

Overcoming Resistance to Endocrine Therapy in Breast Cancer: New Approaches to a Nagging Problem

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Key Words

Breast cancer · Endocrine resistance · Growth factor receptors · MicroRNA · Epithelial-mesenchymal transition · Tamoxifen · Selective oestrogen receptor modulators

Abstract

In the majority of women, breast cancer progresses through increased transcriptional activity due to over-expressed oestrogen receptors (ER). Therapeutic strategies include: (i) reduction of circulating ovarian oestrogens or of peripherally produced oestrogen (in postmenopausal women) with aromatase inhibitors and (ii) application of selective ER modulators for receptor blockade. The success of these interventions is limited by the variable but persistent onset of acquired resistance and by an intrinsic refractiveness which manifests despite adequate levels of ER in about 50% of patients with advanced metastatic disease. Loss of functional ER leads to endocrine insensitivity, loss of cellular adhesion and polarity, and increased migratory potential due to trans-differentiation of the epithelial cancer cells into a mesenchymal-like phenotype (epithelial-mesenchymal transition; EMT). Multiple mechanisms contributing to therapeutic failure have been proposed: (i) loss or modification of ER expression including epigenetic mechanisms, (ii) agonistic actions

of selective ER modulators that may be enhanced through an increased expression of co-activators, (iii) attenuation of the tamoxifen metabolism through expression of genetic variants of P450 cytochromes which leads to more or less active metabolites and (iv) increased growth factor signalling particularly through epidermal growth factor receptor activation of pathways involving keratinocyte growth factor, platelet-derived growth factor, and nuclear factor κ B. In addition, the small non-coding microRNAs, recently recognized as critical gene regulators, exhibit differential expression in tamoxifen-sensitive versus resistant cell lines. Several studies suggest the potential of using these either as targets or as therapeutic agents to modulate EMT regulators as a means of reversing the aggressive metastatic phenotype by reversal of the EMT, with the added benefit of re-sensitization to anti-oestrogens.

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Introduction

Oestrogen, acting through the oestrogen receptor (ER), in conjunction with progesterone and other hormones, is responsible for the normal physiology of the female sex organs, but when a woman develops a neo-

Table 1. Proposed mechanisms of endocrine resistance

Cellular metabolic response	Receptor-related response
Decreased drug uptake	Loss or mutation (possible role of ER β)
Increased activity of p-glycoprotein and other efflux pumps	Aberrant post-translational modification
Intra-cellular drug sequestration	Constitutive activation
Metabolism of tamoxifen to oestrogenic compounds	Agonist action of tamoxifen
Alteration of other transcription factors or ER-associated proteins	Alteration of the oestrogen response element
Modification of signalling pathways	Epigenetic modification
Autonomous growth factor production	

plasm in her breast it assumes an undesired role. Indeed, the ER status is a major prognostic indicator and it is considered to be the primary predictor of the response to endocrine therapy [1–4].

Therapy of breast cancer, in women whose tumours over-express the ER, is based largely on the reduction of circulating or locally synthesized oestrogen or on receptor blockade with selective ER modulators such as tamoxifen [5]. Unfortunately, in addition to the approximately 20–30% of women with clinically ER-negative tumours who will not respond to endocrine agents due to the lack of a target, a significant proportion of ER-positive patients also exhibit *de novo* resistance to anti-oestrogens [6]. In, especially, the latter case, encountering early refractiveness may simply reflect a failure of the staging process to accurately determine the likelihood of success with an endocrine intervention and should be clearly recognized as such. In order to improve the stratification process, there is now the opportunity to perform additional phenotyping such as that afforded by the Oncotype DX and MapQuant Dx screens [7]. These are claimed to indicate distinct groups with differential prognoses. The vast majority of initially responsive patients eventually acquire resistance [8]. Post-menopausal women may benefit from brief periods of remission with aromatase inhibitors (to block peripheral oestrogen production) or other alternative therapies given after selective oestrogen receptor modulator therapy, but most patients experience a relapse and eventually die from metastatic disease [9, 10]. Much effort has been expended to understand the molecular mechanisms of this specific mode of drug resistance in order to find ways to overcome it. Early expectations that it could be explained solely by the loss of ER expression [11] have not been borne out *per se*. Observations from studies utilizing cell lines that have been manipulated to lose oestrogen sensitivity or acquire anti-oestrogen resistance have led to the detailed description

of a number of cellular processes that could be responsible for the defence of the cancer cell [12, 13]. These are listed in table 1, associated either with a metabolic response or with a structural or functional alteration of the receptor. It should be appreciated that resistance by the cancer cell to therapies aimed at antagonizing the action of the oestrogen is effected by making oestrogen (and therefore anti-oestrogens) irrelevant. This can be achieved either by abrogating the need for oestrogen for receptor activation, leading to an enhanced ER constitutive activity, or by activating oestrogen/ER-independent growth pathways. This review presents a brief overview of these mechanisms, which have been discussed in much detail elsewhere in the literature [1–4, 6, 10], and then proposes two other processes [epithelial-mesenchymal transition (EMT) and microRNA (miRNA) dysregulation] whereby the cancer cell may escape from the confines of endocrine control. Addressing these offers a promising new approach to combating endocrine resistance. All references to ER in this review are specifically in relation to ER α unless indicated otherwise.

ER Mutations

ER status is the primary predictor of the response to hormonal therapy, with the loss of expression accounting for *de novo* resistance [14]. Missense mutations in ER genes [15] have been found to result in constitutive ligand-independent activation and hypersensitivity [16]. Several recent studies have shown that ER mutations in the ligand-binding domain may confer endocrine resistance [17, 18] and this may well be due to the clonal expansion of rare mutant clones [19]. Acquisition of resistance during the course of therapy can be attributed to epigenetic changes in the ER gene that modulate transcription (e.g. aberrant methylation of the CpG islands of

