Interactions between the estrogen receptor, its cofactors and microRNAs in breast cancer

Marc P. J. Mccafferty, Roisin E. Mcneill, Nicola Miller, Michael J. Kerin

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REVIEW

Interactions between the estrogen receptor, its cofactors and microRNAs in breast cancer

Marc P. J. McCafferty · Roisin E. McNeill · Nicola Miller · Michael J. Kerin

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Abstract The activity of selective estrogen receptor modulators (SERMs) is not fully explained by an estrogen receptor (ER) switch model that simply turns estrogen activity on or off. A better understanding of the mechanisms involved in estrogen signaling and the development of drug resistance could help stratify patients into more coherent treatment groups and identify novel therapeutic candidates. This review describes how interactions between two novel factors known to influence estrogenic activity: nuclear receptor cofactors—protein partners which modulate estrogen action, and microRNAs—a class of recently discovered regulatory elements, may impact hormone-sensitive breast cancer. The role of nuclear receptor cofactors in estrogen signaling and the associations between ER cofactors and breast cancer are described. We outline the activity of microRNAs (miRNAs) and their associations with breast cancer and detail recent evidence of interactions between the ER and its cofactors and miRNA and provide an overview of the emerging field of miRNA-based therapeutics. We propose that previously unrecognised interactions between these two species of regulatory molecules may underlie at least some of the heterogeneity of breast cancer in terms of its clinical course and response to treatment. The exploitation of such associations will have important implications for drug development.

Keywords Breast cancer · Estrogen receptors · Estrogen receptor cofactors · Endocrine resistance · MicroRNAs

M. P. J. McCafferty · R. E. McNeill · N. Miller (✉) · M. J. Kerin
Department of Surgery, Clinical Science Institute, National University of Ireland, Galway, Galway, Ireland
e-mail: nicola.miller@nuigalway.ie

M. P. J. McCafferty
e-mail: mccaffros@gmail.com

R. E. McNeill
e-mail: roisin.mcneill@nuigalway.ie

M. J. Kerin
e-mail: michael.kerin@nuigalway.ie

Breast cancer is the leading cause of cancer mortality in women worldwide resulting in more than 500,000 deaths annually [1]. The ovarian steroid hormone estrogen, and to a lesser extent progesterone, plays pivotal roles in both normal breast development and in breast cancer [2, see review 3]. Two-thirds of breast cancers are hormone-dependent in that their growth is governed largely by interactions between estrogen and its nuclear receptor, the estrogen receptor (ER). ER overexpression in breast cancer is believed to contribute to tumorigenesis via its stimulatory effect on the proliferation of mammary cells which results in increased cell division and concomitant accrual of mutations. Consequently estrogen has become an obvious therapeutic target resulting in the development of selective estrogen receptor modulators (SERMs) e.g., tamoxifen, raloxifene; aromatase inhibitors; and "pure estradiol analogs like fulvestrant [4].

Nuclear receptor cofactors

Nuclear receptor (NR) cofactors are a structurally and functionally diverse group of proteins, which play key roles in the regulation of NR-mediated gene expression. They navigate the milieu of NR transcription factors, mediating

their partnerships with diverse subsets of genes, coordinating and refining the communications and helping to orchestrate fundamental and diverse cellular processes such as metabolism, growth and morphogenesis [5]. As their actions affect such an expansive battery of genes, they have been called "master genes" [6]. To date 285 NR cofactors have been identified, and these interact with 48 NRs. The majority of those identified are coactivators, recruited by activated, ligand-bound NRs to enhance gene expression, whereas corepressors, of which approximately 40 have been identified, generally interact with unliganded receptors [7] and oppose the actions of coactivators. The balance between coactivators and corepressors defines the outcome of the cellular responses to NR ligands and the switching of corepressors for coactivators can convert a transcription factor from a repressor to an activator of gene expression [8–10].

Coactivators exist with NRs in multiprotein complexes, which act as enzymatic hubs, facilitating a wide range of biochemical reactions along the route from mRNA transcription to protein synthesis. They contribute not only to chromatin remodeling through nucleosome and histone modification and to the recruitment or activation of components of the basal transcription machinery [11–14], but also to the elongation of the nascent RNA, its splicing, processing, termination and transport from the nucleus, protein translation and post-translational protein modification [see reviews 5, 15].

