMed: 20054641]
80. Wang G, et al. RNA Polymerase II Binding Patterns Reveal Genomic Regions Involved in MicroRNA Gene Regulation. *PLoS ONE.* 2010; 5:e13798. [PubMed: 21072189]
81. Rao X, et al. MicroRNA-221/222 confers breast cancer fulvestrant resistance by regulating multiple signaling pathways. *Oncogene.* 2011; 30:1082–1097. [PubMed: 21057537]
82. Qian P, et al. Pivotal Role of Reduced let-7g Expression in Breast Cancer Invasion and Metastasis. *Cancer Res.* 2011; 71:6463–6474. [PubMed: 21868760]
83. Katchy A, et al. Estradiol-activated estrogen receptor α does not regulate mature microRNAs in T47D breast cancer cells. *J Steroid Biochem Mol Biol.* 2011 Epub ahead of print.
84. Bergamaschi A, Katzenellenbogen BS. Tamoxifen downregulation of miR-451 increases 14-3-3[zeta] and promotes breast cancer cell survival and endocrine resistance. *Oncogene.* 2012; 31:39–47. [PubMed: 21666713]
85. Grober O, et al. Global analysis of estrogen receptor beta binding to breast cancer cell genome reveals an extensive interplay with estrogen receptor alpha for target gene regulation. *BMC Genomics.* 2011; 12:36. [PubMed: 21235772]
86. Ring A, Dowsett M. Mechanisms of tamoxifen resistance. *Endocr Relat Cancer.* 2004; 11:643–658. [PubMed: 15613444]
87. Miller TE, et al. MicroRNA-221/222 confers tamoxifen resistance in breast cancer by targeting p27(Kip1). *J Biol Chem.* 2008; 283:29897–29903. [PubMed: 18708351]

88. Xin F, et al. Computational analysis of microRNA profiles and their target genes suggests significant involvement in breast cancer antiestrogen resistance. *Bioinformatics*. 2009; 25:430–434. [PubMed: 19091772]
89. Zhao J-J, et al. MicroRNA-221/222 negatively regulates ERalpha and associates with tamoxifen resistance in breast cancer. *J Biol Chem*. 2008; 283:31079–31086. [PubMed: 18790736]
90. Howe E, et al. The miR-200 and miR-221/222 microRNA Families: Opposing Effects on Epithelial Identity. *J Mammary Gland Biol Neoplasia*. 2012;1–13. [PubMed: 22402939]
91. Cittelly DM, et al. Oncogenic HER2Δ16 Suppresses miR-15a/16 and Dereglates BCL-2 to Promote Endocrine Resistance of Breast Tumors. *Carcinogenesis*. 2010; 31:2049–2057. [PubMed: 20876285]
92. Shi W, et al. MicroRNA-301 Mediates Proliferation and Invasion in Human Breast Cancer. *Cancer Res*. 2011; 71:2926–2937. [PubMed: 21393507]
93. Bracken CP, et al. A Double-Negative Feedback Loop between ZEB1-SIP1 and the microRNA-200 Family Regulates Epithelial-Mesenchymal Transition. *Cancer Res*. 2008; 68:7846–7854. [PubMed: 18829540]
94. Cochrane DR, et al. Loss of miR-200c: A Marker of Aggressiveness and Chemoresistance in Female Reproductive Cancers. *J Oncol*. 2010; 2010:821717. [PubMed: 20049172]
95. Sachdeva M, et al. MicroRNA-101-mediated Akt activation and estrogen-independent growth. *Oncogene*. 2011; 30:822–831. [PubMed: 20956939]
96. Nothnick WB, Healy C. Estrogen induces distinct patterns of microRNA expression within the mouse uterus. *Reprod Sci*. 2010; 17:987–994. [PubMed: 20720260]
97. Pan Q, et al. Differential expression of microRNAs in myometrium and leiomyomas and regulation by ovarian steroids. *J Cell Mol Med*. 2008; 12:227–240. [PubMed: 18182067]
98. Zhang R, et al. Estrogen receptor-regulated microRNAs contribute to the BCL2/BAX imbalance in endometrial adenocarcinoma and precancerous lesions. *Cancer Lett*. 2012; 314:155–165. [PubMed: 22014978]
99. Dai R, et al. Suppression of LPS-induced IFN{gamma} and nitric oxide in splenic lymphocytes by select estrogen-regulated miRNA: A novel mechanism of immune modulation. *Blood*. 2008; 112:4591–4597. [PubMed: 18791161]
100. Pan Q, et al. The expression profile of micro-RNA in endometrium and endometriosis and the influence of ovarian steroids on their expression. *Mol Hum Reprod*. 2007; 13:797–806. [PubMed: 17766684]

