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Review

miRNAs regulated by estrogens, tamoxifen, and endocrine disruptors and their downstream gene targets

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ABSTRACT

MicroRNAs (miRNAs) are short (22 nucleotides), single-stranded, non-coding RNAs that form complimentary base-pairs with the 3′ untranslated region of target mRNAs within the RNA-induced silencing complex (RISC) and block translation and/or stimulate mRNA transcript degradation. The non-coding miRBase (release 21, June 2014) reports that human genome contains ~2588 mature miRNAs which regulate ~60% of human protein-coding mRNAs. Dysregulation of miRNA expression has been implicated in estrogen-related diseases including breast cancer and endometrial cancer. The mechanism for estrogen regulation of miRNA expression and the role of estrogen-regulated miRNAs in normal homeostasis, reproduction, lactation, and in cancer is an area of great research and clinical interest. Estrogens regulate miRNA transcription through estrogen receptors α and β in a tissue-specific and cell-dependent manner. This review focuses primarily on the regulation of miRNA expression by ligand-activated ERs and their *bona fide* gene targets and includes miRNA regulation by tamoxifen and endocrine disrupting chemicals (EDCs) in breast cancer and cell lines.

Contents

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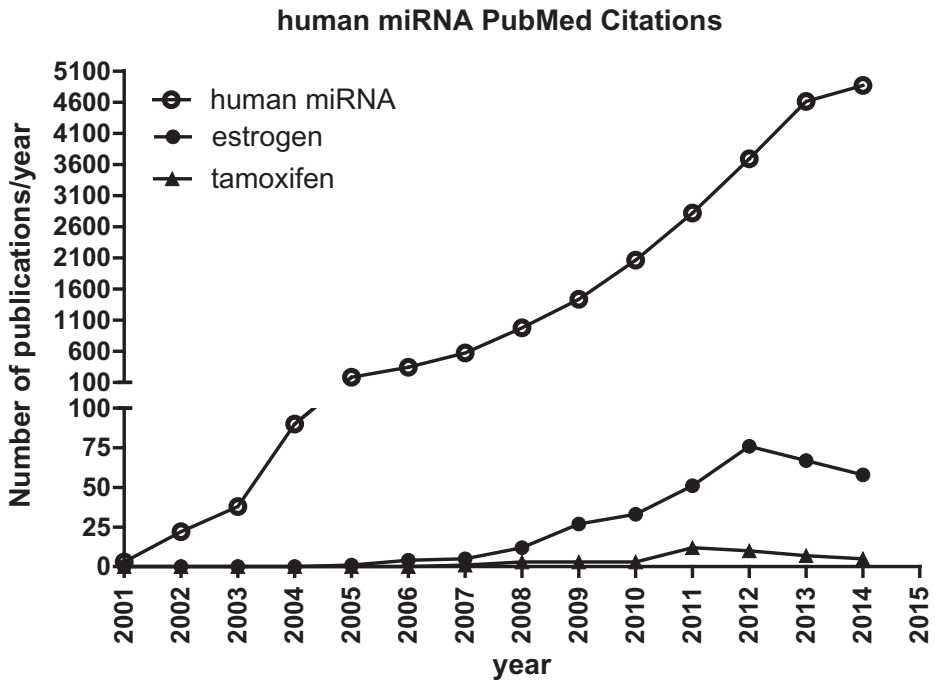

Fig. 1. History of PubMed citations on human miRNA, estrogen AND miRNA, and tamoxifen AND miRNA. The search terms used were human AND miRNA (black closed circles) and human AND miRNA AND estrogen. Each point is the number of publications in the calendar year indicated. The number of citations was taken directly from an advanced search of PubMed and was not hand-curated to remove non-relevant citations.

1. Introduction

The three primary estrogenic steroid hormones: estradiol, estrone, and estriol regulate fertility, development, and homeostasis in various tissues including the brain, breast, cardiovascular system, colon, skin, brain, lung, and reproductive tract in both women and men. The word estrogen is often used in studies when referring to the use of estradiol (E_2), the primary circulating estrogen in premenopausal women which is synthesized from cholesterol in the granulosa cells in the ovary in response to luteinizing hormone (LH). Estrone (E_1) is the primary estrogen in postmenopausal women, synthesized primarily in adipose from adrenal androgens. E_2 and E_1 can also be formed locally, *e.g.*, in breast (To et al., 2014) and lung (James, 2011).

Lifetime estrogen exposure is widely accepted as a major risk factor for the development of breast cancer (Henderson and Feigelson, 2000). Because estrogens have a clear role in the majority of breast cancers and since estrogen receptor α (ERα) is the best prognostic indicator for breast cancer patients and is considered to be the most successful molecular target in the history of cancer drug discovery (Zhou and Slingerland, 2014), much is known about the molecular mechanisms of estrogen regulation of transcription.

Data from ENCODE (Encyclopedia of DNA Elements, http://www.nature.com/encode/) revealed that ~75% of the human genome is transcribed while only ~1% is protein-coding mRNA, suggesting that other RNA transcripts, including long non-coding RNAs (lncRNAs) and small RNAs (85% of which correspond to four major classes: small nuclear (sn)RNAs, small nucleolar (sno)RNAs, micro (mi)RNAs and transfer (t)RNAs), have regulatory functions (Djebali et al., 2012). Next-generation sequencing (NGS) by RNA sequencing (RNA seq), also called 'whole transcriptome shotgun sequencing', is used to identify the transcriptome (Wolf, 2013). The transcriptome includes all the RNAs in that source: mRNA, rRNA, and tRNA; and the non-coding RNAs (ncRNAs): miRNAs, enhancer RNAs (eRNAs), endogenous small-interfering RNAs (siRNAs), Piwi-interacting RNAs (piRNAs), and lncRNAs ranging from 1000 to >90,000 bases (Marrone et al., 2014). Like miRNAs, siRNAs and piRNAs bind Argonaute family members and base pair with target RNA to cause RNA degradation and/or translation repression (Watanabe and Lin, 2014). lncRNAs are involved in assembly of active *e.g.*, Neat1, or repressed, *e.g.*, Xist, nuclear domains for transcription in a cell-dependent manner (Rinn and Guttman, 2014). This review focuses on estrogen regulation of miRNAs.

miRNAs, first described in 1993, are small (22 nucleotides), single-stranded non-coding, evolutionarily conserved RNA molecules that are related to, but distinct from, small interfering RNAs (siRNAs) which regulate mRNA translation or stability (Couzin, 2007; Zamore and Haley, 2005; Zeng, 2006). Comparative genomics analyses have revealed >45,000 miRNA binding sites within human 3′UTRs that are conserved, indicating that >60% of human protein-coding genes have been under selective pressure to maintain pairing to miRNAs (Friedman et al., 2009). Compared to transcriptome or microarray analyses identifying miRNA expression patterns in different human cells, tissues, or with various treatments, there are far fewer published reports of estrogen or tamoxifen regulation of miRNA expression in human cells or tissues (Fig. 1). The pace of publication on miRNAs in humans has slowed since 2013 and publication rate on estrogen and human miRNA peaked in 2012 and is in decline. Given the role of estrogens in stimulating breast cancer, it is not surprising that most studies have examined changes in miRNA expression and their correlation with diagnostic markers used in breast cancer therapies, *e.g.*, ERα and tumor grade (Adams et al., 2008; Blenkiron et al., 2007; Foekens et al., 2008; Iorio et al., 2005; Jiang et al., 2005; Lowery et al., 2008; Mattie et al., 2006; Miller et al., 2008; Si et al., 2007; Tavazoie et al., 2008; Yu et al., 2008). Estrogens regulate miRNA expression by both genomic (transcriptional) and non-genomic/membrane-initiated mechanisms of action. Identification and characterization of estrogen-regulated miRNAs and their targets may provide new biomarkers and therapeutic targets in diseases including breast cancer. There are many online resources about miRNA–mRNA targets recently compiled in http://multimir.ucdenver.edu/ and reviewed in Ru et al. (2015).

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2. Genomic ER activities

Transcription is initiated through a complex series of activities occurring through the cooperative interaction of multiple factors at the target gene promoter in association with interactions with other chromatin regions at great distances from the transcription start site and even on different chromosomes (Nunez et al., 2009). I will use the term ER to refer to either ERα or ERβ or to both subtypes. I will refer to each subtype individually when appropriate to differentiate their established differences. Estrogens bind the ligand binding domains (LBD) of ERα and ERβ which are members of the 48 member steroid/nuclear receptor (NR) superfamily of proteins (Maglich et al., 2001). ERα and ERβ are highly conserved within the DNA binding domain (DBD, C domain), but differ in their N- and C-termini (Klinge, 2001).

Crystal structure studies of the LBD of ERα, excluding the F domain, identified 12 alpha helices and found that E_2 binding repositions helix 12 that acts as a "switch" controlling accessibility of coregulator interaction site: the 'coactivator binding groove' (Ruff et al., 2000).

Chromatin forms a barrier for transcription factor binding. FoxA1, PBX, TLE1, AP2g, and GATA3 act as "pioneer factors" that remodel condensed chromatin to facilitate ERα binding (reviewed in Magnani and Lupien, 2014). ERα interacts directly with high affinity to a specific DNA sequence called the estrogen response element (ERE = 5′-AGGTCAnnnTGACCT-3′) (Klinge, 2001). ER-ERE binding enhances the recruitment of coactivator/chromatin remodeling complexes resulting in histone modifications, nucleosomal repositioning, increased accessibility to the DNA template for RNA polymerase II interaction, and increased target gene transcription (reviewed in Dasgupta et al., 2014; Rosenfeld and Glass, 2001). Chromatin immunoprecipitation (ChIP) of ERα in cell lines, most notably MCF-7 human breast cancer cells, followed by sequencing of the bound DNA (ChIP seq) has established that EREs are located in gene promoters and at great distances from the transcription start site, including in the 3′ flanking regions of regulated genes (Carroll and Brown, 2006; Carroll et al., 2006; Kwon et al., 2007; Lin et al., 2007; Liu et al., 2008; Stender et al., 2010; Welboren et al., 2009). Cell-specific ERα cistromes have been identified in ERα-transfected U2OS cells (Krum et al., 2008), MDA-MB-231 breast cancer cells (Stender et al., 2010), and HeLa cells (Heldring et al., 2011). In another example, ERα overexpression in ERα-HeLa cells identified only 9% of common promoter binding sites with MCF-7.

In addition to direct ER–ERE binding, ER also activates transcription via a "tethering mechanism" whereby ER interacts directly with transcription factors, *e.g.* Sp1 (Porter et al., 1996) and AP-1 (Paech et al., 1997), bound to their response elements. ERβ binding sites appear enriched for AP-1 sites (Zhao et al., 2010). ChIP-seq, ChIP-PET (ChIP for ERα followed by paired-end tag sequencing) and ChIP-chip experiments identified a number of transcription factor binding sites with which ERα interacts in MCF-7 cells including: AP-1, CEBP, FOXA1, PAX6, RORA, PITX2, and GATA2 (Gu et al., 2010).

