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Implications for Hormone-Dependent Cancers**

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Interplay between Steroid Signaling and MicroRNAs: Implications for Hormone-Dependent Cancers

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Abstract

Hormones are key drivers of cancer development. To date, interest has largely focussed on the classical model of hormonal gene regulation but there is increasing evidence for a role of hormone signalling pathways in post-translational regulation of gene expression. In particular, a complex and dynamic network of bi-directional interactions with microRNAs at all stages of biogenesis and during target gene repression is emerging.

MicroRNAs, which act mainly by negatively regulating gene expression through association with 3' untranslated regions of mRNA species, are increasingly understood to be important in development, normal physiology and pathogenesis. Given recent demonstrations of altered microRNA profiles in a diverse range of cancers, their ability to function as oncogenes or tumour suppressors, and hormonal regulation of microRNAs, understanding mechanisms by which microRNAs are generated and regulated is vitally important.

MicroRNAs are transcribed by RNA polymerase II then processed in the nucleus by the Drosha-containing Microprocessor complex and in the cytoplasm by Dicer, before mature microRNAs are incorporated into the RNA-induced silencing complex. It is increasingly evident that multiple cellular signalling pathways converge upon the miR biogenesis cascade, adding further layers of regulatory complexity to modulate miR maturation. This review summarises recent advances in identification of novel components and regulators of the Microprocessor and Dicer complexes, with particular emphasis on the role of hormone signalling pathways in regulating their activity. Understanding hormone regulation of microRNA production and how this is perturbed in cancer is critical for the development of microRNA-based therapeutics and biomarkers.

An Overview of MicroRNA Biogenesis

MicroRNAs were first discovered in 1993, with two independent studies describing the *C. elegans lin-4* gene product as a small non-coding RNA (ncRNA) (Lee *et al.* 1993; Wightman *et al.* 1993). Later it was shown that the *C. elegans let-7* gene product, also a ncRNA, could associate with the 3'UTR of target genes to initiate a cascade of regulatory processes acting on heterochronic genes (Reinhart *et al.* 2000). The first demonstrations of microRNA functionality in other cell systems were by three groups in 2001 (Lagos-Quintana *et al.* 2001; Lau *et al.* 2001; Lee and Ambros 2001). It is now understood that microRNAs act as gene regulators in almost all plant and animal species.

Mammalian microRNAs (miRs) are transcribed by RNA polymerase II (polII), generating a primary microRNA transcript (pri-miR) containing one or more hairpin structures, comprising a stem and terminal loop. Following transcription, pri-miRs are 5' capped and adenylated. The majority are polycistronic and generate several functional mature miRs. In the nucleus, the pri-miR is cleaved into one or more ~70nt precursor miRs (pre-miRs), which have a hairpin structure; this is performed by the Microprocessor complex, which contains the RNase III enzyme, Drosha, and the double-stranded RNA binding domain protein DiGeorge Critical Region 8 (DGCR8)/Partner of Drosha (Pasha). Purified Drosha can cleave pri-miRs *in vitro* (Gregory *et al.* 2004), but requires cofactor proteins for increased efficiency and specificity, hence the Drosha Microprocessor is very large multi-protein complex (>650kD in human cells (Gregory *et al.* 2004)) containing at least 20 different polypeptides, thought to promote fidelity, specificity and/or Drosha cleavage activity and to act as scaffolding proteins. Some general cofactors modulate biogenesis of the majority of miRs, e.g. DGCR8, whilst others promote or inhibit biogenesis of a defined miR subgroup, e.g. KHSRP1, hnRNPA1, and the DEAD box RNA helicases, p68 (DDX5) and p72 (DDX17) (Fukuda *et al.* 2007). The primary function of DGCR8 is to bind to the junction between single-stranded and double-stranded regions of the pri-miR stem via its two RNA binding domains, leading Drosha to cleave approximately 11bp from the junction. DGCR8 ensures correct orientation of Drosha on the miR hairpin (Landthaler *et al.* 2004). Only about 25-100 nucleotides surrounding both ends of the stem-loop are required for Drosha cleavage and pri-miR processing (Lee *et al.* 2003; Zeng *et al.* 2002).

Drosha cleavage results in a product with a 2nt 3' overhang, which is vital both for recognition by Exportin-5, which facilitates Ran-GTP-dependent export of the pre-miR to the cytoplasm, and for Dicer-mediated pre-miR cleavage, since processing occurs at a

defined distance from the free RNA end produced by Drosha. In the cytoplasm, pre-miRs are further processed by another RNase III enzyme, Dicer (enhanced by the accessory dsRBD protein TRBP/PACT), which cleaves the stem-loop to produce a ~22nt miR duplex. The two strands subsequently separate and one strand associates with Argonaute-2 (AGO2), a protein component of the RNA-induced silencing complex (RISC). Unwinding of the duplex to generate the guide strand and the passenger strand is achieved by a combination of helicases associated with the RISC complex (exactly which remains to be determined, although helicases associated with RISC formation and/or activity include p68, p72, RNA helicase A (RHA), TNRC6B and Gemin3/4 (Meister *et al.* 2005; Robb and Rana 2007; Salzman *et al.* 2007)). The mature miR guides RISC to complementary sequences within the 3'UTR of target mRNAs, resulting in translational repression and/or transcript degradation. For a subset of intronic miRs (mirtrons), following transcription, pre-miR hairpins are released from host introns by splicing and debranching, bypassing Drosha-mediated cleavage (Okamura *et al.* 2007; Ruby *et al.* 2007). These pre-miRs re-enter the conventional processing pathway in the cytoplasm, where they are further processed by Dicer. An overview of miR biogenesis is given in Figure 1.

Currently, little is known about factors determining which strand of the miR duplex is incorporated into RISC. One important parameter may be thermodynamic asymmetry of the miR duplex, since the strand with the less stably base-paired 5' end is more frequently incorporated into RISC (Kawamata *et al.* 2009; Pratt and MacRae 2009; Yang *et al.* 2011). The other strand (passenger strand or miR* strand) was thought to be degraded (Yang *et al.* 2011). However, recent deep-sequencing experiments in *Drosophila* revealed that frequently miR*s are not degraded but associate with AGO1 or AGO2, hence both strands of the miR duplex are functional (Mah *et al.* 2010; Okamura *et al.* 2008; Yang *et al.* 2011), and 3'UTR-repressive activities have been assigned to several human miR*s (Kim *et al.* 2011 ; Yang *et al.* 2011). Humans express four different AGO proteins demonstrating similar preferences for miR duplex structures, in that central mismatches promote RISC association (Murphy *et al.* 2008). However, it is likely that many other factors beyond the scope of this review could impinge upon strand selection.

Regulation of MiR Maturation

MiR biogenesis is emerging as a stringently controlled and remarkably complex pathway; coordinated regulation likely serves to prevent mis-expression of miRs both spatially and temporally. MiR levels are thought to be particularly important in development and tumourigenesis. For example, in early development many pri-miRs are expressed but not

efficiently converted into their mature forms, while reduced processing has been shown to contribute to widespread downregulation of many miRs in human cancers. It is widely accepted that miRs can function as oncogenes and tumour suppressors (Shenouda and Alahari 2009). Global miR downregulation has been identified as a general characteristic of a number of human cancers (Kumar *et al.* 2007; Ozen *et al.* 2008), suggesting abrogation of miR biogenesis may induce or maintain tumourigenesis. Indeed, downregulation of Drosha and Dicer and mutations in genes encoding TRBP and Exportin5 have been reported in several cancers (Karube *et al.* 2005; Melo *et al.* 2009; Merritt *et al.* 2008), although overexpression of Dicer is found in precursor lung adenocarcinoma lesions and correlates with poor survival in colorectal cancer (Chiosea *et al.* 2007; Faber *et al.* 2011). Further, dysregulation of miRs as a consequence of activated oncogenic signalling pathways may represent a major driver of tumour development, since oncogenesis-related proteins including p53, SMAD, ER α and AR appear to modulate miR biogenesis. Despite the relative infancy of this research area it is clear that dysregulation of miR biogenesis plays a vital role in a multitude of human pathologies including hormone-dependent malignancies. Important regulators of this process are summarised below and in Figure 1

MiR-Specific Regulation by Microprocessor

The Microprocessor complex contains many proteins and there is evidence that certain of these may be important for processing distinct subsets of miRs, suggesting these proteins can act as specificity factors; for instance, depletion of Microprocessor cofactors p68 or p72 in knockout mice results in reduced levels of a subset of mature miRs whilst others remain unchanged (Fukuda *et al.* 2007). Additionally, the tumour suppressor protein, p53, and TGF β /BMP4-stimulated SMAD are known to enhance processing of distinct subsets of miRs through interaction with p68 and Drosha to increase pri-miR cleavage (see Figure 1) (Davis *et al.* 2008; Suzuki *et al.* 2009). One miR biogenesis of which is enhanced by SMAD is miR-21, which targets several tumour suppressor genes including PTEN, so may act as an oncomiR (Kumarswamy *et al.* 2011). Also BRCA1, which is vital for maintenance of genomic stability, increased levels of mature cancer-associated miRs including let-7a-1, miR-16-1 and miR-34a via a mechanism involving direct BRCA1 association with Drosha and pri-miRs through recognition of branched secondary structures (Kawai and Amano 2012). Processing of the BRCA-1-regulated miR subset is likely to be lost in BRCA-1-mutant cells, perhaps one mechanism by which BRCA1 mutation enhances tumourigenesis.

Self-Regulation of the Microprocessor

Drosha and DGCR8 can mutually regulate each other's function post-transcriptionally: DGCR8 interacts with the Drosha central domain to stabilise Drosha protein (Yeom *et al.* 2006), while the Drosha-DGCR8 complex cleaves hairpin structures in the 5'UTR and coding region of the DGCR8 transcript, leading to degradation of DGCR8 mRNA, generating a negative feedback loop to regulate DGCR8 levels if microprocessor activity is sufficient (Han *et al.* 2009). These data imply collaborative and dynamic mutual regulation of pathways that regulate both mRNA stability and miR maturation.

Regulation of miR maturation by RNA Processing Modulators

mRNA splicing regulators have been shown to preferentially induce maturation and accumulation of one or more miRs from a polycistronic primary miR transcript (Michlewski *et al.* 2008). hnRNPA1 is a multi-functional RNA binding protein involved in many aspects of RNA processing, including alternative splicing. Its direct association with its consensus binding site, UAGGGA/U, in pri-miR stem-loops can either increase Drosha processing by inducing a stem-loop conformational shift, in the case of pri-miR-18a (Guil and Caceres 2007; Newman and Hammond 2010), or repress maturation by inhibiting Drosha association, as is the case for let-7a (Michlewski and Caceres 2010). TAR DNA-binding protein 43 (TDP-43) shares significant sequence homology with hnRNPs and promotes miR biogenesis as a component of both the Microprocessor, where it binds pri-miRs and Drosha, and the cytoplasmic Dicer complex, where it associates with the terminal loop regions of a subset of pre-miRs to enhance their maturation (Kawahara and Mieda-Sato 2012).

The RNA binding protein KH-type splicing regulatory protein 1 (KHSRP1, also known as FBP-2) also regulates miR biogenesis as a component of both the Microprocessor and Dicer complexes (Trabucchi *et al.* 2009). KHSRP1 (previously shown to interact with single-stranded, AU-rich mRNAs to mediate mRNA decay (Gherzi *et al.* 2004)) promotes maturation of a miR subset through association of its KH3 domain with a specific 5' GGG triplet within terminal loops of target pre-miRs, such as pre-let-7a-1 and pre-miR-155 (Ruggiero *et al.* 2009; Trabucchi *et al.* 2009). Further, we recently found that KHSRP protein is upregulated by antiandrogen treatment of prostate cancer cells, i.e. a target of AR signalling hence could mediate androgen regulation of this miR subset (Brooke *et al.* 2014).

TRBP (TAR RNA binding protein) is a key component of the Dicer-containing complex (Melo et al. 2009). Truncating TRBP mutations have been identified in colorectal and endometrial carcinoma cell lines with microsatellite instability, leading to decreased TRBP protein, Dicer destabilisation and defective pre-miR processing. Reintroduction of TRBP into TRBP-deficient colon cancer cells restored processing of known tumour suppressive miRs such as miR-26a (Sander et al. 2008) and miR-125a (Scott et al. 2007), and interestingly, repressed the cells' ability to form tumours in nude mice (Melo et al. 2009), suggesting TRBP may function as a tumour suppressor by maintaining Dicer-mediated biogenesis of tumour suppressive miRs.

Other Factors Regulating MiR Maturation

Enzymes that catalyse RNA/DNA modifications can strongly influence miR biogenesis. For instance, BCDIN3D, an RNA-methyltransferase, O-methylates 5' monophosphates of target pre-miRs, resulting in loss of negative charge and consequently of Dicer binding and miR maturation (Xhemalce et al. 2012). Microprocessor-mediated pri-miR cleavage is regulated by RNA-editing ADARs (adenosine deaminase acting on RNA). ADAR conversion of adenosine (A) to inosine (I) inhibits effective pri-miR processing by Drosha and can alter sequence and structural properties, since base-pairing of inosine is similar to that of guanosine (O'Connell and Keegan 2006; Yang et al. 2006). This editing can target the pri-miR for degradation, produce modified mature miRs that regulate a different set of targets, modify extent of target regulation, or alter pre-miR processing by DGCR8 (O'Connell and Keegan 2006; Yang et al. 2006).

Additionally, an immune regulator ribonuclease, monocyte chemoattractant protein 1-induced protein 1 (MCPIP1), suppresses biogenesis of a subset of miRs through pre-miR terminal loop cleavage, antagonising Dicer activity (Suzuki et al. 2011). Commensurate with a physiological role for this mode of regulation, an inverse correlation was identified between Dicer and MCPIP1 expression in lung cancer, and high MCPIP1 expression was associated with poor prognosis in lung adenocarcinoma patients (Karube et al. 2005; Shedden et al. 2008).

Together, these observations highlight the potential for signalling cascades to regulate the biogenesis of individual miRs or miR subsets, and suggest that interaction of microprocessor cofactors with accessory proteins such as p53, hnRNPA1, KHSRP1 or SMAD can alter the specificity and/or activity of Drosha processing. It may be speculated

that this is mediated through conformational changes to the microprocessor and/or pri-miR, or through displacement of repressive proteins bound to RNA.

Hormonal Regulation of MicroRNA Biogenesis

There are clear indications in the literature to support direct and indirect hormonal regulation of miR biogenesis, with hormone signalling altering miR biogenesis at all stages of maturation, from transcription to control of mature miR stability. Such regulatory mechanisms provide numerous opportunities to impart subtle but stringent control over gene expression in order to maintain appropriate levels of key protein activity during normal cellular homeostasis. Aberrations in these subtle mechanisms of hormone-modulated miR biogenesis may promote disease pathogenesis, particularly in hormone-related cancers.

Estrogen Modulation of MicroRNA Biogenesis

Estrogens have been described as key regulators of miR biogenesis in breast cancer cells, endometrial stromal cells and myometrial smooth muscle cells; these effects are achieved through liganded ER α directly regulating miR transcription, ER α modulation of miR maturation, or via direct or indirect autoregulatory feedback loops, both positive and negative (Castellano *et al.* 2009; Klinge 2009).