Figure 1. The miRNA processing pathway

The figure illustrates the steps in the processing of miRNA, also summarized in the text. miRNAs are transcribed by RNA polymerase II into primary-miRNAs (pri-miRNAs) that are processed into precursor-miRNAs (pre-miRNAs) by the Drosha microprocessor complex which includes DGCR8. The DEAD-box RNA helicases p68 and p72 are components of the Drosha complex. Pre-miRNAs are recognized by exportin-5 and Ran-GTP with GTP hydrolysis allowing export to the cytoplasm. In the cytoplasm, pre-miRNAs are processed by the Dicer complex to mature ~22 nt miRNA. The TRBP/PACT complex interacts with and stabilizes Dicer, and transfers miRNA to Argonaute proteins (Ago1, Ago2, Ago3, and Ago4) in the RNA-induced silencing complex (RISC). miRNA guides the RISC complex to target mRNAs by binding to the 3' UTR or the ORF and represses translation. The silenced

mRNA is directed to the P-bodies for degradation. Interactions of ER α with Drosha, p68, and p77; regulation of Ago2 and Dicer; and possible regulation by nongenomic E₂ signaling (-----) are indicated.

Figure 2. miRNA regulation of ERα and subsequent ERα transcriptional activity

ERα is downregulated by miR-18a,b; miR-193, miR-302c, miR-206, miR-22, miR-221, and miR-222. ERα forms a negative regulatory loop with miR-221, 222, and 206 (red lines). Sp1 increases ERα expression (green arrow) and ERα interacts directly (double headed arrow) with Sp1 to regulate gene transcription. Whether ERα is a *bona fide* target of Let-7a, 7b, and 7i has not been experimentally verified (dotted line). ERα interacts with coregulators including SRC-1/NCOA1, SRC-3/AIB1/NCOA3, RIP140/NRIP, SMRT/NCOR2, and LCoR/LCOR which are downregulated by the miRNAs indicated. The regulation of miRNA gene transcription by ligand-occupied ERα is shown in Table 1 (Stimulation) and Table 2 (Suppression).

Table 1
Estradiol (E₂), tamoxifen, 4-hydroxytamoxifen (4-OHT), or Fulvestrant (ICI 182,780)-
induced miRNAs in animal studies and human cell lines

This table lists miRNAs whose expression is increased by E₂, 4-OHT, or fulvestrant.

miRNA	Comments	Species/tissue/cell line	Ref.
miR-204 miR-451 miR-99b miR-429 miR-720	Peak 2.3-fold at 4 h; 8.5-fold at 2 h, 22-fold at 8 h, 7.5-fold at 30 h ~ 50% increase in 16 h ~ 50% 16–24 h ~ 50% increase in 24 h	CD1 mouse uterus	[96]
miR-202*	Female gonads treated at Embryonic Day (E) 3.5 with an aromatase inhibitor were masculinized by E9.5, and MIR202* expression was increased.	embryonic chicken gonads	[73]
miR-26a	24 h 10 nM E ₂ , also stimulated by ICI or E ₂ + ICI	Primary human myometrial smooth muscle cells (MSMC)	[97]
miR-206	10 nM DPN ~ 1.6-fold	MCF-7 breast cancer cells	[7]
Let-7f, 7a, 7d, 7c, 7g miR-203 Let-7b, 7e miR-98, 21, 200a, 103, 200c, 107, 17-5p, 23a, 200b, 30c, 30b, 424 let-7i	10 nM E ₂ for 4 h Arranged from highest (3.2-) to lowest (1.5-) fold change	MCF-7 cells stably expressing a bicistronic vector control	[29]
miR-520d	4.8-fold 10 nM E ₂ for 4 h	MCF-7 cells expressing a constitutively active AKT, stably	[29]
miR-17-92	10 nM E ₂ for 3, 6, or 12 h 4–5-fold increase (miR-17-92 cluster encodes miR-17, 18, 19, 20, 19b-1, 92-1) E ₂ -induction is mediated by ER α induction of c-Myc expression	MCF-7 cells	[74]
Let-7g miR-15a miR-195 miR-200a miR-203 miR-25 miR-26b miR-27a miR-27b miR-30b miR-365 miR-7 miR-98	10 nM E ₂ for 6 h 1.7-fold 2.32-fold 2.0-fold 2.6-fold 1.8-fold 1.6-fold 2.1-fold 1.7-fold 1.9-fold 2.2-fold 1.5-fold 1.8-fold 1.6-fold	MCF-7 cells	[34]