3. Rapid, membrane-initiated, nongenomic estrogen action

In addition to its classical genomic/transcriptional effects mediated by ER–DNA interaction, described earlier, E_2 has rapid "nongenomic, extra-nuclear, or membrane-initiated" effects that occur very rapidly, *i.e.*, within seconds–minutes after E_2 administration (reviewed in Levin, 2014; Watson et al., 2012) These effects are independent and distinct from the genomic, *i.e.*, ER-mediated transcription, activities reviewed in the preceding section. Rapid estrogen-stimulated intracellular activities are mediated by plasma membrane (PM)-associated ERα, ERβ, ERα splice variants: ERα46, ERα36, and/or by an 'orphan' G-protein coupled estrogen receptor GPR30/ GPER (Cheng et al., 2011; Ignatov et al., 2010; Kolkova et al., 2010; Levin, 2009, 2011; Madeo and Maggiolini, 2010; Prossnitz and Maggiolini, 2009; Recchia et al., 2011; Sandén et al., 2011; Stratton et al., 2010; Wang et al., 2010; Watson et al., 2007). Palmitoylation of ERα46 helps it to localize to the PM (Acconcia et al., 2004; Li et al., 2003; Moriarty et al., 2006; Pedram et al., 2007). ERα36 is also recruited to the PM by palmitoylation (Chaudhri et al., 2014). Evidence of the biological function of PM-associated ERs, including GPER, is supported by experiments in which cell-impermeable E_2–bovine serum albumin (E_2–BSA) or other E_2-conjugates rapidly initiated intracellular kinase cascade activities including MAPK/ERK (p42/p44 MAPKs), endothelial nitric oxide synthase (eNOS), and PI3K/AKT (Belcher et al., 2005; Chen et al., 2004; Filardo et al., 2007; Hisamoto et al., 2001; Jaubert et al., 2007; Mhyre et al., 2006; Monje and Boland, 2002; Razandi et al., 2000; Simoncini et al., 2006; Wang et al., 2008). Increased E_2 during pregnancy activates GPER which, with activation of glucagon-like peptide 1 (GLP1) receptor, increases cAMP-PKA and decreases miR-338-3p resulting in increased expression of proliferation and/or anti-apoptotic genes and β-cell proliferation (Jacovetti and Regazzi, 2013). Overexpression of ERα46 stimulates E_2-induced endogenous miR-21 transcription and reduced miR-21 targets PTEN and PDCD4 in MCF-7 cells (Klinge et al., 2010). ERα36 and miR-210 expression were correlated in TNBC tumors (Pelekanou et al., 2012), but to my knowledge, no mechanistic studies have been performed on ERα36 regulation of miRNA transcription.

4. miRNA processing and general activity

The human genome contains ~2588 mature miRNAs (June 2014, http://www.mirbase.org/) (Kozomara and Griffiths-Jones, 2014). The term miRNome is defined as the full spectrum of miRNAs for a specific genome (Samantarrai et al., 2013). About half of miRNAs are expressed from introns of protein-coding transcripts and miRNAs have 5′ and 3′ sequence features that form boundaries including transcription start sites, CpG islands, and transcription factor binding recognition elements (Saini et al., 2007). miRNAs may be differentially processed from the sense and antisense strands of the same hairpin RNA or transcripts from the same locus (Amaral et al., 2008). miRNAs are produced by canonical miRNA processing or noncanonical pathways (Yang and Lai Eric, 2011).

The canonical and noncanonical pathways of miRNA biogenesis and the regulation of components of this pathway by miRNAs, phosphorylation, and protein:protein interactions and E_2 are depicted in Fig. 2. miRNAs are transcribed as primary-micro-RNAs (pri-miRNAs) by RNA polymerase II either as independent transcription units or are cotranscribed within introns of pre-mRNAs (Macias et al., 2013). pri-miRs are caped and polyadenylated (Verghese et al., 2008). The self-base-pairing stem-loop structure of the pri-miR is cleaved by the microprocessor complex with catalytic Drosha (*RNASEN*), an RNAse III family endonuclease, and its cofactor DGCR8 (DiGeorge syndrome critical region 8 gene) into shorter (60–70 nt) imperfect hairpin-containing precursor-miRNAs (pre-miRNAs) (Thomson et al., 2006). DGCR8 functions as an anchor by binding the pri-miRNA to direct cleavage by Drosha 11 bp from the dsRNA–ssRNA junction (Macias et al., 2013). The Drosha microprocessor also binds and regulates other cellular RNAs (Macias et al., 2013) and includes other proteins and hnRNPs shown in Fig. 2: EWSR1, FUS< Nucleolin, p68, p72 which interacts with YAP2.

Exportin and Ran-GTP or CRM1 export pre-miRNAs from the nucleus. In the cytoplasm, pre-miRNAs are cleaved to the mature ~22 nt transiently double-stranded miRNA duplexes by the RNAse III enzyme Dicer. Dicer with its associated cofactors TRBP (TAR (transactivating response) RNA-binding protein) and PACT (protein activator of the interferon-induced protein kinase) transfers the miRNA to the RNA-induced silencing complex (RISC) containing the catalytic Argonaute proteins (AGO1, AGO2, AGO3, and AGO4; Hock and Meister, 2008) which unwind the duplexes to form single

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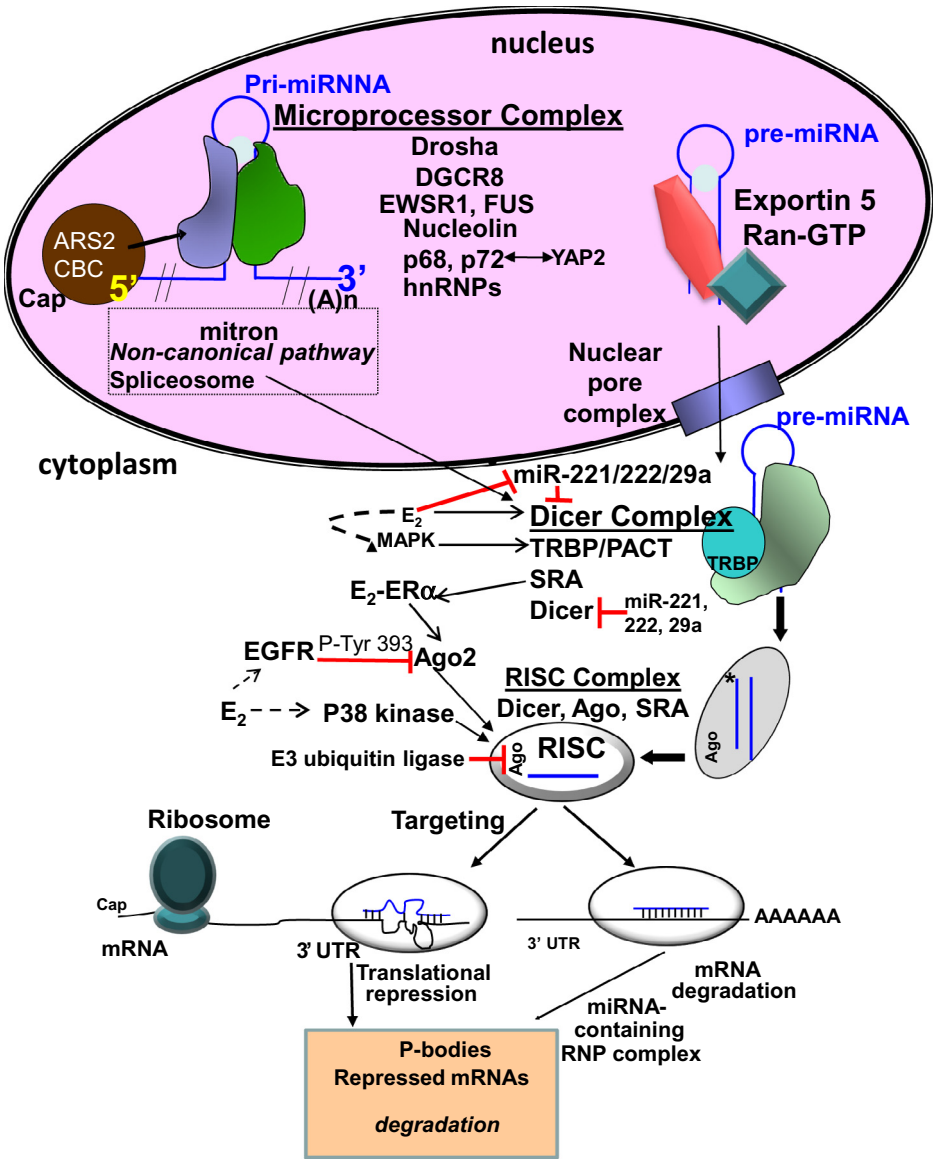

Fig. 2. Model of canonical miRNA biogenesis and function. Primary transcripts of microRNAs (pri-miRNAs) are transcribed by RNA polymerase II, processed by the RNAse III enzyme, Drosha and its cofactor DGCR8, to precursor microRNAs (pre-miRNAs) which are exported from the nucleus by Exportin/RAN-GTP (Verghese et al., 2008). In the cytoplasm, pre-miRNAs are processed by the microprocessor complex that includes Dicer, an RNAse III enzyme, to form mature ~22 nt transiently double-stranded miRNA duplexes that are transferred to Argonaute proteins (most notably AGO2 in the RNA-induced silencing complex (RISC), leading to unwinding of the duplexes to form single stranded miRNAs. The RISC complex binds either to the 3′ untranslated region (3′ UTR) or to the open reading frame (ORF) of its target mRNA. Binding of miRNA/RISC complex with the 3′UTR causes translational repression (Lowery et al., 2008).

stranded miRNAs. One strand miRNA is preferentially selected to bind one of the AGO proteins and by base-pairing directs translational inhibition and/or mRNA degradation by binding either to the 3′ untranslated region (3′ UTR) or to the open reading frame (ORF) of its target mRNA (Berkhout and Jeang, 2007; Cuellar and McManus, 2005; Pasquinelli et al., 2005; Sen and Blau, 2006). AGO2 is the catalytic component of RISC. Dicer binds not only miRNAs but also tRNAs, snoRNAs, mRNA and promoter RNAs (Rybak-Wolf et al., 2014). The widespread reduction of miRNAs in cancers is considered to be the result of defective miRNA processing as reflected in increased pri-miRNAs due to Hippo signaling regulation of p72 nuclear function by YAP sequestering p72 from the Microprocessor in a cell-density-dependent manner (Mori et al., 2014).

The non-canonical pathways of miRNA generation include the generation of mirtrons which are short hairpin pre-miRNAs directly produced by splicing, thus bypassing Drosha-mediated cleavage (Ladewig et al., 2012; Sibley et al., 2012). Some miRNAs function as bimodal miRNAs controlling different target gene sets depending on the region used for interaction. *i.e.*, a canonical seed in positions 2–8 or positions 6–12 nt, *e.g.*, miR-4728-3p, encoded in intron 24 of *HER2* gene (Persson et al., 2011) which downregulates ESR1 expression through an internal seed interaction (Newie et al., 2014).

Just like protein-coding genes, complexity of the miRNome has increased with further research. miRNAs are heterogeneous in length and sequence with isomiRs that are sequence variants of the canonical miRNA currently in the miRBase generated from a single miRNA locus by template and non-template variants (Neilsen et al., 2012). Templated isomiRs match the genomic sequence, but have different 5′-start and/or 3′-ends, resulting from imprecise Drosha or Dicer cleavage (Muller et al., 2014), whereas non-templated isomiRs diverge from the genomic sequence due to post-

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transcriptional enzymatic modification. The most common non-templated modification is adenylation, catalyzed by the adenosine deaminase (ADAR) family of enzymes (Nishikura, 2010). The expression isomiRNAs is dynamic, with differences between cell types and tissues. A tool called IsomiRage http://cru.genomics.iit.it/Isomirage/ is available for profiling the miRNAs/isomiRs and corresponding differential expression patterns using Illumina next-generation sequencing datasets of small RNA (Muller et al., 2014). When applied to primary breast normal and cancer cells the IsomiRage increased the number of detected miRNA species by ~40%, thus revealing additional information "hidden" in sequencing datasets (Muller et al., 2014). These isomiRNAs are effectively loaded on AGO/RISC complexes and thus are thought to function as canonical miRNAs, thus increasing the repertoire of mRNA targets.