High-Throughput Profiling Identifies Estrogen-Regulated MiRs

As for protein-coding genes, hormone stimulation alters transcription of numerous miR-coding genes, and ER α has been demonstrated to associate directly with response elements in promoters of such to initiate gene expression (Bhat-Nakshatri *et al.* 2009). MiRNAome-wide profiling arrays, such as microarrays and qPCR arrays, have helped to define a signature of estrogen-regulated miRs. The first examples of such arrays are limited in their applicability to ER α signalling in humans, being performed in zebrafish, rat breast tissue and mouse spleen (Cohen *et al.* 2008; Dai *et al.* 2008; Kovalchuk *et al.* 2007) and to date only around fifteen studies have examined estrogen regulation of miRs in human cells (Bhat-Nakshatri *et al.* 2009; Castellano *et al.* 2009; Cicatiello *et al.* 2010; Cochrane *et al.* 2010; Di Leva *et al.* 2010; Di Leva *et al.* 2013; Ferraro *et al.* 2012; Hah *et al.* 2011; Iorio *et al.* 2005; Klinge 2009; Maillot *et al.* 2009; Masuda *et al.* 2012; Wickramasinghe *et al.* 2009; Zhao *et al.* 2013). Despite the majority of these being performed in MCF7 breast cancer cells, inconsistencies are evident, presumably attributable to differences in ligand, treatment duration, array platform, statistical

processing and threshold setting as well as lab-specific cell line differences. As such, very few estrogen-regulated miRs have been consistently identified and validated to date. In contrast, considerable efforts have been focussed on identification of miRs that show differential expression in breast cancer, resulting in upwards of 50 publications. Such studies are vital in the identification of novel diagnostic/prognostic biomarkers and therapeutic targets to improve clinical management and treatment of a disease that is a very considerable cause of morbidity and mortality. However, the relationship between miRs that are dysregulated in breast cancer and those that are estrogen-regulated is complex and the degree of overlap is currently unclear.

A summary of the most commonly identified estrogen-regulated miRs is shown in Table 1. In contrast to the identification of numerous estrogen-regulated miRs in MCF7 cells, no miRs demonstrated estrogen regulation following 24h treatment of T47D breast cancer cells with 10nM E₂ (Katchy *et al.* 2012). In one of the most widely-cited studies, Bhat-Nakshatri *et al.* demonstrated estrogen upregulation of 21 miRs (several of which target ER α coactivators *in vitro*) and downregulation of seven in MCF7 cells through microarray analysis (Bhat-Nakshatri *et al.* 2009). Several of the identified miRs were located within intragenic regions of estrogen-regulated genes. Of the identified estrogen-upregulated miRs, let-7f, miR-98 and miR-21 were shown to reduce levels of c-Myc and E2F2 proteins (required for optimal ER α activity and secondary estrogen response respectively), providing evidence for a negative feedback loop to repress ER α activity. Another microarray analysis identified 17 miRs as significantly upregulated by estrogen treatment in MCF7 cells. Interestingly, however, only miR-7 (which targets EGFR in ER α -positive but not ER α -negative breast cancer cells) was subsequently found to be suppressed by treatment with an anti-estrogen, Faslodex (Masuda *et al.* 2012). Reasons for the lack of converse effects of anti-estrogens on estrogen-regulated miRs in this case remain to be identified, but this approach is one means of validating ER-dependent regulation.

As a more direct, and arguably more physiologically relevant method for identification of transcriptionally estrogen-regulated miRs, Hah *et al.* used global nuclear run-on and sequencing (GRO-seq) to map the position and orientation of all RNA polymerases across the MCF7 genome following 100nM E₂ treatment for 10, 60 or 160 minutes. 119 miR-containing transcripts that were regulated by E₂ at one or more time points were identified (Hah *et al.* 2011). Interestingly, MCF7 cells were enriched for 2700 predicted targets of these E₂-regulated miRs compared to other cells, indicating the presence of a complex regulatory network of miRs and their mRNA targets (Hah *et al.*

2011). Other studies have combined microarray expression profiling of miRs with global ER α binding site mapping through ChIP-seq to provide mechanistic insight: Bhat-Nakshatri *et al.* examined functional ER α binding sites within 20kb regions 5' of miR genes following estrogen stimulation that resulted in increased pri-miR transcription. One estrogen-responsive ERE was found in the miR-21 promoter, with five and four EREs identified in the miR-23b~27b~24-1 and miR-23a~27a~24-2 promoters respectively, although different cluster members showed differing extents of regulation (Bhat-Nakshatri *et al.* 2008; Bhat-Nakshatri *et al.* 2009). Ferraro *et al.* used this approach to compare responses in estrogen-treated MCF7 and ZR-75.1 cells (Ferraro *et al.* 2012). They demonstrated that miR-135a-2, miR-181c, miR-26b and the clustered miR-23a, miR-27a and miR-24-2 were estrogen-regulated in both cell lines and located within 10kb of an ER α binding site, while estrogen-regulated miR-25, miR-26a, miR-424, miR-618, miR-760 and miR-942 were located in the intragenic region of estrogen-regulated genes. Further, Di Leva *et al.* demonstrated that binding of ER α to the promoter of the miR-191/425-DALRD3 unit leads to increased levels of miR-191 and miR-425 and loss of DALRD3 levels, with subsequent loss of miR-191 and miR-425 targets that include the tumour suppressor EGR1 (Di Leva *et al.* 2013). Both these studies provide evidence for mutual estrogen-regulation of protein-coding genes and their intragenic miRs.

In a further study, Maillot *et al.* identified 23 miRs as repressed by estrogen in MCF7 breast cancer cells, with at least some of the inhibitory effects attributable to ER α repression of miR cluster transcription (Maillot *et al.* 2009). Most interesting was the observation that a number of estrogen-downregulated miRs reduce DNA synthesis and proliferation of breast cancer cells upon their transient transfection (Maillot *et al.* 2009). Analysis of predicted mRNA targets of these estrogen-downregulated, growth inhibitory miRs demonstrated enrichment for genes with roles in cell growth and proliferation. Of particular interest was the finding that six of seven miRs predicted to target the progesterone receptor (PR) 3'UTR (PR is an ER-upregulated target gene and a vital mediator of ER signalling) are downregulated by estrogen (Maillot *et al.* 2009). These observations provide further evidence for a positive feedback loop, whereby ER α downregulates miRs that repress mediators of estrogen signalling in order to amplify its own activity. Hence, estrogen signalling has two ways to increase activity of a vital mediator of its own activity (PR) – direct and indirect feedback loops.

In addition, hormone treatment can induce recruitment of other transcription factors to miR promoter regions. For example, estrogen-inducible c-Myc is recruited to the promoters of the paralogous miR-17-92 and miR-106a-363 clusters and increases mature

levels of encoded miRs (Castellano et al. 2009). This generates a feedback loop whereby ER α self-regulates through increasing levels of ER α pathway-targeting miRs, since miRs 18a, 19b and 20b directly target and downregulate ER α , whilst miRs 20a, 20b, 17-5p and 106a inhibit translation of the ER α coactivator, AIB1 (Castellano et al. 2009). These constitute a complex and highly sensitive network of interactions for subtle manipulation of ER α signalling in breast cancer cells, and highlight that negative as well as positive feedback loops contribute to this.

It is clear that greater research effort is required to isolate the effects of estrogen on pri-miR transcription from the post-transcriptional effects of liganded ER α to the latter stages of miR biogenesis. As an example, let-7g demonstrated direct regulation by ER α , but not at the levels of transcription, since estrogen treatment reduced levels of mature let-7g, but not pri-let-7g in a MAPK-dependent manner in MCF7 cells (Qian et al. 2011). Although the precise mechanism of such regulation remains unclear, reduction in mature let-7g may be attributable to p68/p72-mediated ER α inhibition of Drosha (discussed below).

ER α Modulation of Microprocessor Activity

A seminal study by Yamagata et al. provided the first *in vivo* evidence for ER α regulation of miR expression. It was also the first to demonstrate ER α modulation of Microprocessor function (Yamagata et al. 2009). Expression of a set of mature miRs was upregulated in female mouse embryos deficient in ER α , whilst estrogen treatment of ovariectomised female mice inhibited production of a subset of mature miRs in the uterus, a target organ of estrogen signalling (Yamagata et al. 2009). Upon further examination it was found that levels of the relevant pri-miRs were unchanged following estrogen treatment, but levels of the cognate mature miRs and pre-miRs was downregulated, suggesting that estrogen-mediated modulation of miR levels occurs at the level of pri-miR to pre-miR processing. The authors hypothesised that estrogen signalling via ER α may modulate Drosha activity through physical association of ER α with the RNA helicases p68 (DDX5) and/or p72 (DDX17), which are important components of the Drosha microprocessor complex and vital to miR maturation. Indeed, it was found that mouse ER α associated with mouse Drosha but only in the presence of E₂ and that this requires p72 and p68, as confirmed by immunoprecipitation assays (Yamagata et al. 2009).

In another study, RNA-ChIP experiments showed dissociation of pri-miRs from Drosha and p72 in the presence of estrogen. Interestingly, the N-terminal of ER α associates with the C-terminal of Drosha, which contains the RNaseIII- and dsRNA-

binding motifs (Macias *et al.* 2009). This could explain the observations of Xi *et al.*, who found the extent of estrogen regulation of pri-miRs was greater than that of their encoded mature miRs (Castellano *et al.* 2009). It is possible that these miRs are also subject to ER α -mediated inhibition of pri-miR processing through repression of Drosha activity in an E₂- and p68/p72-dependent manner, yielding the observed biphasic regulatory pattern.

These data indicate a mechanism whereby ER α inhibits Drosha-mediated processing of a subset of miRs through association with Drosha in a p68/p72- and E₂-dependent manner, resulting in dissociation of the Microprocessor complex from the pri-miR. However, it is likely that further auxiliary factors dictate specificity of estrogen-mediated miR regulation and that liganded ER α acts at other steps of miR biogenesis, for example, pre-miR processing or mature miR stability.

Regulation of MiR Biogenesis Pathway Proteins by Estrogen

Further evidence for estrogen regulation of miR maturation comes from the findings of Nothnick *et al.* (Nothnick *et al.* 2010), who demonstrated that estrogen and progesterone significantly upregulate levels of Exportin-5 mRNA in mouse uterine tissue, an effect abrogated by addition of the anti-estrogen ICI182780. They found that levels of miR-451 were concurrently increased but did not demonstrate that these effects are specifically attributable to the effects of these steroid hormones on Exportin-5 expression, failing to exclude the possibility of estrogen-enhanced transcription of pri-miR-451 independent of Exportin-5. The authors also showed that P₄, but not E₂ increased Dicer expression (Nothnick *et al.* 2010). However, evidence for estrogen regulation of Dicer in human cells comes from Bhat-Nakshatri *et al.* and Lin *et al.*, who demonstrated Dicer protein levels are upregulated by estrogen in MCF7 breast cancer cells (Bhat-Nakshatri *et al.* 2009), with ER α binding to the enhancer region of the Dicer promoter (Lin *et al.* 2007).

In a further study, Cheng *et al.* evaluated expression of miR biogenesis-associated genes in ER-positive and ER-negative cell lines. They found that ER-positive lines had increased levels of TRBP and Dicer transcripts and decreased levels of AGO1 and AGO2 compared to ER-negative (Cheng *et al.* 2009). This is supported by Adams *et al.*, who showed reduced AGO2 expression in ER-positive breast cancer cell lines (Adams *et al.* 2009). It has also been demonstrated that Dicer is regulated by MAPK, and that phosphorylation of TRBP by this kinase pathway increases expression of the growth-promoting miRs 17 and 20 and inhibits maturation of the let-7 tumour suppressor in HeLa cells (Paroo *et al.* 2009). Since it is well established that ER α activates MAPK in several cell lines (Levin 2011), together these data highlight a putative indirect mechanism for

estrogen regulation of Dicer activity through hormone-stimulated MAPK pathway activation. Furthermore, the Dicer-targeting tumour-suppressive let-7, which reduces Dicer protein levels in several cell lines (Tokumaru *et al.* 2008), is downregulated by estrogen treatment (Qian *et al.* 2011), constituting a mechanism for indirect upregulation of Dicer activity by estrogen modulation of miRs. Further, Dicer mRNA is targeted by estrogen-regulated miR-29a, miR-125, let-7 and miR-21, and TRBP by let-7 (Cochrane *et al.* 2010; Shu *et al.* 2011; Tokumaru *et al.* 2008). It is clear from such studies (summarised in Table 2) that a pattern of regulation is increasingly observed whereby feedback loops involving steroid signalling permit maintenance of hormone-regulated miR biogenesis at appropriate levels.

A potential novel role for estrogen in modulating miR:3'UTR interaction

Finally, a novel mechanism has been proposed whereby, complementary to its role in modulating miR levels, ER α may modulate miR activity by altering availability of miR binding sites within target 3'UTRs. One report has demonstrated that ER α signalling can induce alternative polyadenylation and 3'UTR shortening of CDC6, which led to loss of miR binding sites for mRNA repression and increased CDC6 protein levels (Akman *et al.* 2012). This represents a possible additional mechanism for estrogen regulation of gene expression through modulating the potential for repressive miR:3'UTR interactions, however, additional independent studies are required to corroborate these observations, and a defined mechanism for this phenomenon remains to be elucidated. If confirmed, such a mechanism would allow ER α not only to regulate its target genes by altering miR-mediated repression, but also to regulate cellular availability of some miRs in a transcription-independent manner.

Targetting of ER pathway by MiRs - Feedback Circuits Involving Hormone-Regulated MiRs Modulate ER Expression and Activity

MiRs targetting ER (summarised in Table 3)

In 2007, Adams *et al.* demonstrated that miR-206 overexpression targets ER α mRNA and decreases ER α protein levels to reduce growth of breast cancer cells (Adams *et al.* 2007). The tumour suppressive let-7 family of miRs have also been demonstrated to repress ER α expression in MCF7 cells following their transient overexpression (Zhao *et al.* 2011). Leivonen *et al.* employed protein lysate microarray technology to identify ER α -targetting miRs following transfection of 319 pre-miRs into MCF7 and BT-474 cells. Twenty-one miRs were confirmed as downregulating ER α by Western blot and qPCR, and miRs 18a,

18b, 193b, 206 and 302c were confirmed to target ER α 3'UTR in reporter assays (Leivonen *et al.* 2009). Notably, clustering analysis of mRNA targets of these miRs demonstrated enrichment for estrogen-induced genes.

Other miRs identified as directly targeting ER α are miR-221 and miR-222 (Leivonen *et al.* 2009). Indeed, knockdown of either in ER α -negative MDA-MB-468 cells restores ER α expression, and initiates estrogen-responsiveness and anti-estrogen sensitivity (Zhao *et al.* 2008). Again, the authors propose that miR-221/222 also act by additionally targeting downstream components of the ER α signalling pathway. Interestingly, although miR-221/222 decrease ER α protein levels, proliferation of the transfected cells was accelerated through decreased expression of tumour suppressor proteins, thereby highlighting the importance of examining all miR targets and utilising pathway analysis tools to predict effects of miR manipulation (Di Leva *et al.* 2010; Rao *et al.* 2011). In the context of miR-221/222 targetting of ER α , it is interesting that several studies have demonstrated dramatic downregulation of these miRs upon estrogen treatment (Cochrane *et al.* 2010), and that ER α directly inhibits transcription of these miRs by recruiting the transcriptional corepressors, NCoR and SMRT to the defined miR-221/222 promoter (Di Leva *et al.* 2010). Together these data provide further strong evidence for autoregulation of ER α activity through direct downregulation of miRs that directly target its 3'UTR. Similarly, the ER α -targetting miRs 18a and 206 are estrogen-regulated, suggestive of a potential feedback loop to modulate ER α activity. Interestingly, whilst miR-18a is upregulated by estrogen stimulation of MCF7 cells (Castellano *et al.* 2009), miR-206, which also targets the ER α coactivators SRC1 and AIB1, is downregulated via inhibition of Drosha-mediated maturation (Adams *et al.* 2009; Adams *et al.* 2007). The opposite direction of regulation of these ER α -targetting miRs suggests a complex regulatory mechanism that permits subtle yet stringent control of ER α activity, allowing cells to adjust their transcriptional output to varied challenges.