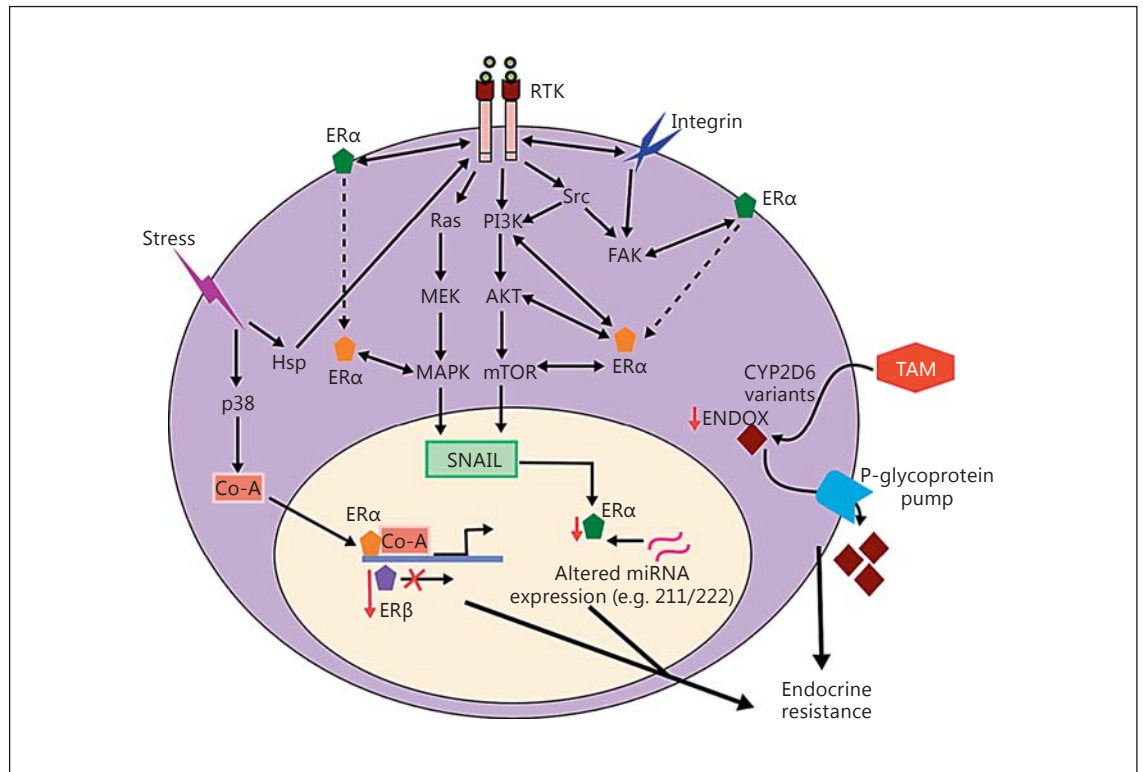


Fig. 1. Mechanisms of endocrine resistance. Various molecules are integrated in a complex network, each playing a role in the development of hormonal therapy resistance in breast cancer. RTK, with their down-stream signalling molecules (e.g. FAK, Src, Akt and PI3K) promote membrane oestrogen-independent ER phosphorylation, leading to constitutive activation, to induce a more invasive and malignant phenotype. Loss/reduction of ER β can also lead to increased ER α -promoted activation. Other cell-signalling receptors such as integrins contribute through synergizing of the action of RTK and the activation of FAK. Environmental stress

leads to an elevated expression of Hsp and p38, nboth of which enhance resistance by maintaining high levels of RTK and over-expression of ER co-activator molecules which stimulate ER signalling. Endocrine resistance can also be due to loss/mutation of ER by promotion of EMT through mediators such as SNAIL. ER α can also be downregulated by specific miRNA. Tamoxifen metabolism to its more active metabolite endoxifen can be reduced by a lower activity of CYP2D6 enzyme variants, as well as by an increased efflux through an upregulated P-glycoprotein pump. TAM = Tamoxifen; ENDOX = endoxifen; Co-A = co-activator.

the ER promoter, as well as histone deacetylation) [20, 21].

Treatment of ER-negative cells with inhibitors of histone deacetylase such as trichostatin A and suberoylanilide hydroxamic acid has been reported not only to restore ER expression but also to re-sensitize cells to endocrine therapy [22, 23]. Moreover, suberoylanilide hydroxamic acid has been found to reduce epidermal growth factor receptor (EGFR) expression and downregulate the activity of downstream effector molecules such as AKT and p38MAPK [24]. In addition, both in vitro and in vivo studies have documented the role of histone deacetylase inhibitors in the upregulation of aromatase expression, which can ultimately restore sensitivity to anti-oestrogens [25, 26].

Growth Factor-Stimulated Pathways

Phosphorylation of sites within the transactivation and DNA-binding domains of the ER can occur without the binding of oestrogen, thereby transforming the receptor into a constitutively activated form [27] abrogating the requirement for oestrogen. Evidence from a variety of experimental settings shows that several growth factor receptors such as EGFR, HER-2, IGF-1R and/or their downstream signalling molecules may be involved in direct ER phosphorylation in the absence of oestrogen binding, effectively rendering the presence of tamoxifen irrelevant [14, 28] (fig. 1).

Mutations and amplifications in the HER family, resulting in unregulated activation, are found in a propor-

tion of solid tumours and have been implicated in cancer progression [29]. The crosstalk between ER and HER-2 is mediated through the PI3K-Akt-mTOR signalling pathway that is frequently deregulated in breast cancer, as well as the Ras-Raf-MEK MAPK pathway [30, 31], leading to enhanced proliferation and survival of malignant cells that mediate resistance to endocrine therapy [32]. The PI3K-Akt pathway leads to ER phosphorylation at certain serine residues, resulting in a ligand-independent ER activation [33]. Additionally the Akt-negative regulator phosphatase and tensin homologue is generally inactive in endocrine resistant breast cancer cells [13]. Human epidermal growth factor 2 (HER2) was also found to increase the expression of the anti-apoptotic protein Bcl-2, leading to enhanced cancer cell survival and oestrogen-independent proliferation [34]. Crosstalk between ER and IGF-1R involves activation of the IGF-1R substrates IRS-1 and IGF-II by the ER and the reciprocal activation of ER through the ability of IGF-1R to phosphorylate ER at certain serine residues [35].

In many pre-clinical studies the use of specific growth factor receptor inhibitors as a beneficial strategy against endocrine resistance has been reported. For instance, inhibition of EGFR re-sensitizes endocrine resistant cells to the inhibitory effect of tamoxifen [13]. The monoclonal antibody trastuzumab (Herceptin) that specifically binds HER-2 has been found to attenuate endocrine resistant breast cancer proliferation [30]. Supplementation of such blockers to aromatase inhibitors or tamoxifen has been suggested to provide a better clinical outcome compared to single-agent therapy [36]. IGF-1R-specific inhibitors lower the basal phosphorylation levels of IGF-1R, EGFR and Akt, and they hinder growth in resistant cells [14]. Moreover, the combination of PI3K pathway inhibitors with tamoxifen enhances its pro-apoptotic effect, with improved clinical outcomes compared to the application of either agent alone [37]. Other combinations that have proved effective are the aromatase inhibitor letrozole and the rapamycin analogue RAD001, or the PI3K and mTOR inhibitor NVP-BEZ235, or the Akt inhibitor for patients who develop resistance to letrozole [37, 38].