Not only are miRNAs active in the cells in which they are transcribed, but also miRNAs circulate in exosomes: 40–100 nM membrane-bound vesicles composed of different growth factors, cytokines, lipids, cytoplasmic proteins, and nucleic acids, including miRNAs, which circulate in the blood and lymph and deliver molecules between tissues (Braicu et al., 2015). The exosomal content is tightly regulated by endosomal sorting complexes required for transport (ESCRT) (Kowal et al., 2014). Specific cell surface markers allow cellular uptake of exosomes with high specificity. The physiological role of exosomes is controversial. Exosomes can facilitate tumor progression by supplying tumor niches with factors that favor proliferation, invasion, drug resistance, and metastasis (Braicu et al., 2015). Circulating miRNAs embedded in exosomes reprogram cellular mechanisms in recipient cells (Turchinovich et al., 2012; Zomer et al., 2010). Whether exosomal miRNAs will be makers in cancer is currently speculative. A recent study appears to be the first comparison between cell-free and exosomal miRNAs in breast cancer patients and healthy women (Eichelser et al., 2014). The authors reported higher exosomal miR-372 and cell free (not exosomal) miR-373 in triple negative breast cancer compared to luminal breast cancer patients and higher cell free miR-101 in both groups (Kowal et al., 2014).

5. miRNA–mRNA interaction

The critical, perfectly complementary base pairing between 7–8 nucleotides at the 5′ end of the miRNA and its target mRNA is referred to as the 'seed sequence'. Base pairing of the miRNA–RISC complex within the ORF requires almost perfect complementarity and the mRNA is either degraded or translation is blocked (Verghese et al., 2008). RNA binding proteins (RBP), *e.g.* HuR, hnRNP E1, and hnRNP L, and miRNAs compete and collaborate to regulate mRNA stability and RBPs can recruit miRNA-containing RICSs to target lncRNAs (Ho and Marsden, 2014). There is evidence that miRNA–mRNA gene silencing occurs in the rough endoplasmic reticulum (RER) by interaction of components of Dicer, TRBP and PACT with the RER (Stalder et al., 2013).

Most commonly, because of imperfect base pairing between the miRNA and the 3′UTR, the RISC complex causes translational repression by interaction with eIF6 which prevents 80S ribosomal assembly (Chendrimada et al., 2007) or by inhibition of translation (Lowery et al., 2008). The exact mechanisms of translational inhibition *versus* mRNA degradation have not yet been fully elucidated (Leung and Sharp, 2013). miRNAs initiate target mRNA degradation by recruiting mRNA decay pathway effectors such as de-adenylation and de-capping enzymes (Behm-Ansmant et al., 2006). The miRNA-containing ribonucleoprotein particle (miRNP)-silenced mRNA is directed to the P-bodies and the mRNA is either released from its inhibition upon a cellular signal and/or actively degraded (Perron and Provost, 2008). Some miRNAs may also increase translation of select mRNAs in a cell cycle-dependent manner (Vasudevan et al., 2007).

miRNAs are considered highly stable, although this is cell-type, cell cycle, and miRNA-specific; further target regulation can promote miRNA's 3′-end uridylation and degradation (Ho and Marsden, 2014). This means that an increase in target mRNA leads to a decrease in its target miRNAs. miRNAs are regulated by competing endogenous RNAs (ceRNAs) (Salmena et al., 2011) which contain miRNA target sites and thus act as miRNA 'sponges' and sequester miRNAs from interaction with target mRNAs. Circular RNAs (circRNAs) are ceRNAs that contain miRNA binding sites and are resistant to miRNA-mediated destabilization (reviewed in Tay et al., 2014). Multiple non-coding RNA species, including sncRNAs, pseudogenes, lncRNAs and circRNAs appear to possess ceRNA activity (Tay et al., 2014).

miRNAs have important roles in regulating cellular processes including replication, differentiation, and apoptosis. In cancer, miRNAs can either act as 'oncosuppressor miRNAs' which are often downregulated in cancer, *e.g.*, the miR29b-1/a in acute myeloid leukemia resulting in upregulation of oncoprotein BCL-2 (Kriegel et al., 2012),or, as 'oncomiRs', by decreasing the levels of tumor suppressor proteins, *e.g.*, miR-21 decreasing PDCD4 (Asangani et al., 2008). miRNAs are expressed in a tissue-specific manner (Volinia et al., 2006). Each miRNA targets ~200 transcripts directly or indirectly (Zhang et al., 2006), but the *bone fide* physiological targets of the vast majority of miRNAs remain to be experimentally verified.

6. HITS-CLIP to identify miRNA–mRNA interaction by Ago2 immunoprecipitation

High-throughput RNA-seq isolated by crosslinking immunoprecipitation (HITS-CLIP) of Argonaute 2 (Ago 2, catalytic component of the RISC complex Kawamata and Tomari, 2010) is used to identify putative miRNA–mRNA ternary complexes (Thomson et al., 2011; Zhang and Darnell, 2011). HITS-CLIP of E_2-treated MCF-7 cells revealed Ago 2 footprints throughout *ESR1* mRNA, including peaks in the 3′UTR and within the coding region, and follow-up experiments identified miR-9-5p binding the 3′ UTR, directly downregulating ERα protein levels (Pillai et al., 2014).

7. Nomenclature of miRNA

miRNAs are preceded a three lettered prefix indicating the species of origin *e.g.*, hsa for *homo sapiens* and mmu for mouse (Griffiths-Jones et al., 2006). miRNAs originating from different genomic loci are assigned a numerical suffix, *i.e.*, hsa-miR-29b-1 and hsa-miR-29b-2. If transcripts are equally expressed they are referred to as miR-21-5p (from the 5′ arm) and miR-21-3p (from the 3′ arm) arise from the same hairpin precursor. Alternatively, miR-21* indicates the less predominant species in RISC (Okamura et al., 2008). miRNAs differing by a few bases are given a lettered suffix, *e.g.*, miR-125a and miR-125b. miRNA families arise from a common ancestor and have similar sequences, *e.g.* miR-221 and miR-222 family. Sixty-one percent of mammalian miRNAs are expressed from polycistronic clusters reflecting shared biological functions for unrelated miRNAs in the same primary transcript (Gurtan and Sharp, 2013). miRNA clusters arise due to gene duplication, *e.g.*, the miR-200 cluster of miRNAs are located in two chromosomes, *i.e.*, miR-200a, miR-200b, and miR-429 are located on chromosome 1 and miR-200c and miR-141 are located on chromosome 12 (Tanzer and Stadler, 2004). Each cluster is transcribed into a common precursor RNA.

8. Regulation of miRNA expression

Levels of mature miRNA are regulated transcriptionally and by processing of pri-miRNAs and pre-miRNAs. In the microprocessor complex the ratio of Drosha and DGCR8 is tightly regulated (Gregory

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et al., 2004). DGCR8 stabilizes Drosha and Drosha cleaves and inactivates DGCR8; providing a tight feedback loop (Han et al., 2009). ERα interacts directly with helicases p68 and p72 (which are established ERα coregulators; Wortham et al., 2009). ERα-p68 interaction was reported to inhibit Drosha complex formation (Yamagata et al., 2009), and thus repress pri-miRNA processing. Importantly, this work was recently retracted (Yamagata et al., 2014). However, another group of investigators also reported that Drosha and p68/DDX5 could be co-purified with ERα in MCF-7 cells, but not with ERβ in ERβ-stably transfected MCF-7 cells (Paris et al., 2012). This report has not been confirmed.

Dicer processes pre-miRNA to mature miRNA. Dicer activity is enhanced by MAPK-phosphorylation of TRBP (Fig. 2) which promotes miRNA processing (Paroo et al., 2009). The RNA coactivator SRA (steroid receptor RNA activator) binds Dicer complex components PACT, TRBP, and PKR in various cell lines and also binds NRs, including ERα (Redfern et al., 2013). Dicer acts as a NR coactivator in MCF-7 cells and is recruited to the PSA gene promoter in DHT-treated LNCaP prostate cancer cells with androgen receptor (AR) (Redfern et al., 2013). These findings suggest that pre-miR processing may be coupled with ERα and AR regulation of gene transcription.

AGO2 is the catalytic component of the RISC complex and serves as a platform to recruit additional regulators of mRNA stability (Gurtan and Sharp, 2013). AGO2 is regulated at the transcriptional and post-transcriptional levels. For example, in MCF-7 breast cancer cells, E_2 inhibits AGO2 expression by activating epidermal growth factor (EGF)-MAPK signaling (Adams et al., 2009). Direct interaction of EGF receptor (EGFR) with AGO2 in the cytoplasm phosphorylates AGO2 at Tyr 393 which reduced AGO2 association with Dicer (Fig. 2) and TRBP suppresses maturation of specific tumor suppressor miRNAs under hypoxic conditions (Shen et al., 2013).

Nucleolin is a multifunctional protein concentrated in the nucleolus, but located throughout the cell, including the plasma membrane, and has roles in transcription, ribosome biogenesis, DNA replication, chromatin remodeling, apoptosis, and macropinocytosis (Bates et al., 2009; Tuteja and Tuteja, 1998). There are several examples of nucleolin functioning as a transcription factor or as a coregulator through its interactions with other proteins (reviewed in Litchfield et al., 2012). Nucleolin was reported to promote the maturation of specific miRNAs implicated in carcinogenesis in MCF-7 and HeLa cells: miR-21, miR-103, miR-221, and miR-222 (Pichiorri et al., 2013).

9. Estrogen regulation of miRNA expression overview

Regulation of miRNA expression by estrogens in animals, fish, and humans has been reviewed by us (Klinge, 2009, 2012) and others (Gupta et al., 2012). Since my previous review, a non-inclusive list of new studies of E_2 regulation of miRNA expression in animals includes: female Fischer 344 rat brain, specifically in the ventral and dorsal hippocampus, central amygdala, and paraventricular nucleus and as a function of aging (Rao et al., 2013); in female ACI rats in an E_2-induced mammary carcinogenesis model (Munagala et al., 2013); mouse aorta (Zhao et al., 2013); mouse liver and primary murine hepatocytes (Zhang et al., 2012); rat cardiac fibroblasts (Queiros et al., 2013). I will not review these studies, but will focus on human cell lines and tissues.

10. ERα and ERβ regulate miRNA expression in a ligand-independent manner

ChIP studies have shown that 'unliganded' ERα (Shang et al., 2000) and ERβ (Vivar et al., 2010) bind DNA in cells grown in serum-free or charcoal-stripped serum medium. Overexpression of ERα in MCF-7 cells upregulated mIR-17 (Liao et al., 2014). Overexpression of ERβ in non-hormone treated MCF-7 and ZR-75.1 human breast cancer cell lines was reported to regulate the expression of >450 miRNAs in next-gen RNA sequencing experiments (Nassa et al., 2014). Here I will focus on updating reports on ER ligand-responsive regulation of miRNA expression in human cell lines and tissues.

11. E_2 and other ER ligands regulate miRNA expression in human cell lines and tissues

The hope of current studies of E_2 regulation of miRNA expression in breast cancer cell lines is that identification of E_2-regulated miRNAs and their gene targets may offer insight into mechanisms of estrogen in breast carcinogenesis and progression and identify targets for therapeutic interference. By far and large, E_2 regulation of the transcriptome, including miRNAs, is best characterized in breast cancer cell lines with MCF-7 studies predominant. This will be apparent in Tables 1 and 2 which summarize the regulation of miRs and their *bona fide* targets by ER ligands including E_2, tamoxifen, 4-OHT, and endocrine disruptors in human cell lines and tissues. It is worth noting that there are conflicting results of E_2 and other ER ligand regulation of miRNAs within cell lines, *e.g.*, MCF-7 and T47D, between reports from different investigators and even within the same lab group in different publications. There are many likely explanations for these differences including cell lines and variations in cell treatment conditions, circadian regulation of ERα expression (Vantaggiato et al., 2014), normalization of data (Katchy et al., 2012), and control genes used for qPCR (Manavalan et al., 2011).