Modulation of ER α Activity Regulators and Synthesis Proteins by MiRs

ER α -mediated gene transcription requires a plethora of accessory factors and comodulators in order to modify chromatin structure such that it is compatible with RNA polymerase binding, and there is increasing evidence that miRs can modulate ER α activity through targeting coregulators. For example, miR-17-5p reduced translation of AIB1 and reduced ER activity on transfected and endogenous target genes by up to 50% in CHO-K1 cells (Hossain *et al.* 2006). In addition, miR-206 was demonstrated to bind to the 3'UTRs of both SRC1, AIB1 and the transcription factor GATA-3 in MCF7 cells (Adams *et al.*

2009). This led to a significant reduction in ERE luciferase reporter activity and inhibition of ER α target gene transcripts, for example, PR (Adams *et al.* 2009). Further, levels of the ER α target gene and effector protein, cyclin D1, were reduced upon infection of MCF7 cells with a retroviral vector containing the miR-17~20 cluster, although effects of cluster expression on ER α activity were not examined (Yu *et al.* 2008). Additional studies have confirmed miR-22 repression of SRC1 through 3'UTR binding in Huh7 hepatocarcinoma cells, whilst miR-140-3p directly inhibits RIP-140 translation through 3'UTR seed region association (Takata *et al.* 2011).

Corepressors of ER α also display miR-mediated regulation. MiRs 10a/b and 284 have been shown to directly suppress SMRT expression through 3'UTR binding and translational inhibition in neuroblastoma and HeLa cells, respectively (Foley *et al.* 2011; Wu *et al.* 2011), although the impact of these miRs on ER α activity was not assessed. Steroid-regulated miR-27a provides a further example of a miR targeting downstream regulators of steroid signalling: miR-27a represses both the steroid receptor corepressor Prohibitin and also ZBTB10, a zinc finger gene which functions to repress Sp transcription factors that have pro-proliferative and angiogenic effects in breast cancer cells (Fletcher *et al.* 2012; He *et al.* 2008; Mertens-Talcott *et al.* 2007). Thus miR-27a is an indirect oncomiR by repressing transcription factor repressors. MiR-27a also indirectly activates ER α , as ER α expression and E2 response are dependent on Sp1, which is derepressed by miR-27a (Li *et al.* 2010). Interestingly, and in keeping with these findings, a single nucleotide polymorphism in the terminal loop of miR-27a that is predicted to inhibit pre-miR-27a processing is associated with reduced risk of familial premenopausal breast cancer (Yang *et al.* 2010). However, the effect of this mutation on the ability of miR-27a to indirectly increase ER α activity through repression of ZBTB10 remains to be evaluated.

It has also been postulated that miRs may modulate estrogen synthesis and release from cells, and there is some limited evidence to support this: transfection of a miR precursor library into primary human granulosa cells (the principal site of pre-menopausal estrogen production) identified 51 miRs that suppress release of estrogen, including miR-15a, miR-24, miR-125b, and members of the let-7 family. Interestingly, no miRs were found to increase estrogen release in this study (Sirotkin *et al.* 2009). Local production of estrogen in breast and other tissues is mediated by aromatase (encoded by *CYP1B1*) and 17 β -hydroxysteroid dehydrogenase. It has been shown that the *CYP1B1* 3'UTR contains putative miR-27b binding sites, and that exogenous miR-27b reduced *CYP1B1* levels and enzymatic activity, thus presumably decreasing local estrogen production (Nakajima and Yokoi 2011; Tsuchiya *et al.* 2006). Of note, miR-27b is widely reported as estrogen-

regulated, although studies are conflicting in the directionality of such regulation (Bhat-Nakshatri et al. 2009; Cochrane et al. 2010; Klinge 2009; Maillot et al. 2009; Mattie *et al.* 2006). It is possible then that this represents a feedback mechanism whereby an ER α -regulated miR can feedback to estrogen synthesis enzymes so indirectly regulate ER activity. Another miR shown to modulate estrogen synthesis is miR-133b, which increases estrogen synthesis in the ovaries by targeting Foxl2, a negative regulator of the steroidogenic enzymes, Steroidogenic Acute Regulation Protein (StAR) and CYP19A1. MiR-133b was shown to associate with Foxl2 3'UTR, reducing Foxl2 protein levels and its binding to StAR and CYP19A1 promoter sequences in granulosa cells, thereby increasing estrogen synthesis and secretion (Dai *et al.* 2013). It is likely that further miRs will emerge as regulating hormone synthesis enzymes, and given the clinical importance of intratumoural steroid synthesis for both breast and prostate cancer it will be of major interest to determine if these are dysregulated in both tumour types and whether they are regulated by steroid signalling themselves.

Androgen Regulation of MicroRNA Biogenesis

Androgens have been demonstrated to alter miR biogenesis in a range of cell lines and *in vivo* models. ChIP-seq data has provided evidence for association of AR with the promoters of a number of miR genes and AR, like ER, demonstrates autoregulation through miRs. A number of studies have described roles for miRs both as mediators or effectors of AR action and regulators of AR activity, discussed below.

Androgen Regulation of MiR Expression

Androgen-responsive tissues express distinct sets of miRs as demonstrated by castration of Sprague-Dawley rats, which resulted in a dramatic reduction in a large subset of miRs in the prostate compared to intact animals, whilst androgen treatment of castrated animals increased miR expression in a tissue-specific manner (Narayanan *et al.* 2010). The miR profile was not altered in the liver upon androgen treatment, but the levator ani muscle (which has an anabolic response to androgens) demonstrated an upregulated miR profile that differed significantly from that of the prostate, preferentially targeting components of the Raf/MEK/ERK signalling pathway (Narayanan et al. 2010). Interestingly, Dicer siRNA completely inhibited DHT induction of the well-characterised androgen responsive gene *PSA* in LNCaP cells, and Dicer^{-/-} mice developed androgen-insensitivity syndrome, indicating that androgen-regulated miRs are vital mediators of AR action *in vivo*

(Narayanan et al. 2010). It was also hypothesised that reduced AR function upon loss of androgen-stimulated miR synthesis may be a result of androgen-regulated miR targeting of AR corepressors. Consistent with this, 75% of predicted NCoR or SMRT-targeting miRs were upregulated in the prostate by DHT, and Dicer siRNA treatment increased transcription of both NCoR and SMRT (Narayanan et al. 2010). Taken together, these innovative data identify androgen-responsive tissue-specific miR profiles, and confirm androgen-upregulated miRs as vital mediators and enhancers of AR signalling through corepressor targeting (see also below).

Takayama *et al.* were amongst the first to describe AR association with miR promoters in prostate cancer cells through integration of 5' Cap analysis of gene expression (CAGE), to identify androgen-regulated transcription start sites, with ChIP on array (ChIP-chip) to identify AR binding sites throughout the human genome. In addition to protein-coding genes, miR-222, miR-221, miR-21, miR-125b, miR-218-1, miR-218-2, let-7c and miR-100 were found to be located adjacent to AR binding sites (Takayama *et al.* 2011). In corroboration, Ribas *et al.* demonstrated androgen-induced AR binding to the miR-21 cluster promoter, which resulted in increased levels of oncomiR-21. This increased androgen-dependent growth and induced androgen-independent growth of LNCaP and LAPC-4 cells (Ribas *et al.* 2009). It should be noted, however, that the CAGE-with-ChIP-chip approach favoured by Takayama *et al.* may not identify all miRs regulated by AR binding to promoter AREs, since the authors only assessed one time point of androgen treatment, thus possibly overlooking earlier or later androgen binding events.

In one of the first miR microarray studies to examine androgen regulation of miRs, Waltering *et al.* showed that seventeen miRs were significantly modulated by DHT treatment in VCaP cells (Waltering *et al.* 2011), at least half of which were confirmed as androgen-regulated in subsequent studies (see Table 4). Later, Mo *et al.* combined time-course microarrays with bioinformatics to identify androgen-regulated miRs and their targets in androgen-stimulated LNCaP cells, focussing on those that showed an early and enduring response to androgen, a subgroup they term 'primary targets' (Mo *et al.* 2013). Amongst 137 miRs primary targets, 22 miRs had existing well-documented roles in prostate cancer (Bonci *et al.* 2008; Ozen et al. 2008; Rokhlin *et al.* 2008; Tong *et al.* 2008). Of these, miR-101, miR-145, miR-34a, miR-182, miR-375, miR-181a, miR-92b and miR-125 were androgen-upregulated, whilst miR-16, miR-126*, miR-23b, miR-100, miR-222, miR-133a, miR-499, and miR-340 were downregulated by androgen, corroborating previous demonstrations of their androgen regulation and altered expression in prostate cancer (Ambs *et al.* 2008 ; Bonci et al. 2008; Tong et al. 2008). Two additional primary

569 targets, miR-19a and miR-27a, showed significant enrichment for roles in key cellular
570 processes upon pathway analysis. Concordant with this, ChIP analysis confirmed AR
571 binding to putative AREs within the promoter regions of these miRs (Mo *et al.* 2013).
572 These data are consistent with our earlier findings, in which we also demonstrated AR
573 binding to the miR-27a promoter after androgen stimulation (Fletcher *et al.* 2012). Similar
574 extents of miR-27a upregulation were observed in the two studies, thereby supporting AR-
575 association with the miR-27a promoter as a *bona fide* physiological event. Pathway
576 analysis of predicted target sets of these miRs showed enrichment for proteins with
577 previously described roles in prostate cancer. For example ABCA1, targeted by miR-27a,
578 inhibits proliferation of prostate cancer cells (Fukuchi *et al.* 2004; Yang *et al.* 2012) whilst
579 RAB13, a GTPase that positively regulates epithelial cell tight junction formation (Kanda *et al.*
580 *et al.* 2007), is a target of miR-19a.

581 Shi *et al.* have demonstrated that miR-125b is significantly transcriptionally
582 upregulated by androgen treatment in LNCaP cells, and its transient transfection increases
583 prostate cancer cell growth whilst reducing Bax1 expression and 3'UTR activity (Shi *et al.*
584 2007). Given that a subset of estrogen-upregulated miRs with putative oncogenic function
585 were demonstrated to target this tumour suppressor in estrogen-treated endometrial
586 carcinoma cells to positively reinforce ER α activity (Zhang *et al.* 2012), it is very interesting
587 that the same phenomenon and mechanism of action was observed for an androgen-
588 regulated miR in prostate cancer (Shi *et al.* 2007).

590 *Positive Feedback Regulation of AR through Androgen-Upregulated MiRs*

591 Several studies have now identified mechanisms whereby AR exploits miRs to maintain
592 high levels of its own expression and activity. Mishra *et al.* demonstrated that AR inhibition
593 reduced levels of miR-21 in AR-positive prostate cancer cells, whilst AR overexpression
594 increases it. Interestingly, miR-21 mimic increased AR mRNA levels, restored AR protein
595 to AR-negative PC3 cells, and enhanced PSA promoter reporter activity. This therefore
596 represents a positive feedback loop whereby androgen-regulated miR-21 increases AR
597 levels to further increase miR-21, which targets several tumour suppressors. The
598 mechanism by which miR-21 increases AR activity was not addressed, although it was
599 hypothesised this may involve miR-21 targetting PTEN, which inhibits AR nuclear
600 translocation and promotes degradation of AR in several cancers (Lin *et al.* 2004;
601 Mulholland *et al.* 2006). Our own studies demonstrated a similar positive feedback loop:
602 androgen-upregulated miR-27a increases AR activity by targeting the AR corepressor and
603 tumour suppressor, PHB, in prostate cancer cells (Fletcher *et al.* 2012). These data

combined suggest that androgen upregulation of miRs that target tumour suppressors and corepressors of AR transcriptional activity to positively reinforce AR signalling may be commonplace in androgen-responsive tissues. Such mechanisms could potentially play a vital role in increasing AR activity in the absence of circulating androgens in cases of advanced, castrate-resistant prostate cancer (CRPC), particularly if such miRs are overexpressed in cancerous tissue, as has been demonstrated for miR-21 (Dart *et al.* 2011; Krichevsky and Gabriely 2009).

Androgen Regulation of Protein Components of the MiR Biogenesis Pathway

Regulation of one or more components of the miR processing complexes is a possible mechanism for androgen regulation of miR biogenesis. For example, Dicer and DGCR8 are upregulated by DHT treatment of LNCaP cells (Mo *et al.* 2013), consistent with the observed increase in Dicer protein levels in prostate cancer (Chiosea *et al.* 2006). In addition, AR was shown to accelerate miR biogenesis through increasing Drosha cleavage of pri-miR species, although the mechanism of androgen-increased Drosha activity remains to be described (Fletcher *et al.* 2012).

Given the general similarities between AR and ER α signalling and since ER α modulates miR biogenesis via Drosha, it may be logical that AR also regulates Drosha activity. Indeed, we demonstrated that androgens increase Drosha reporter activity towards pri-miRs (Fletcher *et al.* 2012). However this effect is opposite to the inhibition of Drosha cleavage by ER α . It may be that both ER α and AR can act to regulate Drosha and Dicer activities, exerting distinct and opposing effects in the nucleus and the cytoplasm to control hormone-stimulated miR biogenesis in different tissues and developmental stages. It may be hypothesised that these regulatory mechanisms are perturbed in cancer, since altered expression of Drosha or Dicer has been reported in a number of cancers (Chiosea *et al.* 2006; Chiosea *et al.* 2007; Faber *et al.* 2011; Merritt *et al.* 2008; Passon *et al.* 2012).

There is also circumstantial evidence for links between androgen signalling and miR processing pathways. The Microprocessor component, p68, was identified as a novel AR-interacting protein in a yeast two-hybrid screen (Clark *et al.* 2008) and demonstrated to be recruited to AREs upon ligand treatment and to act as an AR coactivator in LNCaP cells. Interestingly, mutation of Y⁵⁹³ (a putative phosphorylation site) in p68 abolished AR coactivation, indicating that post-translational modifications of p68 may be vital for this role (Clark *et al.* 2008), although effects of this mutation on RNA helicase activity were not investigated. The above study also found increased p68 immunohistochemical staining in a cohort of prostate cancer patients compared to benign hyperplasia patients (Clark *et al.*

2008). These data potentially support a link between miR processing and AR-mediated transcription, and also suggest that p68-dependent miR processing could be altered in prostate cancer.