miRNA	Comments	Species/tissue/cell line	Ref.
miR-21	100 nM 4-OHT for 6 h	MCF-7 cells	[77]
miR-193b miR-301b	10 nM E ₂ for 4 h Tamoxifen-sensitive (fold not stated)	MCF-7 cells	[80]
miR-130b, 17, 18a, 19a, 19b-1, 20a, 92a-1	10 nM E ₂ for 4 h Tamoxifen-insensitive (fold not stated)	MCF-7 cells	[80]
miR-16-2, 198, 29c	10 nM E ₂ for 24 h LCSciences microarray. Note: not statistically significant in QPCR	T47D breast cancer cells	[83]
miR-342	100 pM E ₂ for 24 h ~ 11-fold increase Not blocked by 1 μ M 4-OHT-	MCF-7-HER2 cells, MCF-7 cells stably overexpressing HER2, but still tamoxifen-sensitive, although	[78]
miR-760 miR-424	10 nM E ₂ , 24 h and 3 d 1.5-fold for each	MCF-7 cells	[76]
miR-17-3p	E ₂ (concentration not given) ~ 2-fold increase	MCF-7 overexpressing the aromatase gene (MCF-7aro), stably	[79]
miR-221 miR-222	200 nM Fulvestrant (not 4-OHT) for 48 h or 4 d	MCF-7	[81]
Let-7a, 7b, 7c, 7d, 7e, 7f, 7g, 7i miR-27a, 320, 424	1 μ M E ₂ 72 h microarray, upregulated > 2-fold	Ishikawa and ECC-7 human endometrial cancer cells	[98]
miR-106a, 19b, 20a,7	10 nM E ₂ for 24 h, all blocked by 1 μ M ICI 182,780	MCF-7 cells	[28]

Table 2
Estradiol- and tamoxifen- inhibited miRNAs

This table lists miRNAs whose expression is decreased by E₂, tamoxifen, or 4-OHT.

miRNA	Comments	Species/tissue/cell line	Ref.
miR-429	~ 50% reduction by 4–8 h (E ₂ 10 mg/kg body weight to ovex CD-1 mice)	Mouse uterus	[96]
miR-181b	~ 50% reduction by 8–16 h	Mouse uterus	[96]
miR-720	~ 50% reduction by 4 h	Mouse uterus	[96]
miR-125a, 16, 195, 143, 145	~ 50 % reduction with 1 µg E ₂ for 24 h in ovex mouse uterus; however, pre-miR-125, 195, 143, & 145 were unaffected, so not transcriptional regulation	Mouse uterus	[23]
miR-146a, 125a,b, 145 Let-7e	8 wk, E ₂ (pellet)-treated C57Bl/6 male mice	Freshly isolated splenic lymphocytes	[99]
miR-202*	<i>In ovo</i> injection of E ₂ at E4.5 caused feminization of male gonads at E9.5 and reduced MIR202* expression to female levels. Reduced MIR202* correlated with reduced expression of the testis-associated genes <i>DMRT1</i> and <i>SOX9</i> , and up-regulation of ovary-associated genes <i>FOXL2</i> and <i>CYP19A1</i> (aromatase).	embryonic chicken gonads	[73]
miR-21 miR-20a	24 h 10 nM E ₂ ~ 70% repression of miR-21 and 30% of miR-20a; blocked by ICI 182,780	isolated human endometrial glandular epithelial cells	[100]
miR-21 miR-26a	24 h 10 nM E ₂ ~ 50% reduction in miR-21 ~ 40% reduction in miR-26a	Primary human leiomyoma smooth muscle cells (LSMC)	[97]
miR-181a- miR-181b miR-181d miR-21 miR-26a, -miR-26b miR-193a miR-98 miR-26b miR-203 miR-200c miR-200a miR-24 miR-27b Let-7g, -7f, -7a, -7c miR-193b miR-23a miR-23b miR-520d miR-499	10 nM E ₂ 48 h Also repressed in T47D, ZR-75-1, BT-474, and BG1, but not SKBR3 breast cancer cells. Applies to all miRs on the left. Range of repression in MCF-7 was 0.36 for miR-181a to 0.82 for miR-499.	MCF-7 cells	[20]