Therefore, the use of combination therapy employing endocrine agents and inhibitors of specifically upregulated molecules represents a potential therapeutic strategy by which endocrine resistance might be prevented or overcome.

Other signalling pathways involving NOTCH and nuclear factor κ B (NF κ B) also control the expression of genes implicated in endocrine resistance [39]. NF κ B expression levels are elevated in ER-negative breast cancer

cells but markedly reduced in ER-positive cells [14], and it is known to engage in crosstalk with the ER [40] and regulate its activity, thus interfering with the response of ER-positive cells to endocrine therapy [41]. NF κ B has been reported by several groups to be over-expressed in endocrine resistant breast tumours [42–44]. This is interesting in view of its role in the inflammatory cascade [45].

Pharmacologic Tolerance of Tamoxifen

Another mechanism of endocrine resistance is related to how the drug (e.g. tamoxifen) is distributed and metabolized within the patient's body. This is regulated through the action of various enzymes. Tamoxifen is metabolized by cytochrome P450 (CYP450) to several compounds. Some of these actually exhibit oestrogen-like activity and would therefore accelerate the onset of endocrine resistance. Others possess enhanced anti-oestrogenic properties; endoxifen (4-hydroxy-N-desmethyl tamoxifen) is the most potent tamoxifen metabolite (with a 100-fold higher affinity to ER than tamoxifen) [46, 47]. The generation of endoxifen is dependent on CYP2D6, for which several allelic variants have been reported [48]. Some of these variants have a reduced activity, resulting in a reduction of the formation of endoxifen, which lessens the antagonistic effect of tamoxifen, thereby allowing a greater degree of drug resistance in such women compared to those with the wild-type alleles generating the most active CYP2D6 enzyme. Another reason for the lower intra-tumoural endoxifen concentrations in women with an acquired resistance is the increased efflux mediated through the action of various membrane pumps, such as P-glycoprotein [47]. The mechanisms by which such pumps are over-expressed include over-expression of the nuclear receptor PXR (in response to extended exposure to tamoxifen) that can enhance the expression of P-glycoprotein, ultimately increasing the endoxifen efflux from tumour cells. Ahmad et al. [49] suggested the administration of oral endoxifen to patients to counter the drug efflux correlated with tamoxifen treatment.

Expression of ER β

The discovery of a second distinct receptor binding to oestrogen (ER β) [50] has led to much speculation regarding the relationship between the two forms. However, there is still a lack of clarity with regard to the precise role

of ER β . It is generally thought to be less expressed in malignant cells compared to normal mammary cells [51], hence indicating a role for ER β as a tumour suppressor as it has been shown to reduce the proliferation effect of ER α , leading to hormonal therapy resistance [52, 53]. It may influence the ER α activity (thereby affecting the clinical outcome) by forming heterodimers. Nonetheless, it has been suggested that ER β could contribute to endocrine resistance through mediation of an agonistic, rather than an antagonistic, effect of tamoxifen if the transcription of target genes is mediated through the AP-1 site rather than an ER-responsive element site [54]. Thus, the particular role that ER β plays during endocrine resistance still needs further investigation.

Altered ER Co-Regulatory Recruitment

The ER co-regulatory molecules are ER-associated proteins that regulate the transcriptional activity of the ER and can be either co-activators or co-repressors. Changes in the level of these co-regulatory proteins can influence the relative balance of action of a mixed agonist/antagonist such as tamoxifen [55]. For instance, AIB-1 is among the most common co-activators, found to be up-regulated in over 50% of breast carcinomas. Webb et al. [56] showed that AIB-1 induces the agonistic, instead of the antagonistic, activity of tamoxifen. On the other hand, the co-repressor N-CoR is correlated with tamoxifen sensitivity, as it recruits histone deacetylase that leads to chromatin condensation, and the subsequent inhibition of gene transcription. Lavinsky et al. [57] reported a decrease in the transcriptional levels of N-CoR in breast cancer cells resistant to endocrine therapy. Thus, the progressive loss of co-repressor activity during the course of endocrine therapy may be a predominant mechanism by which an acquired resistant phenotype might develop.

Tumour Microenvironment

The progression of malignancy, which is characterized by the conversion of cells into more invasive, motile entities resistant to hormonal therapy, depends to a great extent on its interaction with the surrounding microenvironment [58]. Among other molecules, much attention has been focused on integrins [59] as their downstream mediators, particularly FAK, MAPK and PI3K [60], are involved in endocrine resistance. Integrin-activated FAK was found to further facilitate the signalling crosstalk be-

tween integrin and several growth factor receptors that have been implicated in the enhancement of endocrine resistance in breast cancer (e.g. c-erbB2) [61].

Stress is also known to enhance endocrine resistance through the upregulation of stress-related mediators, such as heat shock proteins (Hsp) and p38 MAPK [62, 63]. During endocrine resistance, Hsp90, Hsp70 and Hsp27 play a role in chaperoning the mutated and over-expressed HER-2/ neu, c-Src and IGFR-1, aiding in the emergence of treatment-resistant tumour cells that are significantly dependent on these Hsp for maintenance of high levels of such oncogenes [64]. On the other hand, p38 stimulates endocrine resistance through its phosphorylation of ER α , which enhances interaction with co-activators, specifically increasing the AIB-1 activity, which leads to the ER ligand-independent transactivation [65].