MicroRNA regulation of androgen signalling

MiRs Targetting the Androgen Receptor (Summarised in Table 5)

It is well established that miRs can directly regulate AR signalling through targeting the AR 3'UTR. In one study, Östling *et al.* coupled protein lysate microarrays in LNCaP, LAPC-4, CWR-R1, 22Rv1 and MDA-PCa-2b cells with miR microarray expression analysis in thirteen prostate cell lines to quantify miR-mediated changes to AR protein content in prostate cancer (Östling *et al.* 2011). Seventy-one miRs that altered AR protein levels in all five cell lines were selected for validation, with 52 decreasing and 19 increasing AR protein levels, and 21 AR-targetting miRs were shown to reduce both AR protein levels and androgen stimulated growth upon transient transfection of the miR mimics into LNCaP and 22Rv-1 cells, and MDA-PCa-2b cells respectively. Thirteen of these were then further validated as significantly reducing AR 3'UTR activity: miR-135b, miR-185, miR-297, miR-299-3p, miR-34a, miR-34c, miR-371-3p, miR-421, miR-449a, miR-449b, miR-634, miR-654-5p and miR-9 (Östling *et al.* 2011). Targetting of AR by miR-185 is consistent with the findings of Qu *et al.*, who demonstrated that miR-185 significantly reduces levels of AR protein, but not mRNA, in LNCaP cells, with concomitant decreases in LNCaP cell proliferation and xenograft tumourigenicity upon miR-185 overexpression (Qu *et al.* 2013). Further, targeting of AR by miR-34a is in keeping with data from Kashat *et al.*: in an observational study showing that miR-34a reduced protein and mRNA levels of AR in C4-2B and CWR22Rv-1 cells (Kashat *et al.* 2013).

Other miRs are also reported to target AR directly. Hagman *et al.* demonstrated that ectopic miR-205 overexpression significantly decreased AR protein levels and activity of an AR 3'UTR reporter in VCaP cells, although this was not recapitulated in LNCaP cells (Hagman *et al.* 2013). However, as levels of miR-205 were low in all but one of seven prostate cancer cell lines, the physiological relevance of miR-205 targeting of AR remains to be ascertained. Similarly, AR levels were decreased both at the RNA and protein level following ectopic expression of miR-488* in androgen-dependent and -independent prostate cancer cells, and both AR transcriptional activity and AR 3'UTR reporter activity were also reduced (Sikand *et al.* 2011b).

A potential novel role mechanism for regulation of AR involving miRs involves shortening of the 3'UTR. Recent studies report shortening of 3'UTRs in cancer cells, leading to

enhanced oncogene activation. Indeed, mRNAs with shorter 3'UTRs produced ten-fold more protein, in part due to loss of miR-mediated repression (Mayr and Bartel 2009). In this context, it is interesting that the AR 3'UTR length in several PCa cell lines was found to be approximately 6680bp; far greater than the sequence length of 436bp specified by the AR RefSeq file (NM_0000444) and used by target prediction algorithms. This dramatically increases the potential for direct miR regulation of the AR transcript, although it should be noted that there is evidence to suggest that the extent of regulation of a transcript by a miR is generally greater closer to the 5' end of the 3'UTR (Bartel 2009; Grimson *et al.* 2007). It has been proposed that the AR 3'UTR can function as dynamic entity, and active 3'UTR shortening in response to environmental stimuli may represent a further mechanism of PCa progression (Sikand *et al.* 2011a), permitting AR to evade regulation by tumour suppressive miRs, such as long AR 3'UTR-binding miR-34a and miR-34c (Östling *et al.* 2011), which are proposed potent mediators of p53 tumour suppression. However, very limited information is currently available on miR targeting of the relatively poorly characterised longer AR 3'UTR isoforms. In fact, there remains no definitive evidence for reduced miR binding and consequently higher AR protein levels following AR 3'UTR shortening. Further studies are required to compare AR 3'UTR length in malignant vs benign prostate tissue, and in hormone-naïve PCa vs. CRPC, to assess its potential role in cancer progression.

Inhibition of AR Activity by Tumour Suppressive MiRs

Let-7 expression is frequently decreased, or the gene deleted, in human cancers (Calin *et al.* 2004; Jiang *et al.* 2005; Ozen *et al.* 2008). Its targets include the oncogenes Ras and Myc (Johnson *et al.* 2005; Kumar *et al.* 2007) and it has thus been proposed as a tumour suppressor miR. This tumour suppressor capacity may be achieved in prostate cancer through decreased AR activity, since let-7c was demonstrated to target c-Myc, which is required for transcription of AR (Nadiminty *et al.* 2012). It was found that let-7c reduced AR activity and decreased growth of C4-22B cells; attributable to let-7c association with c-Myc 3'UTR leading to a loss of c-Myc-mediated AR transcription (Nadiminty *et al.* 2012).

In 2009, Epis *et al.* investigated regulation of AR activity by miR-331-3p (Epis *et al.* 2009). It was found that miR-331-3p reduces AR activity in LNCaP cells through targeting the oncogene ERBB2, since AR 3'UTR lacks miR-331-3p binding sites and ERBB2 can activate transcription of AR target genes in the absence of androgen (Craft *et al.* 1999; Mellinghoff *et al.* 2004). ERBB2 was demonstrated to be a direct target of miR-331-3p through 3'UTR reporter assays and protein expression studies in multiple prostate cancer

cell lines. The above theory of indirect miR targeting of AR via ERBB2 is supported by a decrease in PSA promoter activity upon miR-331-3p ectopic expression (Epis et al. 2009).

Such tumour-suppressive, AR-inhibitory miRs could be exploited as potential therapeutics for prostate cancer, either alone or in combination with anti-androgens.

Nuclear RISC Components as Coactivators of ER α and AR

Further evidence for cooperation and functional interaction between the miR biogenesis cascade and steroid signalling pathways has come from the recent study of Redfern *et al.*, which demonstrated that components of the RISC complex, Dicer, PACT and TRBP, bind to an RNA transcript that functions as a coactivator of nuclear receptor (NR) activity, called Steroid Receptor RNA Activator (SRA) (Hube *et al.* 2006; Lanz *et al.* 2002 ; Redfern *et al.* 2013). PACT, TRBP and Dicer increased SRA-enhanced ER α and AR reporter activity, and were recruited to AR target gene promoters, whilst siRNA-mediated depletion of PACT and TRBP reduced expression of AR and ER α target genes (Redfern et al. 2013). Interestingly, RISC protein-bound pre-miRs were identified in the nucleus as well as the cytoplasm, suggesting existence of a nuclear RISC complex in which SRA functions as a scaffold for PACT, TRBP and Dicer (Redfern et al. 2013), raising the possibility of coupling pre-miR processing and NR coregulation of transcription within a single nuclear complex. It is also possible that SRA could sequester RISC components away from their pre-miR substrates to regulate RISC activity. It is feasible that other proteins may fulfil similar roles to SRA in coupling NR transcriptional activity with Drosha Microprocessor-mediated pri-miR cleavage, since both FUS, a microprocessor component that stimulates pri-miR processing (Morlando *et al.* 2012), and p68, known to promote cleavage of a subset of pri-miRs (Fukuda et al. 2007; Kawai and Amano 2012; Salzman et al. 2007; Samaan *et al.* 2013) are known AR cofactors (Brooke *et al.* 2011; Clark et al. 2008; Haile *et al.* 2011).

Conclusions and Summary

The multiple steps of the miR biogenesis cascade provide a plethora of opportunities for hormone regulation of miR synthesis, adding further complexity to allow the most stringent regulation and fine-tuning of a pathway with vital roles in almost all physiological processes, and dysregulation of which has undesirable consequences for the pathogenesis of human cancers. It is clear that distinct subsets of miRs are regulated by estrogen and androgen signalling in responsive tissues, but that these hormones share common mechanisms for regulating activity of miR biogenesis pathway components, altering transcription of miRs, and exploiting target miRs to reinforce their own activity.

From *in vivo* studies, we find evidence that miRs are vital effectors of AR and ER α signalling pathways, and conversely, it is increasingly apparent that multiple miRs can directly target the 3'UTRs of ER α and AR to regulate hormone signalling. Furthermore, NRs preferentially regulate miRs that target their own effectors and cofactors, resulting in feedback that may amplify or restrict their signalling. In addition, several fascinating emerging theories highlighted here may yet have sizeable implications for the bi-directional regulation of miR biogenesis and hormone signalling. We should also remember that miRs themselves have been postulated to function similarly to hormones, influencing gene expression, microenvironment and disease pathogenesis at distant sites, and potentially functioning as readily assayable biomarkers. The interplay between hormone signalling and miR biogenesis, and further characterisation of its components, provides significant opportunity for increased understanding of steroid-related cancer progression and the potential for exploitation of such interactions for the design of novel cancer therapeutics.

Disclosure

The authors have nothing to disclose.

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Figure Legends

Figure 1: Regulators of MicroRNA Biogenesis. Schematic illustration of proteins that modulate miR maturation through multiple mechanisms. MiRs regulated by a particular factor are displayed beside that protein. miR = microRNA, O-methyl = O-methylation, A→I = adenosine to inosine, ADAR = deaminase acting on RNA, pri-miR = primary microRNA, pre-miR = precursor microRNA, ERα = estrogen receptor alpha, hnRNPA1 = heterogeneous nuclear ribonucleoprotein A1, KHSRP1 = KH-type splicing regulatory protein 1, ARS2 = arsenite-resistance protein 2, AR = androgen receptor, BRCA1 = breast cancer type 1 susceptibility protein, TDP-43 = TAR DNA-binding protein 43, DGCR8 = DiGeorge Critical Region 8, TRIM-32 = Tripartite motif-containing protein 32, NHL2 = NDR1/HIN1-Like protein 2, XRN-2 = 5'-3' exoribonuclease 2, GLD2 = defective in Germ Line Development 2, AGO2 = Argonaute 2, TUT4 = Terminal uridylyltransferase 4, TRBP = HIV-1 TAR RNA binding protein, BCDIN3D = BCDIN3 domain-containing protein, MCP1P1 = monocyte chemotactic protein- induced protein 1.

Figure 2: Mutual Regulation by Steroid Hormone Receptors and MicroRNAs. A schematic representation of the mechanisms by which steroid hormone receptors (SHRs) and microRNAs (miRs) mutually regulate each other's activity through feedback loops, permitting a myriad of complex and subtle responses to stimuli. 1) ERα represses miR-206, miR-221 and miR-222, which directly target ERα, thereby reducing levels of ERα inhibitory miRs. 2) ERα represses miR-26a, miR-26b, miR-181a, miR-181b, miR-23a and miR-23b, which target the ERα effector and growth-stimulatory protein, progesterone receptor (PR) to inhibit cell growth and proliferation. 3) A negative feedback loop whereby ERα upregulates c-Myc, a transcription factor required for expression of the miR-17~92 cluster, whose component miRs directly target ERα. This estrogen upregulated cluster also targets the ERα coactivator, AIB1. 4) MiR-18a, miR-18b, miR-193b and miR-302c represses ERα protein translation through direct association with its 3'UTR. 5) ERα has been demonstrated to directly upregulate miR-20a, miR-19a/b, miR-92a-1, miR-21, let-7a, let-7g, miR-203, miR-200a, miR-30b and miR-182, amongst others. 6) ERα represses Drosha cleavage of a subset of pri-miRs through association with p68/p72 to promote Drosha dissociation from the Microprocessor. 7) MiR-140-3p and miR-22 indirectly repress ERα transcriptional activity by targeting the ERα coactivators, RIP-140 and SRC-1, respectively. 8) MiR-27a targets ZBTB10, an inhibitor of Sp transcription factors that

function to enhance ER α transcriptional activity. By repressing an inhibitor of ER α , miR-27a indirectly increases ER α activity. 9) MiR-27b targets CYP1B1, an enzyme responsible for estrogen synthesis, thereby preventing ER α activation. 10) PR inhibits miR-633, miR-29c, miR-29*, miR-193b and miR-29, which targets the PR effector protein, ATP1B1. 11) PR upregulates miR-625*, miR-21, miR-142-5p, miR-146b-5p and miR-513a-5p, which targets PR in a negative feedback loop. 12) AR transcriptionally upregulates miR-141, miR-17-5p, miR-22, miR-27a, miR-29a, miR-29b, miR-32, miR-101, miR-125b, miR-148a, miR-594 and others. 13) MiR-331-3p indirectly reduces AR activity by targeting ERBB2, which can activate transcription of AR target genes in the absence of androgens. 14) AR indirectly maintains its own activity in positive feedback loop whereby AR induces transcription of miR-21, which targets the 3'UTR of PTEN (known to inhibit AR nuclear translocation and promotes its degradation). Androgen-upregulated miR-21 also targets the tumour suppressor, TGFBR2, to stimulate growth. 15) AR directly represses transcription of miR-221 and miR-126*. 16) MiR-205, miR-185, miR-34a/b, miR-135b, miR-297, miR-299-3p, miR-371-3p, miR-421, miR-449a/b, miR-634, miR-654-5p and miR-9 directly repress AR through binding to its 3'UTR. 17) AR indirectly increases its own activity by enhancing transcription of miR-27a, which targets the AR corepressor, PHB. AR also inhibits PHB transcription. 18) Let-7 indirectly inhibits AR by targeting the AR transcription factor, c-Myc. CYP1B1 = cytochrome P450 family 1B1, E₂ = Estradiol, Sp = specificity protein, ZBTB10 = zinc finger and BTB domain containing 10, RIP-140 = receptor interacting protein 140, SRC-1 = steroid receptor coactivator 1, ER α = estrogen receptor alpha, AIB1 = Amplified in breast cancer 1, PR = progesterone receptor, ATP1B1 = ATPase Na⁺/K⁺ transporting beta 1, AR = androgen receptor, ERBB2 = v-erb-b2 erythroblastic leukemia viral oncogene homologue 2, TGFBR2 = transforming growth factor beta receptor 2, PTEN = phosphatase and tensin homologue, PHB = prohibitin.

References

- Adams BD, Cowee DM & White BA 2009 The Role of miR-206 in the Epidermal Growth Factor (EGF) Induced Repression of Estrogen Receptor- α (ER α) Signaling and a Luminal Phenotype in MCF-7 Breast Cancer Cells. *Molecular Endocrinology* **23** 1215-1230.
- Adams BD, Furneaux H & White BA 2007 The Micro-Ribonucleic Acid (miRNA) miR-206 Targets the Human Estrogen Receptor- α (ER α) and Represses ER α Messenger RNA and Protein Expression in Breast Cancer Cell Lines. *Molecular Endocrinology* **21** 1132-1147.
- Akman BH, Can T & Erson-Bensan AE 2012 Estrogen-induced upregulation and 3'-UTR shortening of CDC6. *Nucleic Acids Research* **40** 10679-10688.
- Ambs S, Prueitt RL, Yi M, Hudson RS, Howe TM, Petrocca F, Wallace TA, Liu C-G, Volinia S, Calin GA, *et al.* 2008 Genomic Profiling of MicroRNA and Messenger RNA Reveals Deregulated MicroRNA Expression in Prostate Cancer *Cancer Research* **68** 6162-6170
- Bartel DP 2009 MicroRNAs: Target Recognition and Regulatory Functions. *Cell* **136** 215-233.
- Bhat-Nakshatri P, Wang G, Appaiah H, Luktuke N, Carroll JS, Geistlinger TR, Brown M, Badve S, Liu Y & Nakshatri H 2008 AKT Alters Genome-Wide Estrogen Receptor α Binding and Impacts Estrogen Signaling in Breast Cancer. *Molecular and Cellular Biology* **28** 7487-7503.
- Bhat-Nakshatri P, Wang G, Collins NR, Thomson MJ, Geistlinger TR, Carroll JS, Brown M, Hammond S, Srour EF, Liu Y, *et al.* 2009 Estradiol-regulated microRNAs control estradiol response in breast cancer cells. *Nucleic Acids Research* **37** 4850-4861.
- Bonci D, Coppola V, Musumeci M, Addario A, Giuffrida R, Memeo L, D'Urso L, Pagliuca A, Biffoni M, Labbaye C, *et al.* 2008 The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. *Nat Med* **14** 1271-1277.
- Brooke GN, Culley RL, Dart DA, Mann DJ, Gaughan L, McCracken SR, Robson CN, Spencer-Dene B, Gamble SC, Powell SM, *et al.* 2011 FUS/TLS Is a Novel Mediator of Androgen-Dependent Cell-Cycle Progression and Prostate Cancer Growth. *Cancer Research* **71** 914-924.
- Brooke GN, Gamble SG, Begum S, Dart DA, Odontiadis M, Powell SM, Fioretti FM, Bryan RA, Waxman J, Wait R, *et al.* 2014 Antiandrogens act as selective androgen receptor modulators at the proteome level in prostate cancer cells. *In press*.