Corepressors close the workshop of transcription. They possess histone deacetylase activity, which can inhibit the basal transcriptional machinery and the assembly of preinitiation complexes [10, 16–18]. In contrast to coactivators, comparatively little is known of the actions of specific corepressors, and a definition of their involvement in transcriptional repression is still emerging. Two better characterized corepressors, which serve as repressors for a large number of transcription factors including the steroid hormone receptors, are the structurally related Nuclear Receptor CoRepressor (N-CoR) and Silencing Mediator of Retinoid and Thyroid Receptor (SMRT) [10, 19]. Their structure and function has been comprehensively reviewed elsewhere [15, 20, 21].

Estrogen receptor cofactors and breast cancer

Various cofactors have been associated with ER signaling [22, 23], but it was the cloning and characterization of steroid receptor coactivator-1 (SRC-1) that defined the role of coactivators in ER signaling. Onate et al. [24] showed that SRC-1 interacts with several NRs including ER-alpha, whose transcriptional activity it enhances. These findings were followed by the identification of the structurally and functionally similar p160 family of coactivators, another member of which, SRC-3, is associated with a variety of hormone-dependent and -independent cancers [see review 25] and acknowledged as a potent oncogene in breast cancer [26, 27](Fig. 1).

SRC-3 is involved in normal breast development and implicated in orchestrating the expression of hundreds of genes involved in growth signaling pathways in breast tissue [28]. It is amplified in 5–10% and overexpressed in 30–60% of primary breast cancers [26, 29], and its expression correlates with ERα and PR positivity and tumor size [30].

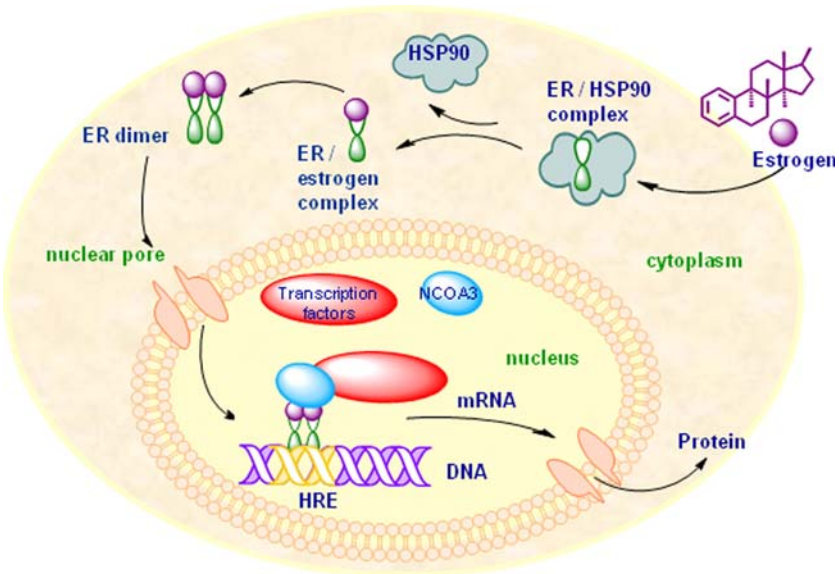

Fig. 1 Activity of ER, a class I nuclear receptor which, in the absence of estrogen is located in the cytoplasm. Estrogen binding to the ER triggers dissociation of heat shock protein 90 (HSP90), homodimerisation and translocation to the nucleus where the ER binds to a specific sequence of DNA known as the hormone response element (HRE). The ER/DNA complex in turn recruits basal transcriptional coregulatory proteins such as NCOA3, which transcribe DNA downstream from the HRE into the mRNA and eventually protein, resulting in changes in cellular function

Another ER coactivator, peroxisome proliferator-activated receptor binding protein (*PPARBP*) is amplified in approximately 24% of human breast tumors and 30% of breast cancer cell lines [31]. The gene encoding PPARBP is localized on chromosome 17q12, in close proximity to the *HER2/neu* amplicon (*ERBB2*), which is frequently amplified in breast cancer [32]. It is suggested that coactivator upregulation and kinase hyperactivation are part of an integrated mechanism in promoting carcinogenesis [33, 34].

It seems highly probable that deregulation of such pervasive gene regulatory elements as the steroid receptor cofactors would lead to significant impacts on the functions of steroid-responsive cells at multiple levels, but most especially at the level of gene expression. The emergence of breast cancer mRNA [35, 36] expression signatures, which reflect distinct breast tumor subsets, raises the question as to what molecular factors orchestrate the presentation of these genetic signatures? In addition to cofactors, the ER has recently been shown to be regulated by a novel class of gene regulatory molecules known as microRNAs (miRNA) [37]. This will be discussed further in detail later.