Identification of E_2- and 4-OHT-regulated miRNAs was originally performed by microarray by us (Manavalan et al., 2011; Wickramasinghe et al., 2009) and others (Bhat-Nakshatri et al., 2009; Ferraro et al., 2012; Papaioannou et al., 2014; Paris et al., 2012; Tilghman et al., 2012; Zhang et al., 2012). These reports are summarized in Tables 1 and 2. An Illumina human MicroRNA Expression Profiling Beadchip was used to identify E_2-regulated miRNAs in MCF-7 and ZR-75.1 cells after 6, 12, 24, and 72 h of treatment following an initial 4 days of 'hormone deprivation' in medium containing 5% dextran-coated charcoal stripped FBS (Ferraro et al., 2012). The authors reported 230 significant miRNA changes (up- and down-regulation) that are summarized in Tables 1 and 2. The authors correlated miRNA expression with ERα *in vivo* binding in published data sets and found ERα binding within 10 kB of miR-125a-2, miR-181c, miR-23a, miR-27a, miR-24-2, and mIR-26 and ERα binding sites within 50 kB of genes in which miRs are embedded: miR-25 in MCM2, miR-26a in CTDSP2, miR-424 in GBC16121, miR-618 in LIN7A, miR-760 in BCAR3, and miR-942 in TTF2 (Ferraro et al., 2012). The authors noted that they found more of miR* strands regulated by E_2 and suggested a possible role of ER in strand selection. Since the * strands are now known to be functional in Ago2-RISC complexes (Xue and He, 2014), these findings appear to reflect the wide range of miRNAs functionally regulating estrogen action *in vivo*.

GRO-seq (global nuclear run-on and sequencing) identified all RNA transcripts in E_2-treated MCF-7 cells (Hah et al., 2011). The authors identified 119 miRNA transcripts as regulated by E_2 at minimally one of the time points (10 and 40 min) examined with half of the miRNAs upregulated and half downregulated, the same as protein-coding transcripts. However, GRO-seq is unable to detect miRNAs that are co-transcribed as a part of their host gene within which they are embedded (Hah and Kraus, 2014). Another genome wide analysis of E_2-regulated miRNA expression was performed in MCF-7 and ZR-75-1 luminal-like breast cancer cells (Cicatiello et al., 2010). In that study, E_2 increased miR-760 and miR-424 and decreased miR-618, miR-570, and miR-107 expression. It will be of interest to correlate binding events, transcriptional regulation, and functional outcome in these large-scale studies.

Aromatase inhibitors are used to inhibit the endogenous synthesis of estrogens in postmenopausal breast cancer patients (Santen et al., 2009). The aromatase inhibitor letrozole (10 nM) stimulated the expression of let-7f, miR-146a, miR-150, miR-27a, miR-263, miR-

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19a, miR-372, miR-23b, miR-203, miR-10b, miR-128a, miR-9, and miR-126 and inhibited miR-134, miR-142-5p, miR-96, miR-148b, and miR-222 expression in MCF-7 cells co-cultured with primary human stromal cells (Shibahara et al., 2012). If these are E_2-regulated miRNAs in MCF-7 cells, then we would expect E_2 to increase miR-134, miR-142-5p, miR-96, miR-148b, and miR-222 and inhibit let-7f, miR-146a, miR-150, miR-27a, miR-263, miR-19a, miR-372, miR-23b, miR-203, miR-10b, miR-128a, miR-9, and miR126. We compared these expected results with published data summarized in Tables 1 and 2. E_2 has not been reported to increase miR-134, miR-148b, or miR-96; however, in agreement with the expected results, E_2 increased miR-142-3p and miR-222 in MCF-7 cells (Table 1). E_2 has not been reported to inhibit miR-146a, miR-150, miR-263, miR-372, miR-10b, miR-9, or miR-126; however, E_2 reduced let-7f, miR-27a, miR-19a, miR-23b, miR-203, miR-128a:9.1, in MCF-7 cells (Table 2).

12. Endocrine disrupting chemicals regulating miRNA expression

Endocrine disrupting chemicals (EDC) are environmental chemicals that mimic or block transcriptional activation elicited by naturally circulating steroid hormones by binding to steroid hormone receptors and either acting as agonists or antagonists of that receptor (Diamanti-Kandarakis et al., 2009; Zoeller et al., 2012). EDC may also affect the levels or activities enzymes involved in steroid hormone synthesis or metabolism, alter the expression or activities of transcriptional coregulators, and cause epigenetic changes (Diamanti-Kandarakis et al., 2009; Knower et al., 2014). The role of EDC in breast cancer is suspected, but not proven (Weyandt et al., 2008). Based on their widespread use, environmental persistence, the possible role of EDC in hormone-related cancers is of keen interest (Casals-Casas and Desvergne, 2011; Diamanti-Kandarakis et al., 2009; Weyandt et al., 2008).

There are few reports examining how EDC affect miRNA expression in fish, animals or animal cell lines (Collotta et al., 2013). Treatment of mouse TM4 Sertoli cells with 10 μg/mL nonylphenol (NP) increased the expression of 47 miRNAs and down-regulated the expression of 100 miRNAs with 24 h of treatment (Choi et al., 2011). Only 10 miRNAs were increased >1.5-fold with mmu-miR-135* being increased ~4-fold. The authors correlated the increase in miR-135* with decreased expression of 18 mRNAs in NP-treated cells, but did not confirm changes at the protein level or whether these are *bona fide* mRNA targets of mmu-miR-135a* (Choi et al., 2011). Neonatal exposure to the estrogenic analog estradiol benzoate (EB) from postnatal days (PND) 1–5 with doses of 0, 0.75, 1.25, 2.5, or 25 μg/d given sc, increased miR-29 (a, b, and c) in adult (PND90) rat testicular tissue with a concordant decrease in miR-29 target Mcl-1 protein (Meunier et al., 2012).

To my knowledge, based on searching PubMed, there are only four studies of the effect of EDC on miRNA expression in human cell lines. One study showed that, like E_2 (Wickramasinghe et al., 2009), 10 μM o,p-dichlorodiphenyltrichloroethane (DDT) and 10 μM bisphenol A (BPA) activate ERα in MCF-7 cells and downregulated miR-21 (Tilghman et al., 2012). In addition, the authors reported that treatment of MCF-7 cells with 1 nM E_2, 10 μM BPA, or 10 μM DDT reduced the expression of let-7a, b, c, d, e, and f, miR-15b, and miR-28b and upregulated miR-638, miR-663, and miR-1915. We reported that the anti-fungal agents fenhexamid and fludioxonil increased miR-21 expression in MCF-7, T47D, and MDA-MB-231 human breast cancer cells and reduced the expression of miR-125b and miR-181a (Teng et al., 2013). In MCF-7 cells, fenhexamid and fludioxonil induction of miR-21 was inhibited by fulvestrant; by AR antagonist, bicalutamide; by actinomycin D and cycloheximide, and by inhibitors of the mitogen-activated protein kinases (MAPK) and phosphoinositide 3-kinase (PI3K) pathways. Fenhexamid activation was inhibited by the arylhydrocarbon receptor antagonist α-napthoflavone.

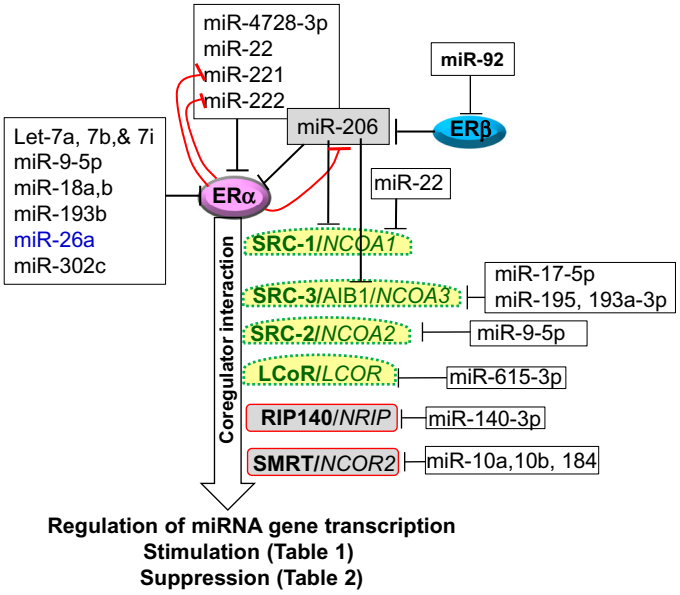

Fig. 3. Overview of miRNAs regulating ERα and ERβ expression and function. miRNAs that inhibit ERα, ERβ, and coregulators involved in gene transcription are indicated as discussed in the text.

The cooking of meat, particularly at high temperature with browning, *e.g.* grilling on a charcoal grill, results in the formation of heterocyclic amines (HCA), including the most abundant: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) which is considered a mammary carcinogen (Nowell et al., 2004). Treatment of MCF-7 cells with 100 nM PhIP decreased miR-21, miR-1, and miR-106b expression and increased miR-923, miR-574-3p, miR-574-5p, and miR-494 (Papaioannou et al., 2014). Other miRNAs regulated by PhIP are listed in Tables 1 and 2.

The antimicrobial agents triclosan (TCS) and triclocarban (TCC) are widely used in many consumer products including soaps, skin creams, toothpastes and deodorants and are present in the aquatic and terrestrial environment (Huang et al., 2014). TCS and TCC are established EDS that compete with E_2 for ERα and ERβ binding, albeit with lower affinity (Gee et al., 2008). TCS and TCC (each at 1 μM) increased the expression of miR-22, miR-206, and miR-193b (two- to threefold) in MCF-7 cells, similar to the stimulation with 1 nM E_2 (Huang et al., 2014).

13. miRNAs regulating ER expression

miRNAs can influence estrogen-regulated gene expression by directly reducing ERα mRNA stability or translation. Nine miRNAs have been reported to reduce ERα protein levels: miR-18a, miR 18b, miR-193b, miR-302c, miR-22 (Pandey and Picard, 2009), miR-201, miR-221, and miR-222 (Klinge, 2012), miR-206 (Adams et al., 2007), miR-222-3p (Liu et al., 2014), miR-4728-3p (Newie et al., 2014), miR-373 (Eichelser et al., 2014); miR-9-5p (Pillai et al., 2014). let-7a, let-7b, and let-7i (Zhao et al., 2011) (Fig. 3). miR-206 is inversely correlated with ERα expression, but not ERβ, in human breast tumors (Kondo et al., 2008). miR-221/222 is higher in ERα negative than ERα positive breast cancer cell lines and human breast tumors (Cochrane et al., 2010; Zhao et al., 2008). Anti-miR-221 suppressed the growth of TAM-resistant breast cancer cells as xenografts in nude mice (Lu et al., 2011). Similarly, the expression of miR-22 was significantly lower in MCF-7, T-47D and BT474 ERα-positive *versus* ERα-negative MDA-MB-231 and SK-BR-3 breast cancer cells

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Table 1
miRNAs upregulated by estradiol (E_2), tamoxifen (TAM), 4-hydroxytamoxifen (4-OHT), Fulvestrant (ICI 182,780), or endocrine-disrupting chemicals (EDC) in animal studies and human cell lines. The *bona fide* targets of the miRNAs are experimentally proven in the reference cited; however, this direct targeting is not necessary substantiated in E_2 regulation in the cells indicated in column 3. DIANA-TarBase v7.0 (Vlachos et al., 2015) web site has a list of *bona fide* targets of miRNAs: http://diana.imis.athena-innovation.gr/DianaTools/.