- 862 Calin GA, Sevignani C, Dumitru CD, Hyslop T, Noch E, Yendamuri S, Shimizu M, Rattan
863 S, Bullrich F, Negrini M, *et al.* 2004 Human microRNA genes are frequently located at
864 fragile sites and genomic regions involved in cancers. . *Proceedings of the National*
865 *Academy of Sciences of the United States of America* **101** 2999-3004
- 866 Castellano L, Giamas G, Jacob J, Coombes RC, Lucchesi W, Thiruchelvam P, Barton G,
867 Jiao LR, Wait R, Waxman J, *et al.* 2009 The estrogen receptor- α -induced microRNA
868 signature regulates itself and its transcriptional response. *Proceedings of the National*
869 *Academy of Sciences* **106** 15732-15737.
- 870 Cheng C, Fu X, Alves P & Gerstein M 2009 mRNA expression profiles show differential
871 regulatory effects of microRNAs between estrogen receptor-positive and estrogen
872 receptor-negative breast cancer. *Genome Biology* **10** R90.
- 873 Chiosea S, Jelezcova E, Chandran U, Acquafondata M, McHale T, Sobol RW & Dhir R
874 2006 Up-Regulation of Dicer, a Component of the MicroRNA Machinery, in Prostate
875 Adenocarcinoma. *The American Journal of Pathology* **169** 1812-1820.
- 876 Chiosea S, Jelezcova E, Chandran U, Luo J, Mantha G, Sobol RW & Dacic S 2007
877 Overexpression of Dicer in Precursor Lesions of Lung Adenocarcinoma. *Cancer Research*
878 **67** 2345-2350.
- 879 Cicatiello L, Mutarelli M, Grober OMV, Paris O, Ferraro L, Ravo M, Tarallo R, Luo S,
880 Schroth GP, Seifert M, *et al.* 2010 Estrogen Receptor α Controls a Gene Network in
881 Luminal-Like Breast Cancer Cells Comprising Multiple Transcription Factors and
882 MicroRNAs. *The American Journal of Pathology* **176** 2113-2130.
- 883 Clark EL, Coulson A, Dalglish C, Rajan P, Nicol SM, Fleming S, Heer R, Gaughan L,
884 Leung HY, Elliott DJ, *et al.* 2008 The RNA Helicase p68 Is a Novel Androgen Receptor
885 Coactivator Involved in Splicing and Is Overexpressed in Prostate Cancer. *Cancer*
886 *Research* **68** 7938-7946.
- 887 Cochrane D, Cittelly D, Howe E, Spoelstra N, McKinsey E, LaPara K, Elias A, Yee D &
888 Richer J 2010 MicroRNAs Link Estrogen Receptor Alpha Status and Dicer Levels in Breast
889 Cancer. *Hormones and Cancer* **1** 306-319.
- 890 Cohen A, Shmoish M, Levi L, Cheruti U, Levavi-Sivan B & Lubzens E 2008 Alterations in
891 Micro-Ribonucleic Acid Expression Profiles Reveal a Novel Pathway for Estrogen
892 Regulation. *Endocrinology* **149** 1687-1696.
- 893 Craft N, Shostak Y, Carey M & Sawyers CL 1999 A mechanism for hormone-independent
894 prostate cancer through modulation of androgen receptor signaling by the HER-2/neu
895 tyrosine kinase. *Nat Med* **5** 280-285.

- 896 Dai A, Sun H, Fang T, Zhang Q, Wu S, Jiang Y, Ding L, Yan G & Hu Y 2013 MicroRNA-
897 133b stimulates ovarian estradiol synthesis by targeting Foxl2. *FEBS Letters* **587** 2474-
898 2482.
- 899 Dai R, Phillips RA, Zhang Y, Khan D, Crasta O & Ahmed SA 2008 Suppression of LPS-
900 induced Interferon- γ and nitric oxide in splenic lymphocytes by select estrogen-regulated
901 microRNAs: a novel mechanism of immune modulation. *Blood* **112** 4591-4597.
- 902 Dart DA, Brooke GN, Sita-Lumsden A, Waxman J & Bevan CL 2011 Reducing prohibitin
903 increases histone acetylation, and promotes androgen independence in prostate tumours
904 by increasing androgen receptor activation by adrenal androgens. *Oncogene*.
- 905 Davis BN, Hilyard AC, Lagna G & Hata A 2008 SMAD proteins control DROSHA-mediated
906 microRNA maturation. *Nature* **454** 56-61.
- 907 Di Leva G, Gasparini P, Piovan C, Ngankeu A, Garofalo M, Taccioli C, Iorio MV, Li M,
908 Volinia S, Alder H, *et al.* 2010 MicroRNA Cluster 221-222 and Estrogen Receptor α
909 Interactions in Breast Cancer. *Journal of the National Cancer Institute* **102** 706-721.
- 910 Di Leva G, Piovan C, Gasparini P, Ngankeu A, Taccioli C, Briskin D, Cheung DG, Bolon B,
911 Anderlucci L, Alder H, *et al.* 2013 Estrogen Mediated-Activation of miR-191/425 Cluster
912 Modulates Tumorigenicity of Breast Cancer Cells Depending on Estrogen Receptor
913 Status. *PLoS Genet* **9** e1003311.
- 914 Epis MR, Giles KM, Barker A, Kendrick TS & Leedman PJ 2009 miR-331-3p Regulates
915 ERBB-2 Expression and Androgen Receptor Signaling in Prostate Cancer. *Journal of*
916 *Biological Chemistry* **284** 24696-24704.
- 917 Faber C, Horst D, Hlubek F & Kirchner T 2011 Overexpression of Dicer predicts poor
918 survival in colorectal cancer. *European journal of cancer (Oxford, England : 1990)* **47**
919 1414-1419.
- 920 Ferraro L, Ravo M, Nassa G, Tarallo R, Filippo M, Giurato G, Cirillo F, Stellato C, Silvestro
921 S, Cantarella C, *et al.* 2012 Effects of Oestrogen on MicroRNA Expression in Hormone-
922 Responsive Breast Cancer Cells. *Hormones and Cancer* **3** 65-78.
- 923 Fletcher CE, Dart DA, Sita-Lumsden A, Cheng H, Rennie PS & Bevan CL 2012 Androgen-
924 regulated processing of the oncomir MiR-27a, which targets Prohibitin in prostate cancer.
925 *Human Molecular Genetics* **21** 3112-3127.
- 926 Foley N, Bray I, Watters K, Das S, Bryan K, Bernas T, Prehn J & Stallings R 2011
927 MicroRNAs 10a and 10b are potent inducers of neuroblastoma cell differentiation through
928 targeting of nuclear receptor corepressor 2. *Cell Death and Differentiation* **18** 1089-1098.

- 929 Fukuchi J, Hiipakka RA, Kokontis JM, Hsu S, Ko AL, Fitzgerald ML & Liao S 2004
930 Androgenic Suppression of ATP-binding Cassette Transporter A1 Expression in LNCaP
931 Human Prostate Cancer Cells. *Cancer Research* **64** 7682-7685.
- 932 Fukuda T, Yamagata K, Fujiyama S, Matsumoto T, Koshida I, Yoshimura K, Mihara M,
933 Naitou M, Endoh H, Nakamura T, *et al.* 2007 DEAD-box RNA helicase subunits of the
934 Drosha complex are required for processing of rRNA and a subset of microRNAs. *Nat Cell*
935 *Biol* **9** 604-611.
- 936 Gherzi R, Lee K-Y, Briata P, Wegmüller D, Moroni C, Karin M & Chen C-Y 2004 A KH
937 Domain RNA Binding Protein, KSRP, Promotes ARE-Directed mRNA Turnover by
938 Recruiting the Degradation Machinery. *Molecular Cell* **14** 571-583.
- 939 Gregory RI, Yan K-p, Amuthan G, Chendrimada T, Doratotaj B, Cooch N & Shiekhattar R
940 2004 The Microprocessor complex mediates the genesis of microRNAs. *Nature* **432** 235-
941 240.
- 942 Grimson A, Farh KK-H, Johnston WK, Garrett-Engle P, Lim LP & Bartel DP 2007
943 MicroRNA Targeting Specificity in Mammals: Determinants beyond Seed Pairing.
944 *Molecular Cell* **27** 91-105.
- 945 Guil S & Caceres JF 2007 The multifunctional RNA-binding protein hnRNP A1 is required
946 for processing of miR-18a. *Nat Struct Mol Biol* **14** 591-596.
- 947 Hagman Z, Hafliadottir BS, Ceder JA, Larne O, Bjartell A, Lilja H, Edsjo A & Ceder Y
948 2013 miR-205 negatively regulates the androgen receptor and is associated with adverse
949 outcome of prostate cancer patients. *Br J Cancer* **108** 1668-1676.
- 950 Hah N, Danko Charles G, Core L, Waterfall Joshua J, Siepel A, Lis John T & Kraus WL
951 2011 A Rapid, Extensive, and Transient Transcriptional Response to Estrogen Signaling in
952 Breast Cancer Cells. *Cell* **145** 622-634.
- 953 Haile S, Lal A, Myung J-K & Sadar MD 2011 FUS/TLS Is a Co-Activator of Androgen
954 Receptor in Prostate Cancer Cells. *PLoS ONE* **6** e24197.
- 955 Han J, Pedersen JS, Kwon SC, Belair CD, Kim Y-K, Yeom K-H, Yang W-Y, Haussler D,
956 Blelloch R & Kim VN 2009 Posttranscriptional Crossregulation between Drosha and
957 DGCR8. *Cell* **136** 75-84.
- 958 He B, Feng Q, Mukherjee A, Lonard DM, DeMayo FJ, Katzenellenbogen BS, Lydon JP &
959 O'Malley BW 2008 A Repressive Role for Prohibitin in Estrogen Signaling. *Molecular*
960 *Endocrinology* **22** 344-360.
- 961 Hossain A, Kuo MT & Saunders GF 2006 Mir-17-5p Regulates Breast Cancer Cell
962 Proliferation by Inhibiting Translation of AIB1 mRNA. *Molecular and Cellular Biology* **26**
963 8191-8201.

964 Hube F, Guo J, Chooniedass-Kothari S, Cooper C, Hamedani M, Dibrov A, Blanchard A,
965 Wang X, Deng G, Myal Y, *et al.* 2006 Alternative splicing of the first intron of the steroid
966 receptor RNA activator (SRA) participates in the generation of coding and noncoding RNA
967 isoforms in breast cancer cell lines. *DNA and Cell Biology* **25** 418-428.

968 Iorio MV, Ferracin M, Liu C-G, Veronese A, Spizzo R, Sabbioni S, Magri E, Pedriali M,
969 Fabbri M, Campiglio M, *et al.* 2005 MicroRNA Gene Expression Deregulation in Human
970 Breast Cancer. *Cancer Research* **65** 7065-7070.

971 Jiang J, Lee EJ, Gusev Y & Schmittgen TD 2005 Real-time expression profiling of
972 microRNA precursors in human cancer cell lines. *Nucleic Acids Research* **33** 5394-5403.

973 Johnson SM, Grosshans H, Shingara J, Byrom M, Jarvis R, Cheng A, Labourier E, Reinert
974 KL, Brown D & Slack FJ 2005 RAS Is Regulated by the let-7 MicroRNA Family. *Cell* **120**
975 635-647.

976 Kanda I, Nishimura N, Nakatsuji H, Yamamura R, Nakanishi H & Sasaki T 2007
977 Involvement of Rab13 and JRAB/MICAL-L2 in epithelial cell scattering. *Oncogene* **27**
978 1687-1695.

979 Karube Y, Tanaka H, Osada H, Tomida S, Tatematsu Y, Yanagisawa K, Yatabe Y,
980 Takamizawa J, Miyoshi S, Mitsudomi T, *et al.* 2005 Reduced expression of Dicer
981 associated with poor prognosis in lung cancer patients. *Cancer Science* **96** 111-115.

982 Kashat M, Azzouz L, Sarkar S, Kong D, Li Y & Sarkar F 2013 Inactivation of AR and
983 Notch-1 signaling by miR-34a attenuates prostate cancer aggressiveness. *Am J Transl*
984 *Res* **4** 432-442.

985 Katchy A, Edvardsson K, Aydogdu E & Williams C 2012 Estradiol-activated estrogen
986 receptor α does not regulate mature microRNAs in T47D breast cancer cells. *The Journal*
987 *of Steroid Biochemistry and Molecular Biology* **128** 145-153.

988 Kawahara Y & Mieda-Sato A 2012 TDP-43 promotes microRNA biogenesis as a
989 component of the Drosha and Dicer complexes. *Proceedings of the National Academy of*
990 *Sciences*.

991 Kawai S & Amano A 2012 BRCA1 regulates microRNA biogenesis via the DROSHA
992 microprocessor complex. *The Journal of Cell Biology* **197** 201-208.

993 Kawamata T, Seitz H & Tomari Y 2009 Structural determinants of miRNAs for RISC
994 loading and slicer-independent unwinding. *Nat Struct Mol Biol* **16** 953-960.

995 Kim T-D, Lee SU, Yun S, Sun H-N, Lee SH, Kim JW, Kim HM, Park S-K, Lee CW, Yoon
996 SR, *et al.* 2011 Human microRNA-27a* targets Prf1 and GzmB expression to regulate NK
997 cell cytotoxicity. *Blood*

- 998 Klinge C 2009 Estrogen Regulation of MicroRNA Expression. *Current Genomics* **10** 169-
999 183.
- 1000 Kovalchuk O, Tryndyak V, Montgomery B, Boyko A, Kutanzi K, Zemp F, Warbritton A,
1001 Latendresse J, Kovalchuk I, Beland F, *et al.* 2007 Estrogen-induced rat breast
1002 carcinogenesis is characterized by alterations in DNA methylation, histone modifications
1003 and aberrant microRNA expression. *Cell Cycle* **6** 2010-2018.
- 1004 Krichevsky AM & Gabriely G 2009 miR-21: a small multi-faceted RNA. *Journal of Cellular*
1005 *and Molecular Medicine* **13** 39-53.
- 1006 Kumar MS, Lu J, Mercer KL, Golub TR & Jacks T 2007 Impaired microRNA processing
1007 enhances cellular transformation and tumorigenesis. *Nat Genet* **39** 673-677.
- 1008 Kumarswamy R, Volkmann I & Thum T 2011 Regulation and function of miRNA-21 in
1009 health and disease. *RNA Biology* **8** 706-713.
- 1010 Lagos-Quintana M, Rauhut R, Lendeckel W & Tuschl T 2001 Identification of Novel Genes
1011 Coding for Small Expressed RNAs. *Science* **294** 853-858.
- 1012 Landthaler M, Yalcin A & Tuschl T 2004 The Human DiGeorge Syndrome Critical Region
1013 Gene 8 and Its D. melanogaster Homolog Are Required for miRNA Biogenesis. *Current*
1014 *Biology* **14** 2162-2167.
- 1015 Lanz RB, Razani B, Goldberg AD & O'Malley BW 2002 Distinct RNA motifs are important
1016 for coactivation of steroid hormone receptors by steroid receptor RNA activator (SRA).
1017 *Proceedings of the National Academy of Sciences* **99** 16081-16086.
- 1018 Lau NC, Lim LP, Weinstein EG & Bartel DP 2001 An Abundant Class of Tiny RNAs with
1019 Probable Regulatory Roles in *Caenorhabditis elegans*. *Science* **294** 858-862.
- 1020 Lee RC & Ambros V 2001 An Extensive Class of Small RNAs in *Caenorhabditis elegans*.
1021 *Science* **294** 862-864.
- 1022 Lee RC, Feinbaum RL & Ambros V 1993 The *C. elegans* heterochronic gene *lin-4*
1023 encodes small RNAs with antisense complementarity to *lin-14*. *Cell* **75** 843-854.
- 1024 Lee Y, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Radmark O, Kim S, *et al.*
1025 2003 The nuclear RNase III Drosha initiates microRNA processing. *Nature* **425** 415-419.
- 1026 Leivonen SK, Makela R, Ostling P, Kohonen P, Haapa-Paananen S, Kleivi K, Enerly E,
1027 Aakula A, Hellstrom K, Sahlberg N, *et al.* 2009 Protein lysate microarray analysis to
1028 identify microRNAs regulating estrogen receptor signaling in breast cancer cell lines.
1029 *Oncogene* **28** 3926-3936.
- 1030 Levin ER 2011 Minireview: Extranuclear Steroid Receptors: Roles in Modulation of Cell
1031 Functions. *Molecular Endocrinology* **25** 377-384.