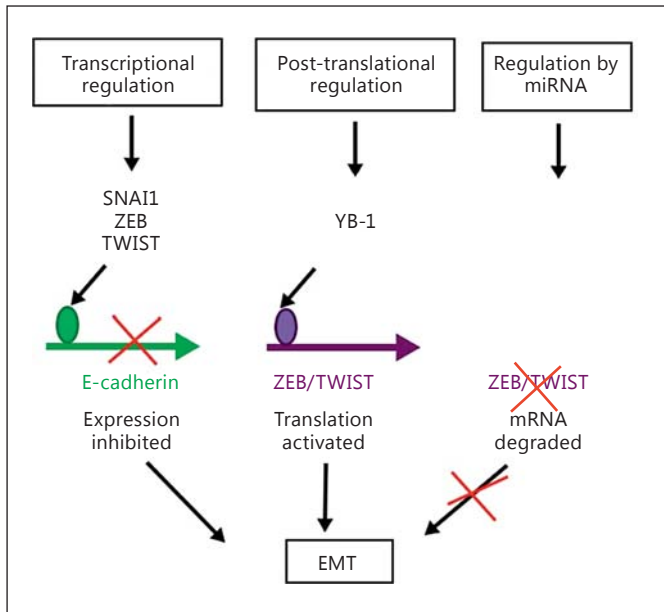
Clinical Correlates

Assessment of the factors responsible for resistance is difficult in a clinical setting. Where the primary tumour has been excised, there is no opportunity for post-treatment molecular analysis. Measurement of the treatment response also relies on monitoring of the survival and relapse, which may be a consequence of the inherent tumour aggressiveness as opposed to acquisition of therapy resistance [66, 67]. However, in the neoadjuvant setting, where the primary tumour remains in situ, there is the opportunity to measure tumour growth with ultrasound or mammography and even the possibility of more than one biopsy to assess gene expression [68] pre- and post-treatment to relate to the clinical response.

Several treatment strategies have employed either combination therapy with anti-oestrogen and aromatase inhibitors to overcome resistance [69, 70] or augmentation of endocrine agents with anti-HER2 monoclonal antibodies [71]. The mTOR inhibitor everolimus and the EGFR inhibitor gefitinib [72, 73] can overcome resistance mediated through the PI3K-AKT-mTOR pathway. Metastatic breast cancer patients have been reported to benefit from combinations of everolimus and fulvestrant following relapse on aromatase inhibitors [74].

Epithelial-Mesenchymal Transition

EMT was first identified as a developmental process in which cells of an epithelial phenotype convert into cells with mesenchymal characteristics, with a significant reduction in cell adhesion points, as well as an increased cell



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Fig. 2. EMT is determined by various molecular events; examples are illustrated. Most commonly, several groups of transcription factors can act to downregulate epithelial markers such as E-cadherin. Regulation of such transcription factors by molecules such as YB-1 can sustain EMT. Conversely, miRNAs can block EMT by targeting its mediators.

migration capacity [75]. EMT is now generally accepted as a prominent hallmark of cancer progression [76].

EMT is driven by several regulatory networks (examples are illustrated in fig. 2), which include a number of nuclear transcription factors. Translational control of the proteins involved in EMT is also regulated by the expression of various small non-coding RNA molecules (miRNA). Proteins that have been linked to the transcriptional control of EMT are SNAIL, SLUG, TWIST, ZEB1, ZEB2, E47, FOXC2 and Krüppel-like factor 8 [77]. They can mediate EMT through binding directly (e.g. SNAIL and ZEB) or indirectly (e.g. TWIST and FOXC2) to the promoter region of E-cadherin, repressing its expression [78]; this is one of the key characteristics of epithelial cells that is lost during EMT. They also modulate the expression of other junction proteins such as claudins and desmosomes, leading to trans-differentiation of epithelial cells into mesenchymal-like cells [79]. Several signalling pathways are linked to the activation of EMT transcription factors, particularly through receptor tyrosine kinase (RTK) and integrin pathways, activating downstream signalling molecules such as Src, MAPK, PI3K, Akt and FAK, leading to an enhanced SNAIL expression [80, 81].

Besides transcriptional regulatory mechanisms, general translational mechanisms have been found to regulate EMT. An example is the enhancement of ZEB and TWIST by the Y-Box binding protein 1 (YB-1) [82]. Moreover, GSK3 β [83] is involved in the maintenance of epithelial differentiation through its ability to phosphorylate SNAIL, targeting it for degradation [84]. Conversely, its downregulation can lead to EMT. Additionally, a number of matrix metalloproteinases have been linked to the stabilization of EMT through their modulation of several signal transduction pathways [77]. In addition, inflammatory responses that are associated with most stages of tumour development [85] have also been implicated as a key inducer of EMT. SNAIL may also induce the expression of pro-inflammatory interleukins (IL-1, IL-6 and IL-8) [86].

A recent paper [87] describes studies with knock-in reporter mouse lines which show that normal gland-reconstituting mammary stem cells as well as tumour-initiating cells can undergo distinct EMT programmes under the influence of SNAIL and SLUG.

EMT and Endocrine Resistance

Development of endocrine insensitivity has been observed in several studies on manipulated cancer cell lines with characteristics similar to those of cells undergoing EMT, indirectly indicating a link between the two processes [88, 89]. Moreover, several growth factor receptors (e.g. EGFR, IGF-1R and FGFR1), which are highly expressed in ER-negative cells, are involved in the EMT process, indicating another link between EMT and endocrine resistance. The c-erbB2 receptor is associated both with acquisition of endocrine resistance in breast cancer cells [90] and with EMT [91]. The expression of E-cadherin is inhibited in cells over-expressing c-erbB2, resulting in a reduction of cell adherence and a gradual loss of epithelial morphology and architecture. Moreover, EGFR/c-erbB2 over-expression has been observed to be localized to sites of membrane protrusion and shape change, leading to a motogenic phenotype through engagement with a pathway linking it to the actin cytoskeleton of cancer cells [92].