miRNA	Ligand	Human cell line/tissue	Comments	*Bona fide* targets
let-7a,b,c,d,e,f,g, i	E2	MCF-7 cells stably expressing a biscistronic vector control (Bhat-Nakshatri et al., 2009). MCF-7 cells (Cochrane et al., 2010; Klinge, 2009) 1 μM E_2 in Ishikawa and ECC-1 ERα+ human endometrial cancer cells (Zhang et al., 2012) let-7a and let-7f-1* were increased at 6, 12, and 72 h but decreased at 24 h with 10 nM E_2 in MCF-7 cells (Ferraro et al., 2012). let-7a* was increased in response to 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012).	Oncosuppressor miR-stimulate apoptosis (White et al., 2011)	DICER1 (Forman et al., 2008); let-7g:COL1A2 (Ji et al., 2010)
miR-7	E2	10 nM E2 MCF-7 cells (Klinge, 2009; Masuda et al., 2012)	oncomiR	XRCC2 (Xu et al., 2014) KLF4 (Meza-Sosa et al., 2014)
miR-10a miR-10b	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)	miR-10b is down-regulated in breast tumors and upregulated in sera (Chan et al., 2013).	BUB1, PLK1, CCNA2 (Biagioni et al., 2012)
miR-15a	E2	10 nM E2 MCF-7 cells (Klinge, 2009)	Upregulated by E2F1 (Ofir et al., 2011).	CCNE1 = CyclinE (Ofir et al., 2011)
miR-16-1*	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-16-2*	E2	10 nM E_2 for 24 h in T47D cells (Katchy et al., 2012)		
miR-17*	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-17-3p	E2	MCF-7 stably transfected to overexpress the aromatase gene (MCF-7aro) (Masri et al., 2010)		
miR-17–92	E2	MCF-7 cells (Castellano et al., 2009; Masuda et al., 2012; Wang et al., 2010)	miR-17–92 cluser encodes miR-17, 18, 19, 20, 19b-1, 92-1	miR-19a and miR-92a: PTEN (Zhu et al., 2014)
miR-18a	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-18a*	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)	miR-18a is higher in ERα- breast tumors (Yoshimoto et al., 2011)	ERα (Castellano et al., 2009)
miR-18b	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012) 10 nM E_2 for 6, 12 h in MCF-7 cells stably overexpressing inducible ERβ or ERα-downregulated at 24 and 72 h (Paris et al., 2012)		
miR-18b*	BPA	10 μM BPA for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-19a, 19b	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012) miR-19a and 19a* were increased by 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012).		
miR-19b-1	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-19b	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-20a*	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-21	Fludioxonil fenhexamid 4-OHT	MCF-7 cells (Teng et al., 2013) MCF-7 cells (Wickramasinghe et al., 2009)	oncomiR Fludioxonil and fenhexamid are endocrine disruptors	NFIB (Hannafon et al., 2011); PTEN, PDCD4 (Wickramasinghe et al., 2009); RASA1 and RASA2 (Queiros et al., 2013)
miR-22	E2 EDC	1 nM E_2, 1 μM triclosan or 1 μM triclocarban for 18 h in MCF-7 cells (Huang et al., 2014)	EDC	
miR-23b*	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ but not ERα (Paris et al., 2012)		
miR-24	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-24-1*	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ but not ERα (Paris et al., 2012)		
miR-25	E2	MCF-7 cells (Klinge, 2009; Masuda et al., 2012)	miR-106b-25 cluster encodes miR-106b, miR-93, and miR-25in the 13th intron of the MCM7 gene (Smith et al., 2012)	BIM (Zhang et al., 2012); DR4 (Razumilava et al., 2012); MCU (Marchi et al., 2013); Smad7 (Li et al., 2013); LATS2 (Feng et al., 2014); RECK (Zhao et al., 2014)
miR-25*	E2	10 nM E_2 12 and 24 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		

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Table 1 (*continued*)

miRNA	Ligand	Human cell line/tissue	Comments	*Bona fide* targets
miR-26a	E2 and fulvestrant	Primary human myometrial smooth muscle cells (MSMC) (Pan et al., 2008)	Oncosuppressor miR	ESR1 (Chen et al., 2011) CHD1, GREB1, and KPNA2 (Tan et al., 2014)
miR-27a	E2	1 μM E_2 in Ishikawa and ECC-1 ERα+ human endometrial cancer cells (Zhang et al., 2012)	OncomiR	EGFR (Zhang et al., 2014)
miR-27b	E2	MCF-7 cells (Masuda et al., 2012)	Oncosuppressor miR	Sp1 (Jiang et al., 2014); LIMK1 (Wan et al., 2014); PPARγ (Lee et al., 2012)
miR-29a	E2	MCF-7 cells (Masuda et al., 2012)	OncomiR: stimulates migration and invasion; repressed by c-myc, YYI, NFκB, CEBPA and stimulated by p53 (Wang et al., 2013)	BCL2, CDC42, CDK6, DNMT, MCL1, Osteonectin, TGFβ3m, TTP, TGF-β1, TGF-β2, TTP (Wang et al., 2013)
miR-29b-2*	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ but inhibited by ERα (Paris et al., 2012)		
miR-29c	E2	10 nM E2 for 24 h in T47D cells (Katchy et al., 2012)		
miR-30b	E2	MCF-7 cells (Klinge, 2009)	Oncosuppressor miR	CCNE2 (Ichikawa et al., 2012); KRAS, PIK3CD and BCL2 (Liao et al., 2014)
miR-30d	E2	1 μM E_2 in Ishikawa ERα+ human endometrial cancer cells (Zhang et al., 2012) 10 μM BPA for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-32	E2	10 nM E_2 72 h in MCF-7 cells stably overexpressing inducible ERβ (Paris et al., 2012)		
miR-33a	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-92	E2	10 nM E_2 24 and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-92a	E2	1 μM E_2 in ECC-1 ERα+ human endometrial cancer cells (Zhang et al., 2012) 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-92a-1*	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-92b	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-93	E2	10 nM E_2 24 h in MCF-10A and T47D cells (Singh et al., 2013). 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-98	E2 BPA	MCF-7 cells (Klinge, 2009) 10 μM BPA for 18 h in MCF-7 cells		
miR-99b	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-101	E2	10 nM E_2 24 h in MCF-7 cells (Kim et al., 2013)		
miR-101*	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ (72 h) but not ERα (Paris et al., 2012)		
miR-103	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-122	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-124	E2	MCF-7 cells (Masuda et al., 2012)	Oncosuppressor miR	Ets1 (Li et al., 2014) miR-124-5p: LAMB1 (Chen et al., 2014) ROCK1 (Gu et al., 2014) FLOT1 (Li et al., 2013) SphK1 (Zhang et al., 2013) CD151 (Han et al., 2013) iASPP (Liu et al., 2013) Slug (Liang et al., 2013)
miR-130b	E2	MCF-7 cells (Wang et al., 2010)		TP53INP1 (Ma et al., 2010); DICER1 (Li et al., 2013)
miR-135a	E2	10 nM E_2 6 h in MCF-7 cells (Kim et al., 2013) 10 nM E_2 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-135b	E2	10 nM E_2 for 6 and 72 h in ZR-75-1 cells, but no change at 12 or 24 h (Ferraro et al., 2012)		
miR-142-3p	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-148	E2	MCF-7 cells (Masuda et al., 2012)	miRNA-148/152 family include miR-148a, miR-148b, miR-152 (Chen et al., 2013)	PXR, DNMT1, CAND1, BCL2, p27, ACVR1, PETN, WNT10B, MSK1, CDC25B, ROCK1, CCKBR, CCK2R, IGF-1R, IRS1 (Chen et al., 2013)
miR-149	E2	MCF-7 cells (Masuda et al., 2012)		GSK3α (Jin et al., 2011) GIT1 (Chan et al., 2014) AKT and E2F1 (Lin et al., 2010)
miR-151-5p	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		

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Table 1 (*continued*)

miRNA	Ligand	Human cell line/tissue	Comments	*Bona fide* targets
miR-155	E2	100 nM E2 for 48 h in MCF-7 cells (Zhang et al., 2013) Higher levels circulating in the serum of breast cancer patients than healthy women (Eichelser et al., 2013)	oncomiR	TRF1 (Dinami et al., 2014). TP53INP1 (Zhang et al., 2013)
miR-181a	E2	1 μM E_2 in Ishikawa ERα+ human endometrial cancer cells (Zhang et al., 2012)		
miR-181d	E2	MCF-7 cells (Masuda et al., 2012)		CCND1 (Hannafon et al., 2011)
miR-186	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012).		
miR-190	E2	10 nM E_2 for 6, 12, and 72 h in ZR-75-1 cells, but not 24 h (Ferraro et al., 2012)		
miR-190a	E2	100 nM E2 in MCF-7 cells increased ERα recruitment to the miR-190a promoter containing a half-site ERE (Chu et al., 2014).		PAR-1 (Chu et al., 2014)
miR-190b	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012).		
miR-191	E2	10 nM E_2 for 6 h in MCF-7 cells (Di Leva et al., 2013) 10 nM E2 (24 h) stimulation was inhibited by 100 nM tamoxifen and by siERα and siERβ in MCF-7 cells (Nagpal et al., 2013). ERα and ERβ ChIPped to the miR-191 promoter in MCF-7 cells (Nagpal et al., 2013). 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		EGR1 (Di Leva et al., 2013) CDK6, BDNF, and SATB1 (Nagpal et al., 2013)
miR-193a-5p	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-193b	E2	MCF-7 cells (Wang et al., 2010)		uPA (Li et al., 2009); YWHAZ, SHMT2, AKR1C2 (Leivonen et al., 2011); miR-193-3p: MYB (Mets et al., 2014)
	EDC	1 nM E_2, 1 μM triclosan or 1 μM triclocarban for 18 h in MCF-7 cells (Huang et al., 2014)		
miR-194	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-195	E2	MCF-7 cells (Klinge, 2009)		CCND1 (Hannafon et al., 2011)
miR-195*	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells highest at 6 h (Ferraro et al., 2012)		ASF1B, BIM, BCL2L2, CCL5, CADM1, EZH2, FGF$1, HDGF, LTF, MAP2K3, NRAS, PTEN, TP53, TWIST1, XBP1 (and others) (Katoh, 2014)
miR-196a2*	E2	10 nM E_2 6 h in MCF-7 cells (Kim et al., 2013)	Mediated by ERα and the protein kinase ERK2 (Kim et al., 2013). By ChIP assay, both ERα and ERK2 were recruited to chromatin with 45 min 10 nM E2 alone with increased pSer5 RNA pol II recruitment (Kim et al., 2013).	TP63 (Kim et al., 2013)
miR-198	E2	10 nM E2 for 24 h in T47D cells (Katchy et al., 2012)		
miR-199a/b-3p	E2	10 nM E_2 for 12, 24, and 72 h in ZR-75-1 cells, but not at 6h (Ferraro et al., 2012)		
miR-199a-5p	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012).		
miR-200a	E2	MCF-7 cells (Klinge, 2009)		BAP1, PTPRD, KLF11, SEPT7, HOX5B, ERBB2IP, RASSF2, ELMO2, SHC1, VAC14 (DIANA)
miR-200c	none	Endogenous ERα in MCF-10A cells ChIPed to the miR-200c promoter and overexpression of ERα in MCF-10A cells increased miR-200c expression (Rokavec et al., 2012). 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-203	E2	MCF-7 cells (Klinge, 2009)		
miR-205	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-206	DPN	ERβ-selective agonist in MCF-7 cells (Adams et al., 2007)	Oncosuppressor miR	
	E2	1 nM E_2, 1 μM triclosan or 1 μM triclocarban for 18 h in		
	EDC	MCF-7 cells (Huang et al., 2014)		
miR-210	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-216a	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-219-5p	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-222	E2	1 nM E_2 or 10 μM BPA for 18 h in MCF-7 cells (Tilghman et al., 2012)		KIT (Osada and Takahashi, 2007); PPP2R2A (Wong et al., 2010); CDKN1C (Qian et al., 2009); CDK1B (Di Leva et al., 2010); DICER1 (Cochrane et al., 2010)
	BPA			