- Li X, Mertens-Talcott SU, Zhang S, Kim K, Ball J & Safe S 2010 MicroRNA-27a Indirectly Regulates Estrogen Receptor α Expression and Hormone Responsiveness in MCF-7 Breast Cancer Cells. *Endocrinology* **151** 2462-2473.
- Lin C-Y, Vega VB, Thomsen JS, Zhang T, Kong SL, Xie M, Chiu KP, Lipovich L, Barnett DH, Stossi F, *et al.* 2007 Whole-Genome Cartography of Estrogen Receptor α Binding Sites. *PLoS Genet* **3** e87.
- Lin H-K, Hu Y-C, Lee DK & Chang C 2004 Regulation of Androgen Receptor Signaling by PTEN (Phosphatase and Tensin Homolog Deleted on Chromosome 10) Tumor Suppressor through Distinct Mechanisms in Prostate Cancer Cells. *Molecular Endocrinology* **18** 2409-2423.
- Macias S, Michlewski G & Cáceres JF 2009 Hormonal Regulation of MicroRNA Biogenesis. *Molecular Cell* **36** 172-173.
- Mah S, Buske C, Humphries R & Kuchenbauer F 2010 miRNA*: a passenger stranded in RNA-induced silencing complex? *Critical Reviews in Eukaryotic Gene Expression* **20** 141-148.
- Maillot G, Lacroix-Triki M, Pierredon S, Gratadou L, Schmidt S, Bénès V, Roché H, Dalenc F, Auboeuf D, Millevoi S, *et al.* 2009 Widespread Estrogen-Dependent Repression of microRNAs Involved in Breast Tumor Cell Growth. *Cancer Research* **69** 8332-8340.
- Masuda M, Miki Y, Hata S, Takagi K, Sakurai M, Ono K, Suzuki K, Yang Y, Abe E, Hirakawa H, *et al.* 2012 An induction of microRNA, miR-7 through estrogen treatment in breast carcinoma. *Journal of Translational Medicine* **10** S2.
- Mattie M, Benz C, Bowers J, Sensinger K, Wong L, Scott G, Fedele V, Ginzinger D, Getts R & Haqq C 2006 Optimized high-throughput microRNA expression profiling provides novel biomarker assessment of clinical prostate and breast cancer biopsies. *Molecular Cancer* **5** 24.
- Mayr C & Bartel DP 2009 Widespread Shortening of 3'UTRs by Alternative Cleavage and Polyadenylation Activates Oncogenes in Cancer Cells. *Cell* **138** 673-684.
- Meister G, Landthaler M, Peters L, Chen PY, Urlaub H, Lührmann R & Tuschl T 2005 Identification of Novel Argonaute-Associated Proteins. *Current Biology* **15** 2149-2155.
- Mellinghoff IK, Vivanco I, Kwon A, Tran C, Wongvipat J & Sawyers CL 2004 HER2/neu kinase-dependent modulation of androgen receptor function through effects on DNA binding and stability. *Cancer Cell* **6** 517-527.
- Melo SA, Roper S, Moutinho C, Aaltonen LA, Yamamoto H, Calin GA, Rossi S, Fernandez AF, Carneiro F, Oliveira C, *et al.* 2009 A TARBP2 mutation in human cancer impairs microRNA processing and DICER1 function. *Nat Genet* **41** 365-370.

- 1067 Merritt WM, Lin YG, Han LY, Kamat AA, Spannuth WA, Schmandt R, Urbauer D,
1068 Pennacchio LA, Cheng J-F, Nick AM, *et al.* 2008 Dicer, Drosha, and Outcomes in Patients
1069 with Ovarian Cancer. *New England Journal of Medicine* **359** 2641-2650.
- 1070 Mertens-Talcott SU, Chintharlapalli S, Li X & Safe S 2007 The Oncogenic microRNA-27a
1071 Targets Genes That Regulate Specificity Protein Transcription Factors and the G2-M
1072 Checkpoint in MDA-MB-231 Breast Cancer Cells. *Cancer Res* **67** 11001-11011.
- 1073 Michlewski G & Cáceres JF 2010 Antagonistic role of hnRNP A1 and KSRP in the
1074 regulation of let-7a biogenesis. *Nat Struct Mol Biol* **17** 1011-1018.
- 1075 Michlewski G, Guil S, Semple CA & Cáceres JF 2008 Posttranscriptional Regulation of
1076 miRNAs Harboring Conserved Terminal Loops. *Molecular Cell* **32** 383-393.
- 1077 Mo W, Zhang J, Li X, Meng D, Gao Y, Yang S, Wan X, Zhou C, Guo F, Huang Y, *et al.*
1078 2013 Identification of Novel AR-Targeted MicroRNAs Mediating Androgen Signalling
1079 through Critical Pathways to Regulate Cell Viability in Prostate Cancer. *PLoS ONE* **8**
1080 e56592.
- 1081 Morlando M, Dini Modigliani S, Torrelli G, Rosa A, Di Carlo V, Caffarelli E & Bozzoni I
1082 2012 FUS stimulates microRNA biogenesis by facilitating co transcriptional Drosha
1083 recruitment. *The EMBO Journal* **31** 4502-4510.
- 1084 Mulholland DJ, Dedhar S, Wu H & Nelson CC 2006 PTEN and GSK3[beta]: key regulators
1085 of progression to androgen-independent prostate cancer. *Oncogene* **25** 329-337.
- 1086 Murphy D, Dancis B & Brown J 2008 The evolution of core proteins involved in microRNA
1087 biogenesis. *BMC Evolutionary Biology* **8** 92.
- 1088 Nadiminty N, Tummala R, Lou W, Zhu Y, Zhang J, Chen X, deVere White RW, Kung H-J,
1089 Evans CP & Gao AC 2012 MicroRNA let-7c Suppresses Androgen Receptor Expression
1090 and Activity via Regulation of Myc Expression in Prostate Cancer Cells. *Journal of*
1091 *Biological Chemistry* **287** 1527-1537.
- 1092 Nakajima M & Yokoi T 2011 MicroRNAs from biology to future pharmacotherapy:
1093 Regulation of cytochrome P450s and nuclear receptors. *Pharmacology & Therapeutics*
1094 **131** 330-337.
- 1095 Narayanan R, Jiang J, Gusev Y, Jones A, Kearbey JD, Miller DD, Schmittgen TD & Dalton
1096 JT 2010 MicroRNAs Are Mediators of Androgen Action in Prostate and Muscle. *PLoS ONE*
1097 **5** e13637.
- 1098 Newman MA & Hammond SM 2010 Emerging paradigms of regulated microRNA
1099 processing. *Genes & Development* **24** 1086-1092.

- 1100 Nothnick W, Healy C & Hong X 2010 Steroidal regulation of uterine miRNAs is associated
1101 with modulation of the miRNA biogenesis components Exportin-5 and Dicer1. *Endocrine*
1102 **37** 265-273.
- 1103 O'Connell MA & Keegan LP 2006 Drosha versus ADAR: wrangling over pri-miRNA. *Nat*
1104 *Struct Mol Biol* **13** 3-4.
- 1105 Okamura K, Hagen JW, Duan H, Tyler DM & Lai EC 2007 The Mirtron Pathway Generates
1106 microRNA-Class Regulatory RNAs in Drosophila. *Cell* **130** 89-100.
- 1107 Okamura K, Phillips MD, Tyler DM, Duan H, Chou Y-t & Lai EC 2008 The regulatory
1108 activity of microRNA species has substantial influence on microRNA and 3' UTR evolution.
1109 *Nat Struct Mol Biol* **15** 354-363.
- 1110 Östling P, Leivonen S-K, Aakula A, Kohonen P, Mäkelä R, Hagman Z, Edsjö A,
1111 Kangaspeska S, Edgren H, Nicorici D, *et al.* 2011 Systematic Analysis of MicroRNAs
1112 Targeting the Androgen Receptor in Prostate Cancer Cells. *Cancer Research* **71** 1956-
1113 1967.
- 1114 Ozen M, Creighton CJ, Ozdemir M & Ittmann M 2008 Widespread deregulation of
1115 microRNA expression in human prostate cancer. *Oncogene* **27** 1788-1793.
- 1116 Paroo Z, Ye X, Chen S & Liu Q 2009 Phosphorylation of the Human MicroRNA-Generating
1117 Complex Mediates MAPK/Erk Signaling. *Cell* **139** 112-122.
- 1118 Passon N, Gerometta A, Puppini C, Lavarone E, Puglisi F, Tell G, Di Loreto C & Damante
1119 G 2012 Expression of Dicer and Drosha in triple-negative breast cancer. *Journal of Clinical*
1120 *Pathology* **65** 320-326.
- 1121 Pratt AJ & MacRae IJ 2009 The RNA-induced Silencing Complex: A Versatile Gene-
1122 silencing Machine. *Journal of Biological Chemistry* **284** 17897-17901.
- 1123 Qian P, Zuo Z, Wu Z, Meng X, Li G, Wu Z, Zhang W, Tan S, Pandey V, Yao Y, *et al.* 2011
1124 Pivotal Role of Reduced let-7g Expression in Breast Cancer Invasion and Metastasis.
1125 *Cancer Research* **71** 6463-6474.
- 1126 Qu F, Cui X, Hong Y, Wang J, Li Y, Chen L, Liu Y, Gao Y, Xu D & Wang Q 2013
1127 MicroRNA-185 suppresses proliferation, invasion, migration, and tumorigenicity of human
1128 prostate cancer cells through targeting androgen receptor. *Molecular and Cellular*
1129 *Biochemistry* **377** 121-130.
- 1130 Rao X, Di Leva G, Li M, Fang F, Devlin C, Hartman-Frey C, Burow ME, Ivan M, Croce CM
1131 & Nephew KP 2011 MicroRNA-221/222 confers breast cancer fulvestrant resistance by
1132 regulating multiple signaling pathways. *Oncogene* **30** 1082-1097.
- 1133 Redfern AD, Colley SM, Beveridge DJ, Ikeda N, Epis MR, Li X, Foulds CE, Stuart LM,
1134 Barker A, Russell VJ, *et al.* 2013 RNA-induced silencing complex (RISC) Proteins PACT,

1135 TRBP, and Dicer are SRA binding nuclear receptor coregulators. *Proceedings of the*
1136 *National Academy of Sciences* **110** 6536-6541.

1137 Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC, Rougvie AE, Horvitz HR &
1138 Ruvkun G 2000 The 21-nucleotide let-7 RNA regulates developmental timing in
1139 *Caenorhabditis elegans*. *Nature* **403** 901-906.

1140 Ribas J, Ni X, Haffner M, Wentzel EA, Salmasi AH, Chowdhury WH, Kudrolli TA,
1141 Yegnasubramanian S, Luo J, Rodriguez R, *et al.* 2009 miR-21: An Androgen Receptor–
1142 Regulated MicroRNA that Promotes Hormone-Dependent and Hormone-Independent
1143 Prostate Cancer Growth. *Cancer Research* **69** 7165-7169.

1144 Robb GB & Rana TM 2007 RNA Helicase A Interacts with RISC in Human Cells and
1145 Functions in RISC Loading. *Molecular Cell* **26** 523-537.

1146 Rokhlin OW, Scheinker V, Taghiyev AF, Bumcrot D, Glover RA & Cohen MB 2008
1147 MicroRNA-34 mediates AR-dependent p53-induced apoptosis in prostate cancer. *Cancer*
1148 *Biol Ther* **7** 1288-1296.

1149 Ruby JG, Jan CH & Bartel DP 2007 Intronic microRNA precursors that bypass Drosha
1150 processing. *Nature* **448** 83-86.

1151 Ruggiero T, Trabucchi M, De Santa F, Zupo S, Harfe BD, McManus MT, Rosenfeld MG,
1152 Briata P & Gherzi R 2009 LPS induces KH-type splicing regulatory protein-dependent
1153 processing of microRNA-155 precursors in macrophages. *The FASEB Journal* **23** 2898-
1154 2908.

1155 Salzman DW, Shubert-Coleman J & Furneaux H 2007 P68 RNA Helicase Unwinds the
1156 Human let-7 MicroRNA Precursor Duplex and Is Required for let-7-directed Silencing of
1157 Gene Expression. *Journal of Biological Chemistry* **282** 32773-32779.

1158 Samaan S, Tranchevent L-C, Dardenne E, Polay Espinoza M, Zonta E, Germann S,
1159 Gratadou L, Dutertre M & Auboeuf D 2013 The Ddx5 and Ddx17 RNA helicases are
1160 cornerstones in the complex regulatory array of steroid hormone-signaling pathways.
1161 *Nucleic Acids Research*.

1162 Sander S, Bullinger L, Klapproth K, Fiedler K, Kestler HA, Barth TFE, Möller P,
1163 Stilgenbauer S, Pollack JR & Wirth T 2008 MYC stimulates EZH2 expression by
1164 repression of its negative regulator miR-26a. *Blood* **112** 4202-4212.

1165 Scott GK, Goga A, Bhaumik D, Berger CE, Sullivan CS & Benz CC 2007 Coordinate
1166 Suppression of ERBB2 and ERBB3 by Enforced Expression of Micro-RNA miR-125a or
1167 miR-125b. *Journal of Biological Chemistry* **282** 1479-1486.

1168 Shedden K, Taylor JMG, Enkemann SA, Tsao M-S, Yeatman TJ, Gerald WL, Eschrich S,
1169 Jurisica I, Giordano TJ, Misek DE, *et al.* 2008 Gene expression-based survival prediction
1170 in lung adenocarcinoma: a multi-site, blinded validation study. *Nat Med* **14** 822-827.

1171 Shenouda S & Alahari S 2009 MicroRNA function in cancer: oncogene or a tumor
1172 suppressor? *Cancer and Metastasis Reviews* **28** 369-378.