The first evidence of a direct link between endocrine insensitivity and EMT came from *in vitro* studies performed in our laboratory on ER-depleted cells. The enforced loss of ER expression by shRNA transfection was found to convert the non-invasive epithelial MCF-7 cell line into a more mesenchymal-like phenotype displaying

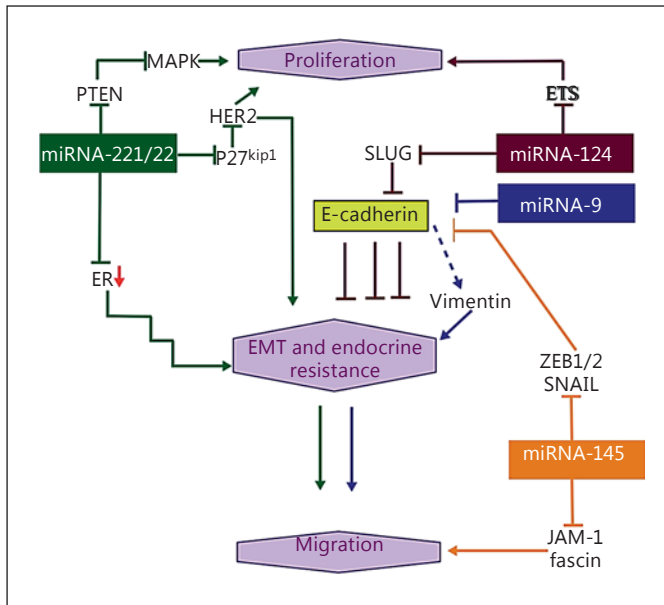


Fig. 3. Integrated miRNA network. miRNAs can be involved in a complex network where they can target the same or different mRNAs affecting different processes. miRNA-221/222 can target and suppress the expression of phosphatase and tensin homologue and p27kip1, thus alleviating the repression of MAPK and HER2, respectively, ultimately leading to a high cell proliferation. miRNA-221/222 also directly targets ER, pushing cancer cells toward EMT and endocrine resistance. On the other hand, miRNA-124 and miRNA-145 attenuate EMT and resistance to hormonal therapy by targeting EMT-related transcription factors (SLUG and SNAIL), thus preventing their inhibition of E-cadherin. miRNA-124 also targets and degrades ETS, which leads to a lower cell proliferation. miRNA-145 represses the migration capacities of cancer cells by targeting JAM-1 and fascin. miRNA-9 plays a crucial role in the induction of EMT through direct repression of E-cadherin and indirect upregulation of vimentin.

highly invasive characteristics [12, 93]. Independently derived ER-silenced lines, using different vectors and variously designated as pII, YS2.5 and IM26, all display remarkably similar features, resembling basal-like metastatic and claudin/occludin-low tumour subtypes that have lost their luminal cell markers. Typically, they all exhibit cadherin switching, i.e. downregulation of E-cadherin and upregulation of mesenchymal N-cadherin (also described by Iseri et al. [89] for their drug-resistant MCF7 lines), with enhanced matrix metalloproteinase production and secretion. Conversion of the cell's structural intermediate filament system from a keratin-based network to a vimentin-rich one is clearly reminiscent of cells undergoing EMT. pII cells show a close similarity to the tumour-derived ER-ve MDA-MB-231 cell line, which is

generally regarded as being mesenchymal like. EMT mediates endocrine resistance through the action of the EMT transcription mediators SNAIL and SLUG. Dhasa-rathy et al. [94] reported the direct repression of ER α expression by SNAIL. SLUG, on the other hand, was reported to be over-expressed in an Src-dependent manner in malignant breast cancer cells and was documented to enhance the anti-apoptotic behaviour of cancer cells, aiding in resistance to anti-cancer therapy [95]. Furthermore, the collective consequence of the action of such transcription factors (i.e. E-cadherin loss), leading to a lower intracellular adhesion and enhanced invasion and motility, was the development of endocrine resistance [96].

Hence, EMT has emerged as a major mediator of endocrine resistance in breast cancer cells [97, 98]. Interestingly, several recent studies have attempted to reverse EMT as a therapeutic strategy to prevent the development of metastatic tumours. For example, in a preclinical study by Yoshida et al. [99] it was found that the anti-tumour effect of the microtubule inhibitor eribulin was facilitated by stimulation of the reversal of EMT (a mesenchymal-epithelial transition in resistant breast cancer cells). Khan et al. [100] recently proposed an oral contraceptive (centchroman) as a candidate drug to prevent breast cancer metastasis by virtue of its ability to reverse EMT. Administration of a TGF- β inhibitor together with doxorubicin was reported to produce a significantly better clinical outcome than that seen with doxorubicin alone [101]. Thus, there is considerable interest in designing strategies to overcome endocrine resistance that are based on the principle of reversing EMT as a means of re-sensitizing the tumour cells to anti-oestrogens.

MicroRNAs and EMT

miRNA and Cancer Progression

A particular mRNA can be targeted by one or several miRNAs, and a single miRNA can target several mRNAs [102], suggesting a highly integrated network of connections synchronizing the phenotype of a cell (some examples are illustrated in fig. 3).

Observations of dysregulation in the expression of miRNAs in cancer have been variously correlated with chromosomal instability, mutations and polymorphisms, epigenetic alterations in coding genes, promoter methylation or modifications in transcription factor activity [103, 104]. Although some miRNAs show an increased expression (oncogenic) [105], it appears that the major-

ity are downregulated in cancer cells and are regarded as tumour suppressors [106, 107]. The interesting point is that miRNAs can be viewed both as prognostic or therapy markers (e.g. detection in blood) [108] and therapeutic targets [109, 110]. Moreover, exogenous miRNA can be used as a therapeutic strategy (discussed further below).

An altered expression of several miRNAs has been implicated in endocrine resistance in breast cancer [111, 112]. Interestingly, a significant number of miRNAs have been particularly linked to EMT through modulation of target mRNAs that play an important role in this process, presenting a new mechanism by which EMT can be modulated.

miRNAs Regulate EMT and Hence Influence Endocrine Resistance

Several miRNAs (miRNA-9, miRNA-24, miRNA-29, miRNA-29a, miRNA-103/107, miRNA-16b-25, miRNA-155 and miRNA-221/222) have been reported to convert breast cancer cells from their epithelial morphology into a more mesenchymal phenotype [113]. For instance, miRNA-9, upregulated in cancer cells [111], induced a mesenchymal appearance coincident with a reduction in E-cadherin and a parallel increase in vimentin [114]. miRNA-221/222, described in various reports as mediators of breast cancer progression, were found to be lower in HER2-negative/ER-positive versus HER2-positive/ER-negative cells and inhibit ER protein expression [115]. Several studies have observed an association between the development of endocrine resistance and over-expression of miRNA-221/222 [116–118]. Elevated miRNA-221/222 leads to downregulation of the cell cycle inhibitor p27^{kip1}, enhancement of β -catenin activation, and suppression of ER expression, which ultimately leads to endocrine resistance. In a contrary report by Pandey and Picard [119], whilst a repressive effect of miR-22 was observed, no repression of ER expression following miRNA-221/222 over-expression was found. Indeed, they observed a positive correlation with miRNA-221/22 and also miR-219 upregulation of the ER 3'UTR. They were unable to reconcile these differences with the earlier reports. Yet another study, by Zhao et al. [116], reported suppression of ER protein expression but not mRNA following miRNA-221/222 transfection into MFC7 cells, while the knockdown of such miRNAs partially restored the ER protein expression in ER-positive cells. Clearly, further investigations are needed to determine the effect of miRNA-221/222 in ER mRNA and protein regulation, which will help to determine the

functional role of such miRNAs and their usefulness in breast cancer therapy.