Endocrine resistance in breast cancer

For twenty-five years, tamoxifen, an orally active SERM, has been the mainstay therapy for the treatment of both early and advanced ER-positive breast cancer in pre and postmenopausal women [38]. In the adjuvant setting, it has been shown to prolong disease free and overall survival and approximately halves the annual incidence rate of contralateral breast cancer [39, 40]. Despite these benefits, only 50% of patients will respond to tamoxifen at first presentation, and all those with metastatic disease will inevitably develop resistance over time [41].

Various mechanisms have been implicated in tamoxifen resistance including alterations in its metabolism, relative or absolute changes in the levels of cofactor proteins, ligand-independent activation of the ER by growth factor signaling pathways and loss of or mutations in the ER leading to increased sensitivity to ligand and/or coactivator recruitment.

Tamoxifen is a prodrug with very little affinity for the ER. It must first be metabolized in the liver by the cytochrome P450 isoform, CYP2D6, into the active metabolites [42]. These metabolites compete with estrogen for the ligand-binding domain (LBD) of the ER, blocking the potential for estrogen stimulation and preventing conformational changes in the receptor critical for the association of cofactors and the transcription of estrogen-responsive genes [43]. Variations in this isoform, whether genetic (e.g., wild-type variant CYP2D61*) or as a result of inhibition by selective serotonin reuptake inhibitors (e.g., fluoxetine and paroxetine) [44], may alter the metabolism of tamoxifen, blunting its antiestrogenic activity [45].

Changes in the absolute or relative levels of coregulatory proteins may also govern tamoxifen action [46, 47]. As a rule, corepressors are recruited to the ER in the presence of an antagonist resulting in repression of transcription [48]. However, in the case of a partial agonist like tamoxifen, corepressors may become rate limiting such that a decrease in their levels may suppress the inhibitory effects of tamoxifen on the ER resulting in development of resistance [48, 49] and shorter disease-free survival [50].

More recently, it is suggested that tumors with increased expression of the coactivator SRC-1 have a greater probability of developing tamoxifen resistance, if they also overexpress the epidermal growth factor receptor, HER2/*neu* [51]. This is also seen in breast tumors expressing high levels of SRC-3 [33]. This action is thought to result after phosphorylation of the ER outside the nucleus i.e., in the cytoplasm or cell membrane, leading to activation of tyrosine kinase receptors such as insulin-like growth factor 1 receptor (IGF-1R), epidermal growth factor receptor (EGFR) and HER2/*neu* [52]. These receptors then initiate the activation of key downstream signaling kinases such as ERK1,2 mitogen-activated protein kinase (MAPK) and AKT, which in turn phosphorylate and activate the ER and/or its coactivators [34]. This ligand-independent cross talk between the ER and growth factor signaling pathways can sustain activation of pathway signaling and the survival of breast cancer by enhancing the estrogen agonist activity of tamoxifen [53].

MicroRNAs and interactions with ER cofactors in breast cancer

MiRNAs are RNA regulatory molecules [54, 55], which have recently come to the fore of molecular research into underlying mechanisms of many diseases and cellular processes, in particular cancer. They exert their functionality by directly binding to 3′ UTR sequences of their target genes resulting in translational repression or degradation of the target sequence. To date, 678 human miRNAs have been identified [56] many of which display highly characteristic temporal and tissue-specific expression patterns. In breast cancer, studies have shown that several miRNAs exhibit dysregulated expression patterns (*miR-10b*, *miR-125b*, *miR-145* and *miR-21*) [57, 58] and that their expression correlates with pathological features such as HER2/*neu* and ER status [59]. More recently, miRNA expression analysis has identified a number of miRNAs that are differentially expressed in the intrinsic breast cancer subtypes [60].

While there is increasing evidence of associations between the dysregulation of nuclear receptor cofactors and cancer and also between miRNAs and cancer, there is a paucity of information about interactions between specific miRNAs and steroid receptor cofactors in normal and malignant breast tissues. MicroRNA repositories, such as miRBase and PicTar, use bioinformatic analysis to identify sequence similarities between miRNAs and their target mRNAs. Consequently, many miRNAs are predicted to target steroid hormone receptor cofactors [61] (Table 1). However, to date only one such interaction has been experimentally demonstrated.