1173 Shi X-B, Xue L, Yang J, Ma A-H, Zhao J, Xu M, Tepper CG, Evans CP, Kung H-J &
1174 deVere White RW 2007 An androgen-regulated miRNA suppresses Bak1 expression and
1175 induces androgen-independent growth of prostate cancer cells *Proceedings of the*
1176 *National Academy of Sciences* **104** 19983-19988

1177 Shu J, Kren BT, Xia Z, Wong PYP, Li L, Hanse EA, Min MX, Li B, Albrecht JH, Zeng Y, *et*
1178 *al.* 2011 Genomewide microRNA down-regulation as a negative feedback mechanism in
1179 the early phases of liver regeneration. *Hepatology* **54** 609-619.

1180 Sikand K, Barik S & Shukla G 2011a MicroRNAs and Androgen Receptor 3' Untranslated
1181 Region: A Missing Link in Castration-resistant Prostate Cancer? *Molecular and Cellular*
1182 *Pharmacology* **3** 107-113.

1183 Sikand K, Slaibi JE, Singh R, Slane SD & Shukla GC 2011b miR 488* inhibits androgen
1184 receptor expression in prostate carcinoma cells. *International Journal of Cancer* **129** 810-
1185 819.

1186 Sirotkin AV, Ovcharenko D, Grossmann R, Lauková M & Mlynček M 2009 Identification of
1187 MicroRNAs controlling human ovarian cell steroidogenesis via a genome-scale screen.
1188 *Journal of Cellular Physiology* **219** 415-420.

1189 Suzuki Hiroshi I, Arase M, Matsuyama H, Choi Young L, Ueno T, Mano H, Sugimoto K &
1190 Miyazono K 2011 MCPIP1 Ribonuclease Antagonizes Dicer and Terminates MicroRNA
1191 Biogenesis through Precursor MicroRNA Degradation. *Molecular Cell* **44** 424-436.

1192 Suzuki HI, Yamagata K, Sugimoto K, Iwamoto T, Kato S & Miyazono K 2009 Modulation of
1193 microRNA processing by p53. *Nature* **460** 529-533.

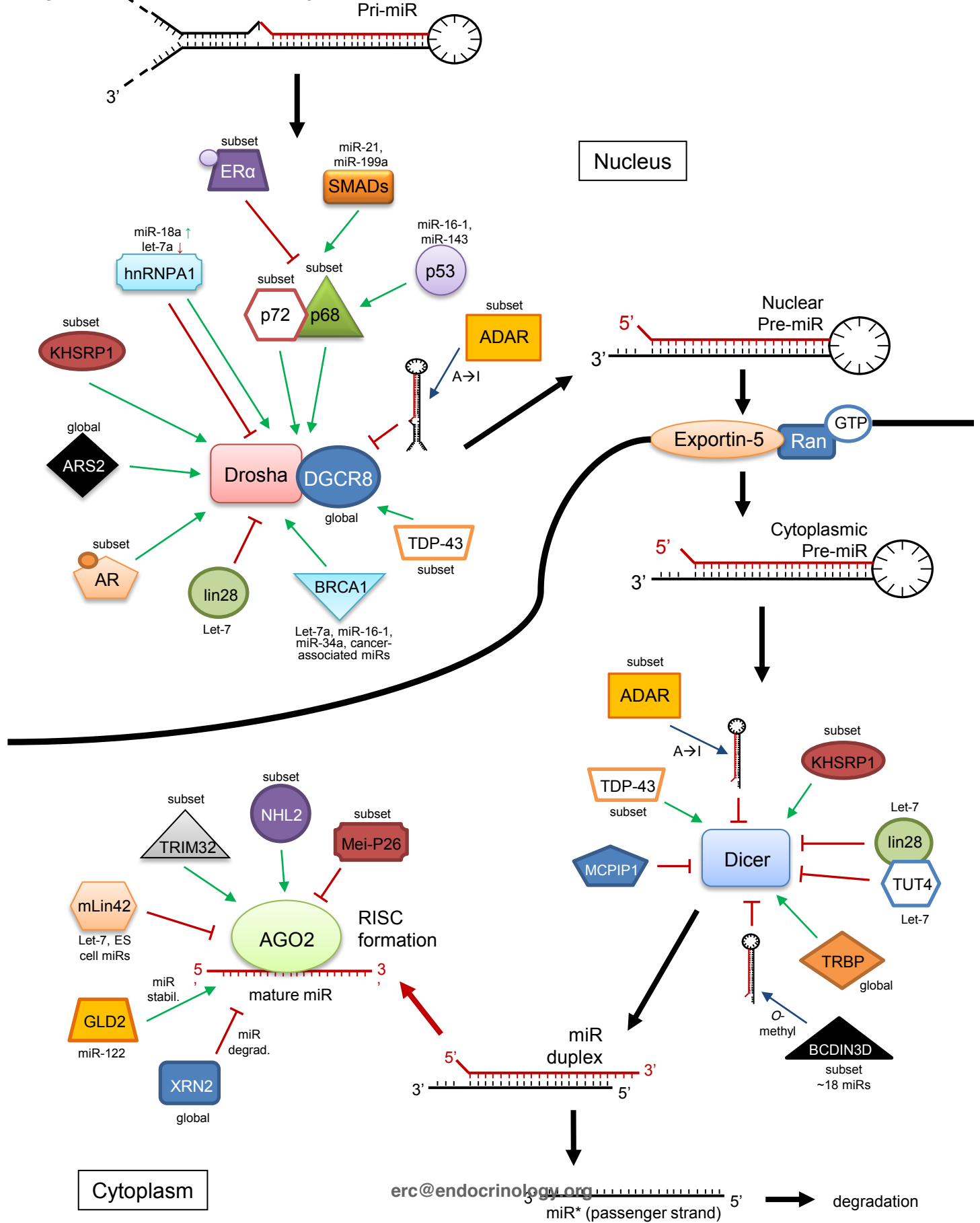
1194 Takata A, Otsuka M, Kojima K, Yoshikawa T, Kishikawa T, Yoshida H & Koike K 2011
1195 MicroRNA-22 and microRNA-140 suppress NF-κB activity by regulating the expression of
1196 NF-κB coactivators. *Biochemical and Biophysical Research Communications* **411** 826-831.

1197 Takayama K, Tsutsumi S, Katayama S, Okayama T, Horie-Inoue K, Ikeda K, Urano T,
1198 Kawazu C, Hasegawa A, Ikeo K, *et al.* 2011 Integration of cap analysis of gene expression
1199 and chromatin immunoprecipitation analysis on array reveals genome-wide androgen
1200 receptor signaling in prostate cancer cells. *Oncogene* **30** 619-630.

1201 Tokumaru S, Suzuki M, Yamada H, Nagino M & Takahashi T 2008 let-7 regulates Dicer
1202 expression and constitutes a negative feedback loop. *Carcinogenesis* **29** 2073-2077.

- 1203 Tong AW, Fulgham P, Jay C, Chen P, Khalil I, Liu S, Senzer N, Eklund AC, Han J &
1204 Nemunaitis J 2008 MicroRNA profile analysis of human prostate cancers. *Cancer Gene*
1205 *Ther* **16** 206-216.
- 1206 Trabucchi M, Briata P, Garcia-Mayoral M, Haase AD, Filipowicz W, Ramos A, Gherzi R &
1207 Rosenfeld MG 2009 The RNA-binding protein KSRP promotes the biogenesis of a subset
1208 of microRNAs. *Nature* **459** 1010-1014.
- 1209 Tsuchiya Y, Nakajima M, Takagi S, Taniya T & Yokoi T 2006 MicroRNA Regulates the
1210 Expression of Human Cytochrome P450 1B1. *Cancer Research* **66** 9090-9098.
- 1211 Waltering KK, Porkka KP, Jalava SE, Urbanucci A, Kohonen PJ, Latonen LM, Kallioniemi
1212 OP, Jenster G & Visakorpi T 2011 Androgen regulation of micro-RNAs in prostate cancer.
1213 *The Prostate* **71** 604-614.
- 1214 Wickramasinghe NS, Manavalan TT, Dougherty SM, Riggs KA, Li Y & Klinge CM 2009
1215 Estradiol downregulates miR-21 expression and increases miR-21 target gene expression
1216 in MCF-7 breast cancer cells. *Nucleic Acids Research* **37** 2584-2595.
- 1217 Wightman B, Ha I & Ruvkun G 1993 Posttranscriptional regulation of the heterochronic
1218 gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. *Cell* **75** 855-862.
- 1219 Wu J, Bao J, Wang L, Hu Y & Xu C 2011 MicroRNA-184 downregulates nuclear receptor
1220 corepressor 2 in mouse spermatogenesis. *BMC Developmental Biology* **11** 64.
- 1221 Xhemalce B, Robson Samuel C & Kouzarides T 2012 Human RNA Methyltransferase
1222 BCDIN3D Regulates MicroRNA Processing. *Cell* **151** 278-288.
- 1223 Yamagata K, Fujiyama S, Ito S, Ueda T, Murata T, Naitou M, Takeyama K-i, Minami Y,
1224 O'Malley BW & Kato S 2009 Maturation of MicroRNA Is Hormonally Regulated by a
1225 Nuclear Receptor. *Molecular Cell* **36** 340-347.
- 1226 Yang C-M, Lu IH, Chen H-Y & Hu M-L 2012 Lycopene inhibits the proliferation of
1227 androgen-dependent human prostate tumor cells through activation of PPAR γ -LXR α -
1228 ABCA1 pathway. *The Journal of Nutritional Biochemistry* **23** 8-17.
- 1229 Yang J-S, Phillips MD, Betel D, Mu P, Ventura A, Siepel AC, Chen KC & Lai EC 2011
1230 Widespread regulatory activity of vertebrate microRNA* species. *RNA* **17** 312-326.
- 1231 Yang R, Schlehe B, Hemminki K, Sutter C, Bugert P, Wappenschmidt B, Volkmann J,
1232 Varon R, Weber B, Niederacher D, *et al.* 2010 A genetic variant in the pre-miR-27a
1233 oncogene is associated with a reduced familial breast cancer risk. *Breast Cancer*
1234 *Research and Treatment* **121** 693-702.
- 1235 Yang W, Chendrimada TP, Wang Q, Higuchi M, Seeburg PH, Shiekhattar R & Nishikura K
1236 2006 Modulation of microRNA processing and expression through RNA editing by ADAR
1237 deaminases. *Nat Struct Mol Biol* **13** 13-21.

- Yeom K-H, Lee Y, Han J, Suh MR & Kim VN 2006 Characterization of DGCR8/Pasha, the essential cofactor for Drosha in primary miRNA processing. *Nucleic Acids Research* **34** 4622-4629.
- Yu Z, Wang C, Wang M, Li Z, Casimiro MC, Liu M, Wu K, Whittle J, Ju X, Hyslop T, *et al.* 2008 A cyclin D1/microRNA 17/20 regulatory feedback loop in control of breast cancer cell proliferation. *The Journal of Cell Biology* **182** 509-517.
- Zeng Y, Wagner EJ & Cullen BR 2002 Both Natural and Designed Micro RNAs Can Inhibit the Expression of Cognate mRNAs When Expressed in Human Cells. *Molecular Cell* **9** 1327-1333.
- Zhang R, He Y, Zhang X, Xing B, Sheng Y, Lu H & Wei Z 2012 Estrogen receptor-regulated microRNAs contribute to the BCL2/BAX imbalance in endometrial adenocarcinoma and precancerous lesions. *Cancer Letters* **314** 155-165.
- Zhao J, Imbrie GA, Baur WE, Iyer LK, Aronovitz MJ, Kershaw TB, Haselmann GM, Lu Q & Karas RH 2013 Estrogen Receptor-Mediated Regulation of MicroRNA Inhibits Proliferation of Vascular Smooth Muscle Cells. *Arteriosclerosis, Thrombosis, and Vascular Biology* **33** 257-265.
- Zhao J-J, Lin J, Yang H, Kong W, He L, Ma X, Coppola D & Cheng JQ 2008 MicroRNA-221/222 Negatively Regulates Estrogen Receptor α and Is Associated with Tamoxifen Resistance in Breast Cancer. *Journal of Biological Chemistry* **283** 31079-31086.
- Zhao Y, Deng C, Wang J, Xiao J, Gatalica Z, Recker R & Xiao G 2011 Let-7 family miRNAs regulate estrogen receptor alpha signaling in estrogen receptor positive breast cancer. *Breast Cancer Research and Treatment* **127** 69-80.