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Table 1 (*continued*)

miRNA	Ligand	Human cell line/tissue	Comments	*Bona fide* targets
miR-223	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-301b	E2	MCF-7 cells (Wang et al., 2010)		
miR-320	E2	1 μM E_2 in Ishikawa and ECC-1 ERα+ human endometrial cancer cells (Zhang et al., 2012)		
miR-320a	E2	1 nM E_2 or 10 μM BPA for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-320c	E2 BPA	1 nM E_2 or 10 μM BPA for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-330-5p	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ not ERα (Paris et al., 2012)		
miR-335	E2	10 nM E_2 for 6, 12 and 72 h in MCF-7 and ZR-75-1 cells, but not at 24 h (Ferraro et al., 2012)		
miR-342	E2; Not blocked by 1 μM 4-OHT	MCF-7-HER2 cells, MCF-7 cells stably overexpressing HER2, but still tamoxifen-sensitive (Cittelly et al., 2010)		
miR-363	E2	10 nM E_2 for 12 and 24 h in ZR-75-1 cells, but not 6 or 72 h (Ferraro et al., 2012)		
miR-365	E2	MCF-7 cells (Klinge, 2009)		
miR-374a*	E2	10 nM E_2 for 6, 12 and 72 h in MCF-7 and ZR-75-1 cells, but repressed >1.5-fold at 24 h (Ferraro et al., 2012)		
miR-375	E2	10 nM E_2 for 24 and 72 h in ZR-75-1 cells, but not 6 or 12 h (Ferraro et al., 2012)		
miR-376b	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells- highest at 6 h (Ferraro et al., 2012)		
miR-423-5p	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-424	E2	MCF-7 cells (Cicatiello et al., 2010) 10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-424*	E2	10 nM E_2 for 6, 12 and 72 h in MCF-7 and ZR-75-1 cells, but not at 24 h (Ferraro et al., 2012)		
miR-425	E2	1 μM E_2 in Ishikawa and ECC-1 ERα+ human endometrial cancer cells (Zhang et al., 2012) 10 nM E_2 for 6 h in MCF-7 cells (Di Leva et al., 2013)		EGR1 (Di Leva et al., 2013)
miR-449a	E2	10 nM E_2 for 6, 12 and 24 h in ZR-75-1 cells, but not 72 h (Ferraro et al., 2012)		
miR-450b-3p,5p	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells- highest at 72 h (Ferraro et al., 2012)		
miR-455-5p, 455-3p	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-484	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-489	E2	10 nM E_2 12, 24, and 72 h in MCF-7 and ZR-75-1 cells, but not at 6 h (Ferraro et al., 2012)		
miR-491-3p	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-499-5p	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-515-5p	Tamoxifen	100 nM tamoxifen for 48 h ~25% decrease in MCF-7 cells (Pinho et al., 2013)		SK1 (Pinho et al., 2013)
miR-520d	E2	MCF-7 cells stably expressing a constitutively active AKT (Bhat-Nakshatri et al., 2009)		
miR-542-5p	E2	10 nM E_2 for 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-542-3p	E2	10 nM E_2 for 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-548d-3p	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ, but not ERα (Paris et al., 2012)		
miR-548e	E2	10 nM E_2 for 6, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-550	E2	10 nM E_2 for 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-556-5p	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells, but not at 24 h (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-560:9.1	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-564	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-574-5p	E2 PhIP	1 μM E_2 in Ishikawa ERα+ human endometrial cancer cells (Zhang et al., 2012) 10 nM E_2 or 100 nM PhIP for 4, 8, 12, or 24 h in MCF-7 cells (Papaioannou et al., 2014)		
miR-574-3p	E2 or PhIP	10 nM E_2 or 100 nM PhIP for 4, 8, 12, or 24 h in MCF-7 cells (Papaioannou et al., 2014)		

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Table 1 (*continued*)

miRNA	Ligand	Human cell line/tissue	Comments	*Bona fide* targets
miR-579	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-590-3p	E2	10 nM E_2 highest stimulation at 6, 12 and 72 h in ZR-75-1 cells with no change detected at 24 h (Ferraro et al., 2012)		
miR-594:9.1	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-615-3p	E2	10 nM E_2 6 h in MCF-7 cells (Kim et al., 2013)		
miR-628-5p	E2	10 nM E_2 for 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-638	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-643	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-651	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-652	E2	10 nM E_2 for 24 and 72 h in ZR-75-1 cells, but not at 6 or 12 h (Ferraro et al., 2012)		
miR-653	E2	10 nM E_2 for 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-653:9.1	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-660	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-663	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-663b	E2	10 nM E_2 for 6 and 24 h in ZR-75-1 cells (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-708	E2	10 nM E_2 for 12, 24, and 72h in ZR-75-1 cells, but not at 6 h (Ferraro et al., 2012)		
miR-720	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-760	E2	24 h and 3 d in MCF-7 cells (Cicatiello et al., 2010) 10 nM E_2 for 24 and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-886-3p	E2	10 nM E_2 for 24 h in MCF-7 and ZR-75-1 cells, but not at 6, 12, or 72 h (Ferraro et al., 2012)		
miR-938	E2	10 nM E_2 for 6 h in MCF-7 cells (Razandi et al., 2000)		
miR-939	E2	10 nM E_2 for 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-940	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-942	E2	10 nM E_2 for 72 h in MCF-7 and ZR-75-1 cells, but not 6, 12, or 24 h (Ferraro et al., 2012)		
miR-944	E2	10 nM E_2 for 6 h in MCF-7 cells (Razandi et al., 2000)		
miR-1206	E2	10 nM E_2 for 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-122	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-1248	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-1268	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-1275	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-1305	E2	10 nM E_2 for 12 and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-1323	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-1826	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-1915	E2 BPA	1 nM E_2 for 10 μM BPA for 18 h in MCF-7 cells (Tilghman et al., 2012)		

(Xiong et al., 2010). A protein lysate microarray (LMA)-based strategy in which a library of pre-miRs was transiently transfected into MCF-7 and BT-474 cells in 384-well plates and ERα protein was subsequently analyzed in protein lysates that were printed on nitrocellulose-coated slides (Leivonen et al., 2009). miR-18a, miR-18b, miR-193, miR-206, and miR-302c reduced ERα by directly binding sites in the 3′UTR of ERα. Further, the authors reported an inverse correlation between the expression of miR-18a, -18b and ERα-negative breast tumor samples (Leivonen et al., 2009). ERα is upregulated during breast carcinogenesis and cancer stem cells (CSCs) isolated from MCF-7 and T47D cells had increased ERα and decreased let-7a, let-7b, let-7c, let-7d, let-7g levels (Sun et al., 2013). miR-873 was reported to inhibit E_2-ERα-regulated gene transcription and cell proliferation by directly targeting CDK3, thus inhibiting ERα phosphorylation (Ser104, 106, and 118) and thus, ERα activity in MCF-7 cells (Cui et al., 2014). Stable overexpression of miR-873 in tamoxifen-resistant MCF-7 cells sensitized cells to tamoxifen (Cui et al., 2014).

14. miRNAs that regulate ER coregulators

miRNAs may also affect estrogen-regulated gene expression by reducing the expression of ER-interacting coactivators. miR-17-5p inhibited translation of coactivator SRC-3/AIB1/NCOA3 and reduced E_2–ERα–ERE–luciferase activity in transfected cells (Hossain et al., 2006). miR-195 inhibited SRC-3 expression in HepG2 cells by direct

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Table 2
Estradiol- and tamoxifen-inhibited miRNAs. This table lists miRNAs whose expression is decreased by E_2, tamoxifen, or 4-OHT. MCF-7, T47D, ZR-75-1, BT-474, and BG1 are ERα positive breast cancer cells.

miRNA	Ligand	Species/tissue/cell line	Comments	*Bona fide* targets
let-7g, -7f, -7a, -7c	E2	10 nM E_2 48 h in MCF-7 cells; also repressed in T47D, ZR-75-1, BT-474, and BG1, but not SKBR3 breast cancer cells (Maillot et al., 2009).10 nM E_2 6 h in MCF-7 cells (Klinge, 2009) let-7g in MCF-7 cells (Qian et al., 2011) 10 nM letrozole stimulated Let-7 expression in MCF-7 cells co-cultured with primary human stromal cells (Shibahara et al., 2012) 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)	Blocked by fulvestrant	GAB2; FN1 (Qian et al., 2011)
let-7b	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
let-7f	4-OHT	1 μM 4-OHT for 1 month in MCF-7 cells (Ujihira et al., 2015)		
let-7i	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-7-1	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-9, miR-9-d	E2	10 nM E_2 for 24 h in ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-15a*	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-16	E2	10 nM E2 for 6, 24, and 48 h in MCF-7 cells; blocked by pretreatment with 1 μM ICI 182,780 (Yu et al., 2012) 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-16-1*	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-17	E2	10 nM E_2 for 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)	Oncosuppressor miR206	
miR-17*	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ but increased by ERα (Paris et al., 2012)		
miR-18a, miR-18b	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-19a, 19b	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-20a	E2	24 h 10 nM E_2 in isolated human endometrial glandular epithelial cell; blocked by ICI 182,780 (Pan et al., 2007) 10 nM E_2 for 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-21	E2	24 h 10 nM E_2 in isolated human endometrial glandular epithelial cells and in primary human leiomyoma smooth muscle cells (LSMC) (Pan et al., 2008) 10 nM E_2 for 48 h in MCF-7 cells (Adams et al., 2007; Maillot et al., 2009). 10 nM E_2 6 h: ~60% reduction in miR-21 in MCF-7 cells (Wickramasinghe et al., 2009) 10 nM E_2 for 12 or 24 h in MCF-7 cells (Kim et al., 2013). 10 μM E_2 for 24 h in MCF-7 cells, no effect in MDA-MB-231 cells (Selcuklu et al., 2012). 10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012). 10 nM E_2 or 100 nM PhIP for 24 h in MCF-7 cells (Papaioannou et al., 2014). 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012).	Blocked by ICI 182,780 isolated human endometrial glandular epithelial cells ERα or ERK2 knock-down reduced E2-downregulation of miR-21 expression (Kim et al., 2013)	PTEN, PDCD4 (Wickramasinghe et al., 2009) JAG1 (Selcuklu et al., 2012)
miR-22, 22*	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-23a. 23b	E2	10 nM E_2 48 h in MCF-7 cells; also repressed in T47D, ZR-75-1, BT-474, and BG1, but not SKBR3 breast cancer cells (Maillot et al., 2009) miR-23a: 10 nM 3 h in MCF-7 cells (Saumet et al., 2012) and 10 nM E_2 for 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-24	E2	10 nM E_2 48 h in MCF-7 cells; also repressed in T47D, ZR-75-1, BT-474, and BG1, but not SKBR3 breast cancer cells (Maillot et al., 2009)		
miR-25	E2	10 nM E_2 for 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-26a	E2	24 h 10 nM E_2 LSMC (Pan et al., 2008) 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-26a-2*	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-26b	E2	10 nM E_2 48 h in MCF-7 cells; also repressed in T47D, ZR-75-1, BT-474, and BG1, but not SKBR3 breast cancer cells (Maillot et al., 2009) 10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012) 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		