A number of other miRNAs have been implicated in EMT reversal. These include the miR-7, miR-124, miR-145 and miR-200 family and miR-205, miR-375 and miR-448 [113, 120].

In vivo studies have shown reduced invasion and lung metastasis in MDA-MB-231 cells with an elevated miR-124 expression [121]. Both in vitro and in vivo studies have attributed the anti-invasive capacity of miR-124 to its ability to target the 3' UTR region of SLUG, reducing its expression [122], while also enhancing E-cadherin levels, or to target flotillin-1, RhoG or ROCK [123, 124].

Similarly, miR-145 over-expression also inhibits EMT by reducing ZEB1/2 and SNAIL expression levels while enhancing E-cadherin expression [125]. Direct links have been reported between negative regulators of EMT, SOX2 and KLF4 and miRNA-145 [126, 127]. miRNA-145 also enhanced EMT reversal through targeting of Oct4.

These and several preclinical studies clearly indicate that miRNA-mediated reversal of EMT, utilizing an endogenous pathway, could be an effective therapeutic strategy for breast cancer patients with metastatic disease.

miRNAs in Cancer Proliferation and Invasion

Besides their role in EMT, several miRNAs (e.g. miR-10b, miR-21, miR-27a, miR-221/222, miR-301a and miR-495) have been linked to either enhancement [113, 128] or inhibition (e.g. miR-22, miR-31, miR-93, miR-145, miR-206, miR-335, miR-486-p and miR-769-3p) of cancer proliferation and metastasis [113].

Both miR-21 and miR-221/222 are involved in cancer progression through upregulation of HER2 and MAPK [102] and inhibition of p27^{kip1} and the phosphatase and tensin homologue [113, 129, 130]. Conversely, miR-124 over-expression is also associated with a reduced cancer proliferation as it causes cell cycle arrest at G₀ and G₁ [131]; its anti-proliferative effect is mediated by targeting of the E26 transformation specific-1 gene. miRNA-145 has an anti-metastatic effect mediated through the reduced expression of JAM-A and fascin, both of which have been reported to enhance the migration capacities of cancer cells [132, 133]. It also suppresses the mucin 1 gene, leading to downregulation of β -catenin and cadherin-11 [134].

Therapeutic Utility of miRNAs

Several miRNAs have been described as markers for distant metastases in both ER-positive [135] and triple-negative breast cells [136, 137], and some (miR-128a and miR-210) have been identified as markers for disease-free survival [138, 139]. They are also potentially useful as markers for the therapeutic response to tamoxifen in advanced ER-positive patients or to trastuzumab treatment in ER-negative patients [140, 141].

miRNA-based drugs could also be used either to target specific oncogenes (to suppress their expression) or to replace downregulated miRNAs that function as tumour suppressors. The ability of some miRNAs to simultaneously target several different mRNA molecules is also an attractive feature for the treatment of multifactorial diseases [142]. Of course, on the flip side, the pleiotropic nature of these miRNAs means that greater care needs to be exercised to identify any undesired potential targets.

miRNA Mimics

With the increasingly rapid identification and sequencing of the miRNA population of many cell types, a large number of miRNA mimics are now commercially available. Thus miRNAs that are downregulated in breast tumours may be restored to normal levels by introduction of such constructs (i.e. replacement therapy). These mimics would have the same sequence as the absent naturally occurring miRNA. Typically, they may be introduced through viral or liposomal delivery [143] as with other nucleic acids.

Antagomirs

Antagomirs are small oligonucleotides that inhibit the miRNA-target mRNA interaction by binding to the appropriate miRNA molecules [144–146]. For example, the anti-miR-21 oligonucleotide was found to suppress the growth and migration effects of miR-21 in both ER-positive and ER-negative cells in vitro, and it also suppressed tumour growth in xenograft mouse models in vivo [147, 148]. Moreover, this anti-miR-21 also restored breast cancer sensitivity to topotecan and taxol. Combination therapy with both anti-miR-21 and taxol achieved a 50% therapeutic reduction in cancer cell viability and invasion over taxol alone [148].

Prior to clinical usage, it is also important to determine the extent, if any, of interaction with conventional drugs. To date, the most developed miRNA-based agent is anti-miR-122, which is used for the treatment of hepatitis C infection [149].

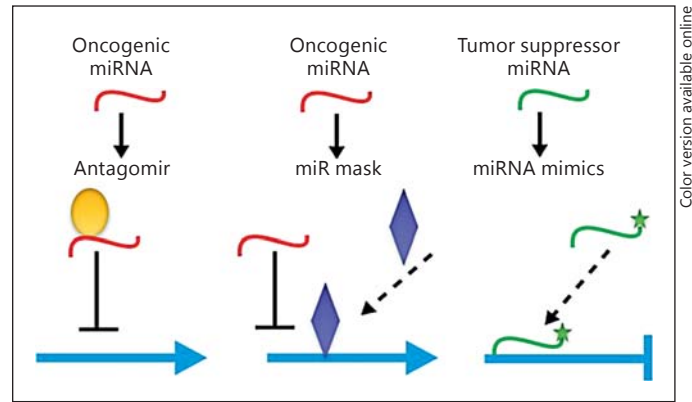


Fig. 4. miRNA manipulation as a treatment modality. miRNA binds to a specific target mRNA to facilitate the downregulation of target proteins. miRNA mimics are synthetic oligonucleotide duplexes that have the same sequence as the endogenous miRNA and function as replacements for the downregulated miRNAs (acting as tumour suppressors). Antagomirs (also called antimiRs) are oligonucleotides that have complementarity with the target miRNA, thereby binding and preventing them from interacting with the target mRNA, allowing a normal translation. An miR mask is complementary to a sequence in the mRNA and binds to it without affecting the mRNA integrity or translation, yet it prevents the endogenous miRNA from binding. A sponge miR mask differs from the miR mask in that it binds to any mRNA with a similar target sequence and is therefore miRNA seed specific and not gene specific.

miR Masks

miR masks are a range of oligonucleotide compounds currently under development that are designed to bind to either a specific miRNA or its target mRNA. The miR masks that bind to a target mRNA would potentially prevent the binding of a specific miRNA seed family, thus stopping only one miRNA from interacting with its target mRNA [94].