miRNA	Comments	Species/tissue/cell line	Ref.
miR-206	1 nM E ₂ or 10 nM PPT (an ER α -selective agonist) 80% reduction in expression with 24 h treatment	MCF-7 cells	[7]
miR-21	10 nM E ₂ 6 h ~ 60% reduction in miR-21	MCF-7 cells	[77]
Let-7a	10 nM E ₂ 6 h (log ₂ (E ₂ /EtOH)) -0.3	MCF-7 cells	[34]
Let-7f	-0.25		
miR-149	-3.17		
miR-200c	-0.42		
miR-328	-3.92		
miR-342	-0.26		
miR-302b*	10 nM E ₂ for 4 h 0.4-fold	MCF-7 cells stably expressing a biscistronic vector control	[29]
miR-506	0.5-fold		
miR-524*	0.5-fold		
miR-27a	0.6-fold		
miR-270	0.6-fold		
miR-143	0.6-fold		
miR-9	0.7-fold		
miR-424	10 nM E ₂ for 4 h 0.3-fold	MCF-7 cells stably expressing a constitutively active AKT	[29]
miR-518d	0.3-fold		
miR-518e	0.3-fold		
miR-506	0.3-fold		
miR-409-5p	0.3-fold		
miR-216	0.3-fold		
miR-518c	0.5-fold		
miR-526b	0.5-fold		
miR-24b	0.5-fold		
miR-337	0.5-fold		
miR-146	0.5-fold		
miR128b	0.5-fold		
miR-124a	0.6-fold		
miR-211	0.6-fold		
miR143	0.6-fold		
miR-128a	0.6-fold		
miR-126 & 126*	0.6-fold		
miR-1	0.6-fold		
miR-10b	0.7-fold		

miRNA	Comments	Species/tissue/cell line	Ref.
miR-181a	100 nM E ₂	MCF-7 cells	[75]
miR-181b	Fold repression not indicated		
miR-221	siER α in MCF-7 or 10 d treatment of MDA-MB-231 cells with the demethylating agent 5-AZA-2'-deoxycytidine (10 μ M. Restored ER α expression)	MCF-7 and T47D cells	[37]
miR-222	10 nM E ₂ for 24 h ~ 80% reduction in MCF-7 and T47D cells		
miR-221, miR-222	10 nM E ₂ for 48 h ~ 90% reduction	MCF-7 cells	[81]
miR-339-5p	10 nM E ₂ for 24 h LCSciences microarray	T47D	[83]
miR-220c	Not statistically significant in QPCR		
miR-650			
miR-107	10 nM E ₂ , 6, 12, 24 h and 3 d	MCF-7 cells	[76]
miR-618	1.5-fold for all three miRNAs		
miR-570	Repression of miR-570 was ablated at 3d		
Let-7g	10 nM E ₂ , maximally reduced at 6 h. Repression was blocked by Fulvestrant. E ₂ action was ER and MAPK-mediated. In turn, the decrease in let-7g directly increased GAB2 and FN1 expression, which, in turn, increased MAPK, MMP-2, and MMP-9 activity.	MCF-7 cells	[82]
miR-181	100 nM 4-OHT for 6 h in MCF-7 cells~ 50% reduction	MCF-7 cells	[46]
miR-451	1 μ M tamoxifen- repressed by 45% at 4 h and 90% at 24 h Expression ~ 2-fold lower in tamoxifen- resistant MCF-7 cells	MCF-7 cells	[84]