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Table 2 (*continued*)

miRNA	Ligand	Species/tissue/cell line	Comments	*Bona fide* targets
miR-27a*	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-27b	E2	10 nM E_2 48 h in MCF-7 cells; also repressed in T47D, ZR-75-1, BT-474, and BG1, but not SKBR3 breast cancer cells (Maillot et al., 2009) 10 nM E_2 for 72 h in MCF-7 cells (Ferraro et al., 2012)	Oncosuppressor miR	
miR-29a	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-29a*	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-29b-1*, 29b-2*	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-30a	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ (Paris et al., 2012)	ERβ ChIPed to the promoter (Paris et al., 2012).	
miR-30c-2*	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012).		
miR-30d	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012). ERα was more inhibitory than ERβ.		
miR-34a	E2	10 nM E_2 for 24 h MCF-7 cells (Zhao et al., 2013) 10 nM E_2 for 6 h in HUVEC, LNCaP, C38IM, and C27IM human prostate cancer cells (Nanni et al., 2013) Higher levels circulating in the serum of breast cancer patients than healthy women (Eichelser et al., 2013) 10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)	Oncosuppressor miR-stimulate apoptosis (White et al., 2011)	LMTK3 (Zhao et al., 2013) SIRT1 (Yamakuchi et al., 2008)
miR-92a	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-99a	E2	10 nM E_2 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-99b	E2	10 nM E_2 for 6,12, 24 and 72 h in ZR-75-1 cells, most repressed at 72 h (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-105-2	4-OHT	1 μM 4-OHT for 1 month in MCF-7 cells (Ujihira et al., 2015)		
miR-106	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-106b	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012) 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-107	E2	10 nM E_2, for 6, 12, 24 h and 3 d in MCF-7 cells (Cicatiello et al., 2010) 10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-125a-3p	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)	Oncosuppressor miR	
miR-125a	4-OHT	1 μM 4-OHT for 1 month in MCF-7 cells (Ujihira et al., 2015)		
miR-125b-2*	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)	Oncosuppressor miR	BAK1, BCL2, DICER1, ERBB2, ERBB3, ETS1, FGFR2, IL6R, JUN , LIN28A, LIN28B, MCL1, MUC1, NCOR2, SIRT7, STAT3, TNF, TP53 (and others) (Katoh, 2014)
miR-128a:9.1	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)	oncomiR	
miR-130b*	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-132*	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-135a	E2	10 nM E_2 for 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013) 10 nM E_2 24 h in MCF-7 cells (Razandi et al., 2000)		
miR-139-5p	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-140	E2	10 nM E2 for 24 h in ERα-stably transfected MCF-10A cells (Zhang et al., 2012). ERα binds the miR-140 promoter in E2 or BPA-treated MCF_7 cells.		SOX2 (Zhang et al., 2012)

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Table 2 (*continued*)

miRNA	Ligand	Species/tissue/cell line	Comments	*Bona fide* targets
miR-140-5p	E2	10 nM E_2 for 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-141	E2 or PhIP	10 nM E_2 or 100 nM PhIP for 24 h in MCF-7 cells (Papaioannou et al., 2014).		
miR-142-3p	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-143	E2	10 nM E_2 for 6, 24, and 48 h in MCF-7 cells; blocked by pretreatment with 1 μM ICI 182,780 (Yu et al., 2012)		
miR-148b*	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-149	E2	10 nM E2 6 h in MCF-7 cells (Klinge, 2009)		
miR-142-3p	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-146b-5p	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-181a, 181b, 181d	E2	10 nM E_2 48 h in MCF-7 cells; also repressed in T47D, ZR-75-1, BT-474, and BG1, but not SKBR3 breast cancer cells (Maillot et al., 2009) miR-181a and 181b inhibited by 100 nM E_2 in MCF-7 cells (Hah et al., 2011)		
miR-181	4-OHT	100 nM 4-OHT for 6 h in MCF-7 cells (Manavalan et al., 2011)		
miR-181a*, 181c*	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012) miR-181c* 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-181c	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-183	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013) 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-185*	E2	10 nM E_2 for 12 and 72 h in ZR-75-1 cells, but not 6 or 24 h (Ferraro et al., 2012)		
miR-186	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ, but increased by ERα (Paris et al., 2012)		
miR-192	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-193a	E2	10 nM E_2 48 h in MCF-7 cells; also repressed in T47D, ZR-75-1, BT-474, and BG1, but not SKBR3 breast cancer cells (Maillot et al., 2009)		
miR-193a-3p	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-193b*	E2	10 nM E_2 for 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-194	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-194b*	E2	10 nM E_2 for 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-196a	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-196b	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-199a/b-3p	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ but increased by ERα (Paris et al., 2012)		
miR-199b-5p	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα- except that 24 h of E2 increased miR-199b-5p in ERα-MCF-7 cells (Paris et al., 2012)		
miR-200a	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013) 10 nM E2 6 h MCF-7, LCC1, and LCC2 breast cancer cells (Manavalan et al., 2013)		
miR-200b	E2 4-OHT	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013) 10 nM E2 6 h MCF-7, LCC1, LCC2, and LCC9 breast cancer cells (Manavalan et al., 2013) 500 nM 4-OHT for h in ECC-1 and Ishikawa endometrial cancer cells (Bai et al., 2013)	4-OHT induced c-Myc that inhibited miR-200a, miR-200b, and miR-429 transcription (Bai et al., 2013). miR-200b promoter P2 is hypermethylated in primary breast tumors and ERα-negative cell lines (Wee et al., 2012).	ZEB2 (Bai et al., 2013)
miR-200c	E2 4-OHT	10 nM E_2 for 6 h in MCF-7 cells (Klinge, 2009) 10 nM E_2 for 6 h MCF-7, LCC1, LCC2, and LCC9 breast cancer cells (Manavalan et al., 2013). 500 nM 4-OHT for h in ECC-1 and Ishikawa endometrial cancer cells (Bai et al., 2013)		ZEB2 (Bai et al., 2013)

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Table 2 (*continued*)

miRNA	Ligand	Species/tissue/cell line	Comments	*Bona fide* targets
miR-203	E2	10 nM E_2 for 6, 24, and 48 h in MCF-7 cells; blocked by pretreatment with 1 μM ICI 182,780 (Yu et al., 2012). 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-204	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-205	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)	Oncosuppressor miR	
miR-206	1 nM E_2 or 10 nM PPT (an ERα-selective agonist)	MCF-7 cells (Adams et al., 2007)	80% reduction in expression with 24 h treatment	
miR-218	E2	10 nM E_2 for 24 and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-220c	E2	10 nM E2 for 24 h in T47D cells (Katchy et al., 2012)		
miR-221	E2	10 nM E_2 for 24 h ~80% reduction in MCF-7 and T47D cells (Di Leva et al., 2010) Repressed by ERα knockdown 10 nM E2 48 h in MCF-7 cells (Rao et al., 2011) 10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013) 1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)	Pro-metastatic/pro-proliferative	*ESR1* = ERα (reviewed in Guttilla et al., 2012)
miR-221*	E2	10 nM E_2 for 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-222	E2 BPA	10 nM E_2 for 24 h ~80% reduction in MCF-7 and T47D cells (Di Leva et al., 2010) Repressed by ERα knockdown 10 nM E2 for 48 h in MCF-7 cells (Rao et al., 2011)		
miR-223	E2	10 nM E_2 for 3 h in MCF-7 cells (Saumet et al., 2012)		
miR-301a	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-320b miR-320d	E2	1 nM E_2 for 18 h in MCF-7 cells (Tilghman et al., 2012)		
miR-328	E2	10 nM E_2 6 h in MCF-7 cells (Klinge, 2009) 10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-330-5p	E2 PhIP	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012) 10 nM E_2 or 100 nM PhIP for 4, 8, 12, or 24 h in MCF-7 cells (Papaioannou et al., 2014)		
miR-338-3p	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012). 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-342	E2	10 nM E_2 for 6 h in MCF-7 cells (Klinge, 2009)		
miR-345	E2	10 nM E_2 for 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-362-5p	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-365	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-374b*	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-375	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-376a	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-377	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-379	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-429	4-OHT	500 nM 4-OHT for h in ECC-1 and Ishikawa endometrial cancer cells (Bai et al., 2013)		
miR-451	Tamoxifen	1 μM tamoxifen repressed by 4 h and 90% at 24 h (Bergamaschi and Katzenellenbogen, 2012)	Expression approximately twofold lower in tamoxifen-resistant MCF-7 cells (Bergamaschi and Katzenellenbogen, 2012)	
miR-487b	E2	10 nM E_2 for 6, 12, and 72 h in ZR-75-1 cells, but no significant expression at 24 h (Ferraro et al., 2012)		
miR-499	E2	10 nM E_2 for 48 h in MCF-7 cells; also repressed in T47D, ZR-75-1, BT-474, and BG1, but not SKBR3 breast cancer cells (Maillot et al., 2009)		

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Table 2 (*continued*)

miRNA	Ligand	Species/tissue/cell line	Comments	*Bona fide* targets
miR-504	E2	10 nM E_2 for 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-	E2	10 nM E_2 for 24 h in MCF-7 cells (Ferraro et al., 2012)		
miR-515-5p	E2	10 nM E_2 48 h in MCF-7 cells mediated by ERα binding (Pinho et al., 2013)		SK1 (Pinho et al., 2013)
miR-518c*	E2 or PhIP	10 nM E_2 or 100 nM PhIP for 4, 8, 12, or 24 h in MCF-7 cells (Papaioannou et al., 2014)		
miR-520d	E2	10 nM E_2 48 h in MCF-7 cells; also repressed in T47D, ZR-75-1, BT-474, and BG1, but not SKBR3 breast cancer cells (Maillot et al., 2009)		
miR-548g	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-570	E2	10 nM E_2 for 6, 12, 24 h and 3 d in MCF-7 cells (Cicatiello et al., 2010) 10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-574-3p	4-OHT	1 μM 4-OHT for 1 month in MCF-7 cells (Ujihira et al., 2015)		Clathrin heavy chain (CLTC) (Ujihira et al., 2015)
miR-579	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-582-3p	E2	10 nM E_2 for 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-583-5p	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-584	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-589	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-590-5p	E2	10 nM E_2 24 h ERβ stably expressing SW480 colon cancer cells (Edvardsson et al., 2013)		
miR-610	E2	10 nM E_2 for 6,12, 24 and 72 h in ZR-75-1 cells, most repressed at 72 h (Ferraro et al., 2012)		
miR-615-5p	E2 or PhIP	10 nM E_2 or 100 nM PhIP for 24 h in MCF-7 cells (Papaioannou et al., 2014)		
miR-616	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-618	E2	10 nM E_2 for 6, 12, 24 h and 3 d in MCF-7 cells (Cicatiello et al., 2010). 10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-632	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-638	E2 or PhIP	10 nM E_2 or 100 nM PhIP for 4, 8, 12, or 24 h in MCF-7 cells (Papaioannou et al., 2014)		
miR-646	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-650	E2	10 nM E_2 for 24 h in T47D cells (Katchy et al., 2012)		
miR-663	E2 or PhIP	10 nM E_2 or 100 nM PhIP for 4, 8, 12, or 24 h in MCF-7 cells (Papaioannou et al., 2014)		
miR-671:9-1, 671-3p	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-708*	E2	10 nM E_2 for 6, 24, and 72 h in ZR-75-1 cells, but not 12 h (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-874	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-877	4-OHT	1 μM 4-OHT for 1 month in MCF-7 cells (Ujihira et al., 2015)		
miR-935	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012) 10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-938	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		
miR-1225	E2	10 nM E_2 for 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-1228	E2	10 nM E_2 for 24 h in T47D cells (Katchy et al., 2012)		
miR-1229	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-1234	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-1238	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 and ZR-75-1 cells (Ferraro et al., 2012)		
miR-1257	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		

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Table 2 (*continued*)

miRNA	Ligand	Species/tissue/cell line	Comments	*Bona fide* targets
miR-1267	E2	10 nM E_2 in MCF-7 cells stably overexpressing inducible ERβ or ERα (Paris et al., 2012)		
miR-1301	E2	10 nM E_2 for 6, 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-1303	E2	10 nM E_2 for 12, 24, and 72 h in ZR-75-1 cells (Ferraro et al., 2012)		
miR-1468	E2	10 nM E_2 for 6, 12, 24, and 72 h in MCF-7 cells (Ferraro et al., 2012)		

interaction with the 3′UTR region (Jiang et al., 2014). There are 3 reports on miRNA regulation of corepressors that target ERα. miR-10a and -10b repress SMRT/NCOR2 (Foley et al., 2011). miR-184 (Wu et al., 2011) and miR-16 (Zhou et al., 2012) represses SMRT/NCOR2 translation, but how they affect ER activity is unknown. MTA1 (metastatic tumor antigen 1) repressed miR-661, but the effect on ERα transcription was not evaluated (Bui-Nguyen et al., 2010). miR-615-3p repressed LCoR expression (Jiang et al., 2011), but whether this affects ERα was not studied. Clearly, little is known about regulation of ER coactivators and corepressors by miRNAs.