The different treatment strategies mentioned above are illustrated in figure 4.

Conclusion

Endocrine resistance presents as a highly complex network of events that requires multiple therapeutic interventions to combat it. In addition to the now well-trodden linear strategies of inhibition of growth factor receptors that appear to propagate resistance (with monoclonal antibodies and small-molecule inhibitors), the increasing evidence linking resistance to EMT provides possibilities for a different approach. In this case, the aim is to reverse

EMT and force the cell back to an epithelial phenotype with a reduced migratory capacity and possibly re-sensitized to anti-oestrogens. This may be achieved either with the application of synthetic antagomirs to block the action of endogenous miRNAs that restrict the expression of epithelial genes or miRs that can downregulate specific EMT mediators to suppress or reverse the mesenchymal-like phenotype typical of metastasizing cells.

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Disclosure Statement

The authors declare that no conflicts of interest exist.

References

- Massarweh S, Schiff R: Unraveling the mechanisms of endocrine resistance in breast cancer: new therapeutic opportunities. *Clin Cancer Res* 2007;13:1950–1954.
- Ring A, Dowsett M: Mechanisms of tamoxifen resistance. *Endocr Relat Cancer* 2004;11:643–658.
- Mouridsen HT, Rose C, Brodie AH, et al: Challenges in the endocrine management of breast cancer. *Breast* 2003;12:S2–S19.
- Musgrove EA, Sutherland RL: Biological determinants of endocrine resistance in breast cancer. *Nat Rev* 2009;9:631–643.
- Katzenellenbogen BS, Katzenellenbogen JA: Estrogen receptor transcription and transactivation: estrogen receptor alpha and estrogen receptor beta – regulation by selective estrogen receptor modulators and importance in breast cancer. *Breast Cancer Res* 2000;2:335–344.
- Dixon MJ: Endocrine resistance in breast cancer. *New J Sci* 2014;2014:1–27.
- Sparano JA, Paik S: Development of the 21-gene assay and its application in clinical practice and clinical trials. *J Clin Oncol* 2008;26:721–728.
- Massarweh S, Schiff R: Resistance to endocrine therapy in breast cancer: exploiting estrogen receptor/growth factor signaling crosstalk. *Endocr Relat Cancer* 2006;13:S15–S24.
- Osborne CK: Tamoxifen in the treatment of breast cancer. *N Engl J Med* 1998;339:1609–1168.
- Normanno N, Maio MD, Maio ED, et al: Mechanisms of endocrine resistance and novel therapeutic strategies in breast cancer. *Endocr Relat Cancer* 2005;12:721–747.
- Jaiyesimi IA, Buzdar AU, Decker DA, et al: Use of tamoxifen for breast cancer: twenty-eight years later. *J Clin Oncol* 1995;13:513–529.
- Al Saleh S, Al Mulla F, Luqmani YA: Estrogen receptor silencing induces epithelial to mesenchymal transition in human breast cancer cells. *PLoS One* 2011;6:1–10.
- Austreid E, Lonning PE, Eikesdal HP: The emergence of targeted drugs in breast cancer to prevent resistance to endocrine treatment and chemotherapy. *Expert Opin Pharmacother* 2014;15:681–700.
- Gracia-Becerra R, Santos N, Diaz L, et al: Mechanisms of resistance to endocrine therapy in breast cancer: focus in signaling pathways, miRNAs and genetically based resistance. *Int J Mol Sci* 2013;14:108–145.
- MacGregor SJ, Liu H, Bentrem DJ, et al: Allosteric silencing of activating function 1 in the 4-hydroxytamoxifen estrogen receptor complex is induced by substituting glycine for aspartate at amino acid 351. *Cancer Res* 2000;60:5097–5105.
- Fuqua SA, Wiltschke C, Zhang QX, et al: A hypersensitive estrogen receptor-alpha mutation in premalignant breast lesions. *Cancer Res* 2000;60:4026–4029.
- Segal CV, Dowsett M: Estrogen receptor mutations in breast cancer—new focus on an old target. *Clin Cancer Res* 2014;20:1724–1726.
- Li S, Shen D, Shao J, et al: Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. *Cell Rep* 2013;4:1116–1130.
- Jeselson R, Buchwalter G, De Angelis C, et al: ESR1 mutations: a mechanism for acquired endocrine resistance in breast cancer. *Nat Rev Clin Oncol* 2015;12:573–583.
- Yang X, Phillips DL, Ferguson AT, et al: Synergistic activation of functional estrogen receptor (ER)-α by DNA methyltransferase and histone deacetylase inhibition in human ER-α-negative breast cancer cells. *Cancer Res* 2001;61:7025–7029.
- Parl FF: Multiple mechanisms of estrogen receptor gene repression contribute to ER-negative breast cancer. *Pharmacogenomics J* 2003;3:251–253.
- Marks PA: The mechanism of the anti-tumor activity of the histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA). *Cell Cycle* 2004;3:534–535.
- Fan J, Yin WJ, Lu JS, et al: ER alpha negative breast cancer cells restore response to endocrine therapy by combination treatment with both HDAC inhibitor and DNMT inhibitor. *J Cancer Res Clin Oncol* 2008;134:883–890.
- Zhou Q, Shaw PG, Davidson NE: Inhibition of histone deacetylase suppresses EGF signaling pathways by destabilizing EGFR mRNA in ER-negative human breast cancer cells. *Breast Cancer Res Treat* 2009;117:443–451.
- Sabnis GJ, Goloubeva O, Chumsri S, et al: Functional activation of the estrogen receptor-α and aromatase by the HDAC inhibitor entinostat sensitizes ER-negative tumors to letrozole. *Cancer Res* 2011;71:1893–1903.
- Giacinti L, Giacinti C, Gabellini C, et al: Scriptaid effects on breast cancer cell lines. *J Cell Physiol* 2012;227:3426–3433.
- Ali S, Coombes RC: Endocrine-responsive breast cancer and strategies for combating resistance. *Nat Rev Cancer* 2002;2:101–112.
- Musgrove EA, Sutherland RL: Biological determinants of endocrine resistance in breast cancer. *Nat Rev Cancer* 2009;9:631–643.
- Hynes NE, MacDonald G: ErbB receptors and signaling pathways in cancer. *Curr Opin Cell Biol* 2009;21:1777–1784.
- Shou J, Massarweh S, Osborne CK, et al: Mechanisms of tamoxifen resistance: increased estrogen receptor-HER2/neu crosstalk in ER/HER2-positive breast cancer. *J Natl Cancer Inst* 2004;96:926–935.
- Azim HA Jr, Piccart MJ: Simultaneous targeting of estrogen receptor and HER2 in breast cancer. *Expert Rev Anticancer Ther* 2010;10:1255–1263.
- Stemke-Hale K, Gonzalez-Angulo AM, Lluch A, et al: An integrative genomic and proteomic analysis of PIK3CA, PTEN, and AKT mutations in breast cancer. *Cancer Res* 2008;68:6084–6091.
- Campbell RA, Bhat-Nakshatri P, Patel NM, et al: Phosphatidylinositol 3-kinase/AKT-mediated activation of estrogen receptor alpha: a new model for anti-estrogen resistance. *J Biol Chem* 2001;276:9817–9824.
- Dowsett M: Overexpression of HER-2 as a resistance mechanism to hormonal therapy for breast cancer. *Endocr Relat Cancer* 2001;8:191–195.
- Becker MA, Ibrahim YH, Cui X, et al: The IGF pathway regulates ERalpha through a S6K1-dependent mechanism in breast cancer cells. *Mol Endocrinol* 2011;25:516–528.
- Evans AH, Pancholi S, Farmer I, et al: EGFR/HER2 inhibitor AEE788 increases ER-mediated transcription in HER2/ER-positive breast cancer cells but functions synergistically with endocrine therapy. *Br J Cancer* 2010;102:1235–1243.