Hossain et al. demonstrated that SRC-3 expression is down regulated by *miR-17-5p*, thereby regulating the growth of breast cells [62]. Loss-of-function experiments in which *miR-17-5p* sequences were transfected into MCF-7 cells to specifically target SRC-3 expression reduced estrogen-stimulated growth, primarily due to the translational inhibition of SRC-3 (Fig. 2). Reciprocal experiments whereby transfection of sequences to inhibit *miR-17-5p* resulted in enhanced estrogen-stimulated growth [62]. Thus, cross talk between an ER coactivator and a miRNA mediates breast cancer proliferation in a cell-specific manner, responsive to the transcriptome as well as external stimuli. Since the *miR-17-92* polycistronic cluster of which *miR-17-5p* is a member is deleted in over a fifth of breast cancers [63], and SRC-3 is frequently upregulated in breast cancers [26], further studies to determine the precise nature and effects of the relationship between these genes are warranted. There can be little doubt about the existence

Table 1 Estrogen receptor coregulator genes and their partner regulatory miRNA

Cofactor	Synonyms/ isoforms	Chromosome location	Predicted partner miRNAs[a]
Coactivators			
NCOA1	SRC-1	2p23	*miR-557*, *miR-492*, *miR-23b*, *miR-23a*, *miR-219*, *miR-216*, *miR-148b*, *miR-148a*, *miR-130b*, *miR-130a let-7d*
NCOA2	GRIP1, TIF2	8q12	*miR-200b*, *miR-200c*, *miR-137*, *miR-181a*, *miR-181b*, *miR-181c*, *miR-556*, *miR-330*, *miR-199a*, *miR-199b*, *miR-299-5p*, *miR-186*, *miR-377*
NCOA3	AIB1, SRC-3	20q12	*miR-489*, *miR-767-3p*, *miR-140-5p*, *miR-615-5p*, *miR-675*, *miR-454*, *miR-320*, *miR-133a*, *mir-133b*, *miR-17-5p*[a,b]
PPARBP	MED1, TRIP2	17q12	*miR-568*, *miR-587*, *miR-618*, *miR-200a*, *miR-604*, *miR-611*, *miR-217*, *miR-212*, *miR-141*, *miR-221*, *miR-105*
CREBBP	CBP	16p13.3	*miR-153*, *miR-548b-3p*, *miR-628-3p*, *miR-130a*, *miR376c*, *miR-548d-3p*, *miR-200c*, *miR-582-3p*
EP300	p300	22q13.2	*miR-591*, *miR-132*, *miR-583*, *miR-574*, *miR-148a*, *miR-600*, *miR-609*, *miR-154*
Corepressors			
NCOR1	TRAC1	17p11.2	*miR-579*, *miR-548b*, *miR-573*, *miR-452*, *miR-556*, *miR548a*, *miR-185*, *miR-641*, *miR-154*, *miR-136*, *miR-135a*, *miR-135b*, *miR-10a*
NCOR2	SMRT	12q24	*miR-184*, *miR-572*, *miR-30a-5p*, *miR-34b*, *miR-34c*, *miR-29a*, *miR-29b*[b], *miR-16*, *miR-10b*[b], *miR-30d*, *miR-10a*, *miR195*, *miR-27b*[b]
NRIP1	RIP140	21q11.2	*miR-769-3p*, *miR-587*, *miR-186*, *miR-455*

[a] miRBase: miRNA sequences, targets and gene nomenclature

[b] Experimentally supported [62]

[c] Previously shown to have increased/decreased expression breast cancer

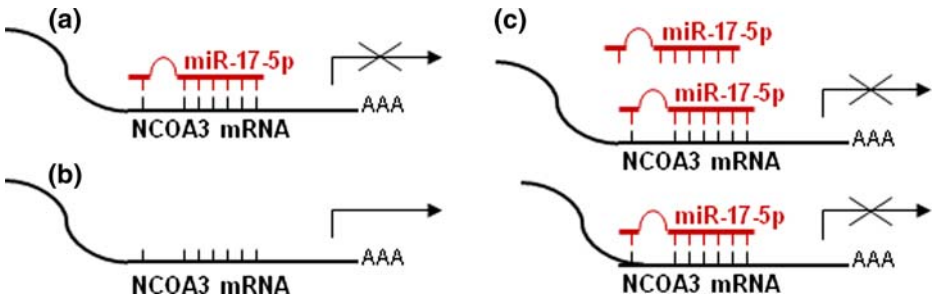

Fig. 2 **a** Transcriptional modulation of *NCOA3* by *miR-17-5p* is as yet the only experimentally demonstrated partnership between a miRNA and its nuclear receptor cofactor target gene. Disruption of this interaction either by loss (**b**) or gain (**c**) of miR-17-5p sequences could significantly disrupt the balance of *NCAO3* gene expression, with cascade effects on ER expression

further, as yet undiscovered, interactions between miRNA and ER cofactors.