15. E_2 regulation of AGO2 in human breast cancer cell lines

The expression of Argonaut-2 (Ago2), the catalytic subunit of the RISC complex that mediates miRNA-dependent cleavage/degradation in mammals, is higher in ERα-negative, HER2-positive than ERα-positive/HER2 negative (luminal) human breast cancer cell lines and tumors (Adams et al., 2008). However, E_2 and the ERα-agonist PPT, but not the ERβ-agonist DPN, increased AGO2 protein expression in MCF-7 cells (Adams et al., 2008). Further studies showed that EGF acts through the MAPK pathway to increase Ago2 protein stability, but there were no studies examining the mechanism by which E_2 and PPT, presumably through ERα, increase Ago2 protein levels. Surprisingly, Ago2 overexpression in MCF-7 cells increased ERα protein levels by threefold, despite also increasing miR-206 that reduces ERα (Adams et al., 2008). The authors concluded that this "discordant" finding indicates that there is a greater concentration of miRNAs than target proteins involved in ERα suppression than those that target ERα itself (Adams et al., 2008). Microarray profiling shows that the expression of Ago1 and Ago2 proteins is higher while Dicer and TRBP1 are lower in ERα-negative *versus* ERα-positive breast cancer cells (Cheng et al., 2009).

16. MicroRNA and endocrine-resistant breast cancer

Altered miRNA expression is likely to play a role in endocrine-resistance in breast cancer. A PubMed search for 'MicroRNA and endocrine resistance in breast cancer' generated nine new publications since my previous review (Klinge, 2012). A recent review of mechanisms of endocrine resistance includes a paragraph on the upregulation of miR-221, miR-222, and miR-181b and downregulation of miR-21, miR-342, and miR-489 in tamoxifen-resistant breast cells (Zhao and Ramaswamy, 2014). miR-221/222 promoted TAM-resistance by targeting ERα and the cell cycle regulator p27 (also known as Kip1) (Zhao et al., 2008). Overexpression of miR-221/222 also associates with Fulvestrant-resistance (Rao et al., 2011). miR-221/222 is also increased in $CD44^{+}CD24^{-/low}$ human breast cancer stem cells, indicating a role for these stem cells in endocrine resistance (Shimono et al., 2009). miRNAs in CSCs and their role in chemoresistance have been recently reviewed (Sun et al., 2014).

My laboratory identified miRNAs that are differentially regulated by TAM in endocrine-sensitive MCF-7 and endocrine-resistant LY2 human breast cancer cells (Manavalan et al., 2011). LY2 cells were derived from MCF-7 by serial passage in the antiestrogen LY 117018, a precursor to Raloxifene (RAL) (Bronzert et al., 1985), and express wild-type ERα mRNA levels similar to MCF-7 cells (Mullick and Chambon, 1990), but are resistant to TAM, RAL, and Fulvestrant (ICI 182,780) (Crawford et al., 2010). We identified 97 miRNAs regulated in the opposite direction in MCF-7 and LY2 cells. Quantitative real-time PCR (qPCR) selectively confirmed higher miR-200a, miR-200b, and miR-200c in MCF-7 than LY2 cells and higher miR-10a, miR-22, miR-29a, miR-125b, and miR-222 in LY2 than in MCF-7 cells (Manavalan et al., 2011). Some of the mRNA targets include *PDCD4*, *BCL2*, *CYP1B1*, and *ERBB3*.

Members of the miR-200 family and miR-221/222 are implicated in epithelial–mesenchymal transition (EMT) and metastasis (Sreekumar et al., 2011). Many studies have identified an inverse relationship between the expression of the miR-200 family and its targets ZEB1/2 in cells (Bracken et al., 2008; Burk et al., 2008; Hurteau et al., 2007; Korpal et al., 2008; Park et al., 2008). *ZEB1*, a target of miR-200 family of miRNAs and a promoter of EMT, was found to be overexpressed in LY2 cells when compared to MCF-7 cells (Manavalan et al., 2011). We observed a progressive decrease in the expression of miR-200a, miR-200b, and miR-200c in an MCF-7-derived cell line model of TAM/endocrine resistance, *i.e.*, decreasing from MCF-7, LCC1 (E2-independent, but TAM-sensitive; to the TAM-resistant LCC2, LCC9, and LY2 cell lines, respectively (Manavalan et al., 2013)). Concurrently, we detected an increase in ZEB1 expression in LCC9 and LY2 cells. Overexpression of miR-200b and miR-200c enhanced the sensitivity of LY2 breast cancer cells to growth inhibition by antiestrogens 4-OHT and fulvestrant. These data are in agreement with other reports showing an inverse correlation between miR-200 family and ZEB1 expression in basal-like, triple negative breast cancer (TNBC) cells such as MDA-MB-231 and BT549 (Burk et al., 2008; Gregory et al., 2008; Hurteau et al., 2007; Park et al., 2008). CpG island methylation of miR-200c/miR-141 promoter has been reported in breast and prostate cancer cells (Davalos et al., 2012; Neves et al., 2010; Vrba et al., 2010). Treatment of MDA-MB-231 and BT549 breast and PC3 prostate cancer cells with 5-aza-2′-deoxycytidine (5-aza-dC), a demethylating agent, increased miR-200c and miR-141 expression (Vrba et al., 2010). Our study agrees with these reports of epigenetic silencing of the miR-200 family, because we demonstrated that treatment of LY2 cells with 5-aza-dC + histone deacetylase inhibitor trichostatin A (TSA) increased miR-200b and miR-200c expression (Manavalan et al., 2013). There was a concomitant decrease in the expression of ZEB1 mRNA and protein and the LY2 cells appeared more epithelial in morphology and were sensitized to TAM and fulvestrant inhibition. Likewise, knockdown of ZEB1 increased antiestrogen sensitivity of LY2 cells resulting in inhibition of cell proliferation (Manavalan et al., 2013).

Global miRNA analysis of 153 ERα+ primary breast tumors from women who subsequently took tamoxifen as an adjuvant monotherapy revealed that no single miRNA profile was predictive of patient outcome (Lyng et al., 2012). Decreased expression of miR-190b, miR-339-5p, miR-520c-3-, miR-520g, miR-520h, miR-139-3p, miR-204, miR-502-5p, miR-365, and miR-363 in the primary

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tumors was associated with recurrence in tamoxifen-treated patients (Lyng et al., 2012).

miR-342 was downregulated in two TAM-resistant cell lines derived from MCF-7 cells called LCC2 and TAMR1 (Cittelly et al., 2010). Overexpression of miR-342 conferred TAM-sensitivity and increased apoptosis. miR-451, an oncosuppressor miRNA, was downregulated in TAM-resistant breast cancer cells (Bergamaschi and Katzenellenbogen, 2011). miR-451 targets 14-3-3ζ an anti-apoptotic gene that is overexpressed in TAM-resistant tumors and is associated with lower survival (Bergamaschi and Katzenellenbogen, 2011). Increased expression of ERα36, a truncated form of the full length ERα66, that blocks ERα66 genomic activity while activating MAPK signaling, has been reported in TAM-resistant breast tumors (Fowler et al., 2009). let-7a targets ERα36 and loss of let-7 family members conferred TAM-resistance by activating non-genomic estrogen signaling mediated by ERα36 (Zhao et al., 2011).

miRNA microarray profiling identified 10 miRNAs downregulated in a TAM-resistant MCF-7 cell line compared with wt MCF-7 cells: miR-125a, miR-489, miR-375, miR-653, miR-135b, miR-556-3p, miR-190b, miR-556-5p, miR-561, and miR-548h; while 12 miRs were upregulated: miR-551b, miR-519a, miR-376a*, miR-31, miR-224, miR-521, miR-31*, miR-655, miR-205, miR-518f, miR-520h, miR-455-3p (Ward et al., 2013). Transfection of TAM-resistant MCF-7 cells with pre-miR-375 re-sensitized the cells to ~15% growth inhibition by 5 μM TAM, reduced mRNA expression of EMT markers: FN1, ZEB1, and SNAI2, and reverted EMT-like invasive appearance of the cells (Ward et al., 2013). MTDH was identified as a direct target of miR-375 and siMTDH in TAM-resistant MCF-7 cells partially sensitized the cells to tamoxifen and higher TDFH was correlated with reduced disease-free survival in tamoxifen-treated breast cancer patients (Ward et al., 2013).

The miRNA cluster C19MC, encoding 59 miRNAs spanning ~100 kB (Flor and Bullerdiek, 2012), is the largest known cluster of miRNAs in the human genome (Bortolin-Cavaille et al., 2009). Many miRNAs of C19MC are oncomiRs when re-expressed in tissues (Flor and Bullerdiek, 2012). miRNA microarray profiling revealed that 18 miRNAs in the C19MC cluster were upregulated in a TAM-resistant MCF-7 cell line compared with wt MCF-7 cells including miR-520c-3p, miR-519d, miR-518b, miR-520h, miR-521, miR-518f, miR-520b, miR-518c, miR-512-5p, miR-512-3p, miR-518e*, miR-515-5p, miR-517c, miR-522, and miR-519a (Ward et al., 2014). Overexpression of a miR-519a mimic in MCF-7 cells resulted in TAM-resistance and transfection of TAM-resistant MCF-7 cells with a miR-519a inhibitor restored TAM-growth inhibition on the cells (Ward et al., 2014). The authors verified CDKN1A, RB1, and PTEN as *bona fide* targets of miR-519a and correlated increased miR-519a expression with poorer disease-free survival in ERα+ breast cancer patients (Ward et al., 2014).

17. Conclusion

Estrogens, most commonly E_2, and other ER ligands including tamoxifen and endocrine disruptors regulate diverse physiological effects through genomic and nongenomic/membrane-initiated mechanisms that alter cellular expression of miRNAs. miRNAs are post-transcriptional regulators of mRNA translation and stability. Although miRNA changes in fish, mice, rats, and human breast cancer cells in response to E2 and tamoxifen have been reported, there are relatively few studies examining the detailed mechanisms for these responses and their downstream *bona fide* targets. The effect of E_2 varies between and within cell lines depending on the ratio of ERs, including GPER, expressed, coregulators, chromatin structure, cell cycle, circadian rhythms, and numerous other physiological parameters. Future HITS-CLIP and global high-throughput studies are needed to elucidate the general principles while detailed biochemical/molecular studies are required to dissect the specific mechanisms involved in ER/miRNA interactions and their roles in human health and disease.

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