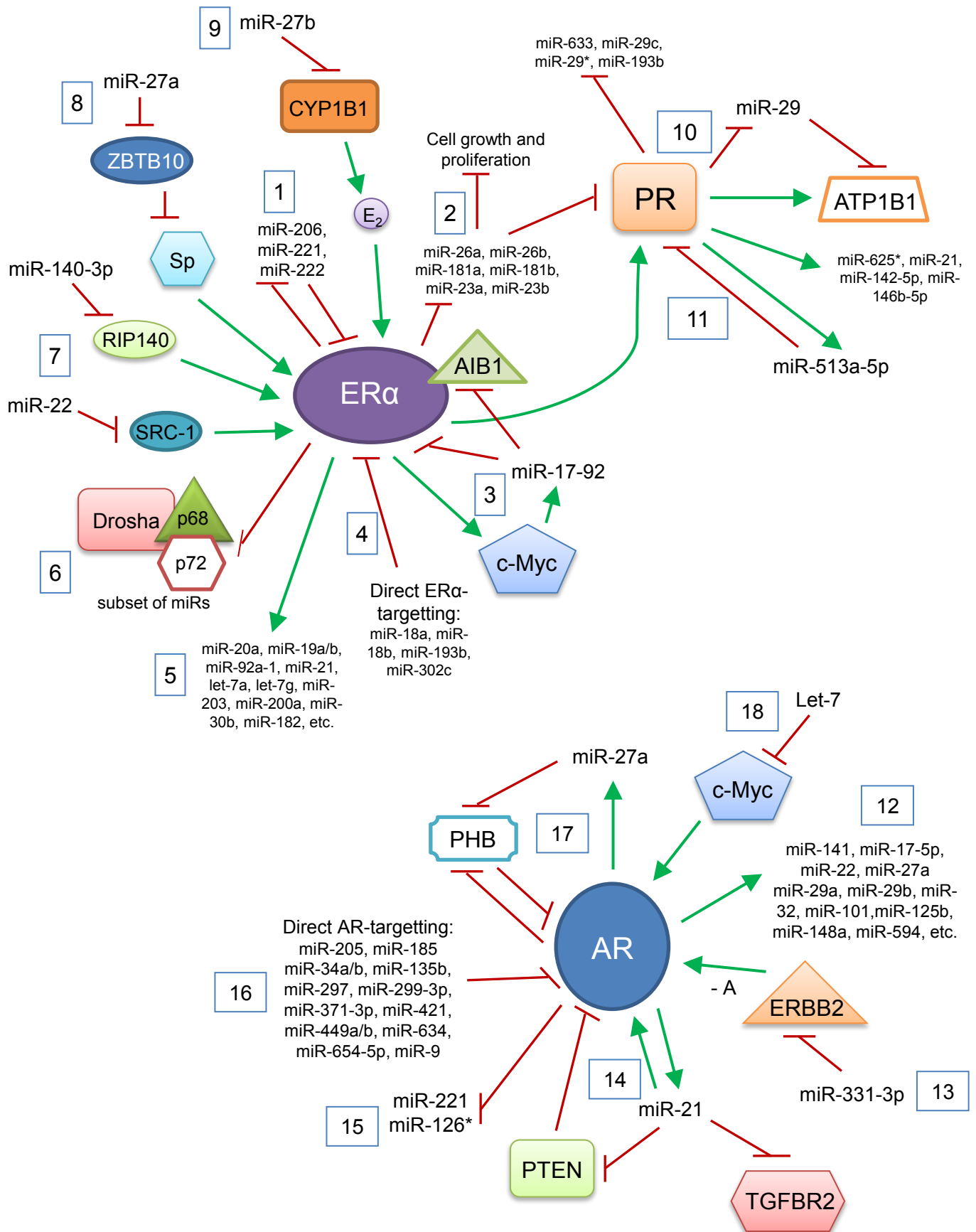


Table 1: MicroRNAs Regulated by Estrogen Receptor Alpha in at Least Two Studies

MiR	Regulation	Reference
17	↑/↑/↑	Castellano <i>et al.</i> 2009, Bhat-Nakshatri <i>et al.</i> 2009, Di Leva <i>et al.</i> 2010
19a	↑/↑/↑	Castellano <i>et al.</i> 2009, Ferraro <i>et al.</i> 2012, Di Leva <i>et al.</i> 2010
20a	↑/↑/↑/↑	Castellano <i>et al.</i> 2009, Klinge <i>et al.</i> 2009, Kovalchuk <i>et al.</i> 2007, Ferraro <i>et al.</i> 2012, Di Leva <i>et al.</i> 2010
19b	↑/↑/↑	Castellano <i>et al.</i> 2009, Ferraro <i>et al.</i> 2012, Di Leva <i>et al.</i> 2010
92a-1	↑/↑/↑/↑	Castellano <i>et al.</i> 2009, Klinge <i>et al.</i> 2009, Mattie <i>et al.</i> 2006, Kovalchuk <i>et al.</i> 2007, Di Leva <i>et al.</i> 2010
206	↓/↓	Adams <i>et al.</i> 2007, Munagala <i>et al.</i> 2013
221	↓/↓	Cochrane <i>et al.</i> 2010, Di Leva <i>et al.</i> 2010
222	↓/↓	Cochrane <i>et al.</i> 2010, Di Leva <i>et al.</i> 2010
21	↑/↓/↑/↑/↓/↑	Bhat-Nakshatri <i>et al.</i> 2009, Klinge <i>et al.</i> 2009, Mattie <i>et al.</i> 2006, Kovalchuk <i>et al.</i> 2007, Hu <i>et al.</i> 2013, Maillot <i>et al.</i> 2009, Wickramasinghe <i>et al.</i> 2009, Di Leva <i>et al.</i> 2010
Let-7a	↑/↑/↓/↓/↑/↑	Bhat-Nakshatri <i>et al.</i> 2009, Blenkiron <i>et al.</i> 2007, Klinge <i>et al.</i> 2009, Maillot <i>et al.</i> 2009, Mattie <i>et al.</i> 2006, Di Leva <i>et al.</i> 2010
Let-7b	↑/↑	Bhat-Nakshatri <i>et al.</i> 2009, Mattie <i>et al.</i> 2006
Let-7c	↑/↑/↓	Bhat-Nakshatri <i>et al.</i> 2009, Klinge <i>et al.</i> 2009, Maillot <i>et al.</i> 2009
Let-7d	↑/↑/↑	Bhat-Nakshatri <i>et al.</i> 2009, Klinge <i>et al.</i> 2009, Di Leva <i>et al.</i> 2010
Let-7f	↑/↑/↓/↓/↑/↑	Bhat-Nakshatri <i>et al.</i> 2009, Blenkiron <i>et al.</i> 2007, Klinge <i>et al.</i> 2009, Kovalchuk <i>et al.</i> 2007, Maillot <i>et al.</i> 2009, Di Leva <i>et al.</i> 2010
Let-7g	↑/↑/↓/↑	Bhat-Nakshatri <i>et al.</i> 2009, Klinge <i>et al.</i> 2009, Maillot <i>et al.</i> 2009, Di Leva <i>et al.</i> 2010
Let-7i	↑/↑	Bhat-Nakshatri <i>et al.</i> 2009, Klinge <i>et al.</i> 2009
203	↑/↑/↓/↑/↑	Bhat-Nakshatri <i>et al.</i> 2009, Klinge <i>et al.</i> 2009, Maillot <i>et al.</i> 2009, Zhao J <i>et al.</i> 2013, Di Leva <i>et al.</i> 2010
98	↑/↓/↑	Bhat-Nakshatri <i>et al.</i> 2009, Maillot <i>et al.</i> 2009, Di Leva <i>et al.</i> 2010
200a	↑/↑/↑/↓/↑	Bhat-Nakshatri <i>et al.</i> 2009, Klinge <i>et al.</i> 2009, Mattie <i>et al.</i> 2006, Maillot <i>et al.</i> 2009, Di Leva <i>et al.</i> 2010
103	↑/↑/↑	Bhat-Nakshatri <i>et al.</i> 2009, Mattie <i>et al.</i> 2006, Di Leva <i>et al.</i> 2010
200c	↑/↓/↓/↑	Bhat-Nakshatri <i>et al.</i> 2009, Klinge <i>et al.</i> 2009, Maillot <i>et al.</i> 2009, Di Leva <i>et al.</i> 2010
107	↑/↑	Bhat-Nakshatri <i>et al.</i> 2009, Zhao J <i>et al.</i> 2013
23a	↑/↑/↓	Bhat-Nakshatri <i>et al.</i> 2009, Klinge <i>et al.</i> 2009, Maillot <i>et al.</i> 2009
200b	↑/↑/↑/↑	Bhat-Nakshatri <i>et al.</i> 2009, Klinge <i>et al.</i> 2009, Zhao J <i>et al.</i> 2013, Di Leva <i>et al.</i> 2010
30c	↑/↑/↑	Bhat-Nakshatri <i>et al.</i> 2009, Mattie <i>et al.</i> 2006, Ferraro <i>et al.</i> 2012
30b	↑/↑/↑/↑	Bhat-Nakshatri <i>et al.</i> 2009, Iorio <i>et al.</i> 2005, Mattie <i>et al.</i> 2006, Klinge <i>et al.</i> 2009
506	↓/↓	Cochrane <i>et al.</i> 2010, Bhat-Nakshatri <i>et al.</i> 2009
27a	↓/↑/↓/↓	Cochrane <i>et al.</i> 2010, Klinge <i>et al.</i> 2009, Maillot <i>et al.</i> 2009, Bhat-Nakshatri <i>et al.</i> 2009
27b	↓/↑/↑/↓/↓	Cochrane <i>et al.</i> 2010, Mattie <i>et al.</i> 2006, Klinge <i>et al.</i> 2009, Maillot <i>et al.</i> 2009, Bhat-Nakshatri <i>et al.</i> 2009
143	↓/↓/↑	Cochrane <i>et al.</i> 2010, Bhat-Nakshatri <i>et al.</i> 2009, Mattie <i>et al.</i> 2006
9	↓/↓	Cochrane <i>et al.</i> 2010, Bhat-Nakshatri <i>et al.</i> 2009
106b	↑/↓/↑/↑	Klinge <i>et al.</i> 2009, Blenkiron <i>et al.</i> 2007, Kovalchuk <i>et al.</i> 2007, Di Leva <i>et al.</i> 2010
15a	↑/↑	Klinge <i>et al.</i> 2009, Mattie <i>et al.</i> 2006
149	↓/↑	Klinge <i>et al.</i> 2009, Kovalchuk <i>et al.</i> 2007
16	↑/↑	Klinge <i>et al.</i> 2009, Mattie <i>et al.</i> 2006
182	↑/↑/↑/↑	Klinge <i>et al.</i> 2009, Mattie <i>et al.</i> 2006, Munagala <i>et al.</i> 2013, Zhao J <i>et al.</i> 2013
183	↑/↑/↑	Klinge <i>et al.</i> 2009, Munagala <i>et al.</i> 2013, Zhao J <i>et al.</i> 2013
23b	↑/↓	Klinge <i>et al.</i> 2009, Maillot <i>et al.</i> 2009
25	↑/↑/↑/↑	Klinge <i>et al.</i> 2009, Kovalchuk <i>et al.</i> 2007, Mattie <i>et al.</i> 2006, Di Leva <i>et al.</i> 2010
26a	↑/↑/↑/↓/↑	Klinge <i>et al.</i> 2009, Mattie <i>et al.</i> 2006, Iorio <i>et al.</i> 2005, Maillot <i>et al.</i> 2009, Ferraro <i>et al.</i> 2012
26b	↑/↑/↓/↑/↑	Klinge <i>et al.</i> 2009, Mattie <i>et al.</i> 2006, Maillot <i>et al.</i> 2009, Ferraro <i>et al.</i> 2012, Di Leva <i>et al.</i> 2010
342	↓/↑/↓	Klinge <i>et al.</i> 2009, Mattie <i>et al.</i> 2006, Zhao J <i>et al.</i> 2013
365	↑/↓	Klinge <i>et al.</i> 2009, Kovalchuk <i>et al.</i> 2007
489	↑/↑/↑	Klinge <i>et al.</i> 2009, Ferraro <i>et al.</i> 2012, Di Leva <i>et al.</i> 2010
7	↑/↑	Klinge <i>et al.</i> 2009, Masuda <i>et al.</i> 2012
181a	↓/↑	Maillot <i>et al.</i> 2009, Ferraro <i>et al.</i> 2012
24	↓/↑	Maillot <i>et al.</i> 2009, Mattie <i>et al.</i> 2006
196	↑/↑	Mattie <i>et al.</i> 2006, Munagala <i>et al.</i> 2013
205	↑/↓/↑/↑	Mattie <i>et al.</i> , Munagala <i>et al.</i> , Zhao <i>et al.</i> , Ferraro <i>et al.</i> 2012
99a	↑/↑	Mattie <i>et al.</i> , Ferraro <i>et al.</i>

Table 2: Steroid Hormone Regulation of MicroRNA Biogenesis Pathway Components

MiR Biogenesis Protein	Regulating SHR	Direction of Regulation	Reference(s)
Exportin-5	ER α , PR	↑	Nothnick <i>et al.</i> 2010
Dicer	ER α , PR	↑	Bhat-Nakshatri <i>et al.</i> 2009, Lin CY <i>et al.</i> 2007, Cheng C <i>et al.</i> 2009
TRBP	ER α	↑	Cheng C <i>et al.</i> 2009
p68	AR	↑	Clark <i>et al.</i> 2008a, Clark <i>et al.</i> 2008b
AGO2	ER α	↓	Cheng C <i>et al.</i> 2009, Adams <i>et al.</i> 2007

Table 3: MicroRNAs Targetting Estrogen Receptor Alpha

MiR	ERα Activity	Reference
206	↓	Adams <i>et al.</i> 2007, Ioria <i>et al.</i> 2005, Kondo <i>et al.</i> 2008, Leivonen <i>et al.</i> 2009
221/222	↓	Zhao <i>et al.</i> 2008, Leivonen <i>et al.</i> 2009
22	↓	Pandey <i>et al.</i> 2009, Leivonen <i>et al.</i> 2009
17~92 cluster	↓	Castellano <i>et al.</i> 2009, Leivonen <i>et al.</i> 2009
Let-7	↓	Zhao <i>et al.</i> 2010
27a	↑	Li <i>et al.</i> 2010, Mertens-Talcott <i>et al.</i> 2007
219	↓	Leivonen <i>et al.</i> 2009
9	↓	Leivonen <i>et al.</i> 2009
517a/c	↓	Leivonen <i>et al.</i> 2009
373	↓	Leivonen <i>et al.</i> 2009
181a/c/d	↓	Leivonen <i>et al.</i> 2009
302c	↓	Leivonen <i>et al.</i> 2009
301	↓	Leivonen <i>et al.</i> 2009
372	↓	Leivonen <i>et al.</i> 2009
193b	↓	Leivonen <i>et al.</i> 2009
130a/b	↓	Leivonen <i>et al.</i> 2009
93	↓	Leivonen <i>et al.</i> 2009
520d	↓	Leivonen <i>et al.</i> 2009

Table 4: MicroRNAs Identified as Androgen Receptor-Regulated in at least Two Studies

MiR	Direction of Regulation	Reference
221	↓/↓/↓	Waltering <i>et al.</i> 2011, Ambs <i>et al.</i> 2008, Sun <i>et al.</i> 2009
141	↑/↑	Waltering <i>et al.</i> 2011, Murata <i>et al.</i> 2010
21	↑/↑/↑/↑	Waltering <i>et al.</i> 2011, Ribas <i>et al.</i> 2009, Takayama <i>et al.</i> 2011, Sun <i>et al.</i> 2009
22	↑/↑/↑/↑	Waltering <i>et al.</i> 2011, Murata <i>et al.</i> 2010, Mo <i>et al.</i> 2013, Wang Q <i>et al.</i> 2009
29a	↑/↑/↑	Waltering <i>et al.</i> 2011, Ribas <i>et al.</i> 2009, Mo <i>et al.</i> 2013
29b	↑/↑/↑/↑	Waltering <i>et al.</i> 2011, Murata <i>et al.</i> 2010, Ribas <i>et al.</i> 2009, Wang Q <i>et al.</i> 2009
32	↑/↑	Waltering <i>et al.</i> 2011, Jalava <i>et al.</i> 2012
148a	↑/↑/↑	Murata <i>et al.</i> 2010, Ribas <i>et al.</i> 2009, Jalava <i>et al.</i> 2012
125b	↑/↑/↑/↑/↑	Murata <i>et al.</i> 2010, Shi <i>et al.</i> 2007, Mo <i>et al.</i> 2013, Takayama <i>et al.</i> 2011, Sun <i>et al.</i> 2009
17-5p	↑/↑	Murata <i>et al.</i> 2010, Ribas <i>et al.</i> 2009
27a	↑/↑/↑	Murata <i>et al.</i> 2010, Fletcher <i>et al.</i> 2012, Mo <i>et al.</i> 2013
101	↑/↑	Cao <i>et al.</i> 2010, Mo <i>et al.</i> 2013
594	↑/↑	Ribas <i>et al.</i> 2009, Mo <i>et al.</i> 2013
Let-7c	↑/↓	Takayama <i>et al.</i> 2011, Mo <i>et al.</i> 2013
23a	↑/↓	Fletcher <i>et al.</i> 2012, Mo <i>et al.</i> 2013
126*	↓/↓	Mo <i>et al.</i> 2013, Ambs <i>et al.</i> 2008

Table 5: MicroRNAs Targetting the Androgen Receptor

MiR	Direction of AR Activity	Reference
205	↓	Hagman <i>et al.</i> 2013
185	↓/↓	Qu <i>et al.</i> 2013, Östling <i>et al.</i> 2011
34	↓/↓	Kashat <i>et al.</i> 2013, Östling <i>et al.</i> 2011
135b	↓	Östling <i>et al.</i> 2011
297	↓	Östling <i>et al.</i> 2011
299-3p	↓	Östling <i>et al.</i> 2011
371-3p	↓	Östling <i>et al.</i> 2011
421	↓	Östling <i>et al.</i> 2011
449a/b	↓	Östling <i>et al.</i> 2011
634	↓	Östling <i>et al.</i> 2011
654-5p	↓	Östling <i>et al.</i> 2011
9	↓	Östling <i>et al.</i> 2011
124	↓	Shi <i>et al.</i> 2013
488*	↓	Sikand K <i>et al.</i> 2011
141	↓ (indirect via targetting Shp)	Xiao J <i>et al.</i> 2012
Let-7c	↓ (indirect via targetting Myc)	Nadiminty <i>et al.</i> 2012
17-5p	↓ (indirect by targetting PCAF)	Gong <i>et al.</i> 2012
27a	↑ (indirect via targetting AR corepressor PHB)	Fletcher <i>et al.</i> 2012
331-3p	↓ (indirect via targetting ERBB2)	Epis <i>et al.</i> 2009