MiRNA-based therapeutics

The potential scale of miRNA–mRNA interactions is vast and current bioinformatic analyses predict that miRNAs may target 30–50% of all known human protein coding genes [64]. Single miRNAs may target up to several hundred mRNAs and multiple miRNAs may converge on individual mRNAs, some transcripts having in excess of one hundred binding sites for different miRNA. Such scope for interactions between miRNAs and mRNAs opens up very exciting therapeutic avenues for multigenic diseases such as cancer and makes miRNAs compelling candidates for the identification of novel diagnostic and prognostic indicators. The latter point is borne out by findings that miRNA expression differs between tumor and normal tissues and that miRNA profiles can distinguish and classify tumors [see reviews 65, 66]. Indeed, in certain cases miRNA profiles provide more accurate prognostication than mRNA-based disease signatures, which have been shown to correlate and define breast cancer subtypes [67]. Various miRNA-targeting genes such as *ESR1* (*miR-206*), *ERBB2* (*miRs-125a* and *b*) and *SRC-3* (*miR-17-5p*) have been suggested as therapeutic targets [see review 68] in breast cancer.

Whether miRNA-based RNAi therapeutics will develop at similar speed to siRNA therapeutics [69, 70] remain to be seen. The challenges to development of siRNA-based therapeutics such as stable delivery and specificity of action [69, 71–73] will be revisited in the development of miRNA-based therapeutics. If these challenges can be overcome, miRNA-based therapeutics are likely to develop rapidly.

MicroRNA and response to therapy

MiRNA-mediated response to treatment is presently the focus of much research. A recent study by Miller et al. [74] showed a significant upregulation of eight miRNAs (*miR-221*, *miR-222*, *miR-181*, *miR-375*, *miR-32*, *miR-171*, *miR-213*, *miR-203*) and downregulation of seven miRNAs (*miR-342*, *miR-489*, *miR-21*, *miR-24*, *miR-27*, *miR-23*, *miR-200*) in a tamoxifen resistant cell line compared to a tamoxifen sensitive cell line. They reported increased expression of *miR-221* and *miR-222* in HER2/*neu* positive primary breast tumors, typically resistant to endocrine therapy, compared to HER2/*neu* negative tumors and suggested a relationship between tamoxifen resistance and reduced levels of the cell cycle inhibitor p27(Kip1) by augmenting *miR-221/222* expression [74]. Interestingly, the miRNA repositories miRBase and PicTar have identified potential cofactor targets for a number of these differentially expressed miRNAs; *miR-221* (PPARBP), *miR-222* (N-CoR), *miR-375* (SMRT), *miR-23* (SRC-1) and *miR-200* (SRC-2).

Podrigny et al. [75] demonstrated that prolonged exposure of rats to tamoxifen was associated with altered expression of known tumor-associated miRNAs and their protein targets including *miR-16* (BCL2), *miR-17-5p* (E2F1), *miR-20* (E2F1), *miR-106a* (RB1) and *miR-34* (NOTCH1).

Several reports also describe the involvement of miRNAs in response to treatments such as radiation and chemotherapy. For example, members of the *let-7* family exhibit rapidly altered expression following irradiation [76]. A decrease in the expression of *let-7* prior to radiation affects cell survival, increasing radioresistance, possibly through an interaction with survival genes and DNA damage response pathways. It has also been demonstrated that the miRNA expression profiles of cholangiocarcinoma cells change in response to the chemotherapy drug gemcitabine, and that the inhibition of *miR-21*, previously identified as an antiapoptotic miRNA in many cancers [57, 77–80], increases cellular sensitivity to this drug [81]. Also, *miR-214* has been shown to confer cisplatin resistance in ovarian cancer by targeting the PTEN/Akt pathway [82]. While considerable work remains to be done to elucidate the molecular mechanisms underlying possible miRNA-mediated response to treatment, these early studies hold promise of a place for miRNA therapeutics in augmenting existing treatments.

Conclusion

Transcriptional activation by the ER is a complex, multistep process regulated by coactivator and corepressor proteins. This process is further complicated with the recent discovery of miRNAs. Although many interactions between miRNAs and ER have been predicted, as of yet, few have been shown experimentally. The current rapid pace of miRNA research and profiling should provide deeper insight into these complex interactions, improve our knowledge of endocrine resistance and potentially identify a powerful tool for tumor prevention and therapy.

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