- 37 Cavazzoni A, Bonelli MA, Fumarola C, et al: Overcoming acquired resistance to letrozole by targeting the PI3K/AKT/mTOR pathway in breast cancer cell clones. *Cancer Lett* 2012; 323:77–87.
- 38 Vilquin P, Villedieu M, Grisard E, et al: Molecular characterization of anastrozole resistance in breast cancer: pivotal role of the Akt/mTOR pathway in the emergence of de novo or acquired resistance and importance of combining the allosteric Akt inhibitor MK-2206 with an aromatase inhibitor. *Int J Cancer* 2013;133:1589–1602.
- 39 Magnani L, Stoeck A, Zhang X, et al: Genome-wide reprogramming of the chromatin landscape underlies endocrine therapy resistance in breast cancer. *Proc Natl Acad Sci USA* 2013;110:E1490–E1499.
- 40 Sas L, Lardon F, Vermeulen PB, et al: The interaction between ER and NFκB in resistance to endocrine therapy. *Breast Cancer Res* 2012; 14:212–216.
- 41 Zheng LH, Zhao YH, Feng HL, et al: Endocrine resistance in breast cancer. *Climacteric* 2014;17:522–528.
- 42 Zhou Y, Yau C, Gray JW, et al: Enhanced NFκB and AP-1 transcriptional activity associated with antiestrogen resistant breast cancer. *BMC Cancer* 2007;7:59.
- 43 deGraffenried LA, Chandrasekar B, Friedrichs WE, et al: NF-κB inhibition markedly enhances sensitivity of resistant breast cancer tumor cells to tamoxifen. *Ann Oncol* 2006;15: 885–890.
- 44 Zhou Y, Eppenberger-Castori S, Eppenberger U, et al: The NFκB pathway and endocrine-resistant breast cancer. *Endocr Relat Cancer* 2005;12(suppl 1):S37–S46.
- 45 Hagemann T, Wilson J, Kulbe H, et al: Macrophages induce invasiveness of epithelial cancer cells via NF-κB and JNK. *J Immunol* 2005;175:1197–1205.
- 46 Briest S, Stearns V: Tamoxifen metabolism and its effect on endocrine treatment of breast cancer. *Clin Adv Hematol Oncol* 2009;7:185–192.
- 47 Teft WA, Mansell SE, Kim RB: Endoxifen, the active metabolite to tamoxifen, is a substrate of efflux transporter P-glycoprotein (multidrug resistance 1). *Drug Metab Dispos* 2011; 39:558–562.
- 48 Ring A, Dowsett M: Mechanisms of tamoxifen resistance. *Endocr Relat Cancer* 2004;11: 643–658.
- 49 Ahmad A, Ali SM, Ahmad MU, et al: Orally administered endoxifen is a new therapeutic agent for breast cancer. *Breast Cancer Res Treat* 2010;122:579–584.
- 50 Nilsson S, Gustafsson J-A: Estrogen receptor transcription and transactivation: Basic aspects of estrogen action. *Breast Cancer Res* 2000;2:360–366.
- 51 Lazennec G, Bresson D, Lucas A, et al: ERβ inhibits proliferation and invasion of breast cancer cells. *Endocrinology* 2001;142:4120–4130.
- 52 Hopp TA, Weiss HL, Parra IS, et al: Low levels of estrogen receptor β protein predict resistance to tamoxifen therapy in breast cancer. *Clin Cancer Res* 2004;10:7490–7499.
- 53 Liu M-M, Albanese C, Anderson CM, et al: Opposing action of estrogen receptor α and β on cyclin D1 gene expression. *J Biol Chem* 2002;277:24353–24360.
- 54 Paech KM, Webb P, Kuiper GG, et al: Differential ligand activation of estrogen receptors ERα and ERβ at AP-1 sites. *Science* 1997;277:1508–1510.
- 55 Shim W-S, DiRenzo J, DeCaprio JA, et al: Segregation of steroid receptor coactivator-1 from steroid receptors in mammary epithelium. *Proc Natl Acad Sci USA* 1999;96:208–213.
- 56 Webb P, Nguyen P, Shinsako J, et al: Estrogen receptor activation function 1 works by binding p160 co-activator proteins. *Mol Endocrinol* 1998;12:1605–1618.
- 57 Lavinsky RM, Jepsen K, Heinzel T, et al: Diverse signaling pathways modulate nuclear receptor recruitment of N-CoR and SMRT complexes. *Proc Natl Acad Sci USA* 1998;95: 2920–2925.
- 58 Catalano V, Turdo A, Franco SD, et al: Tumor and its microenvironment: a synergistic interplay. *Semin Cancer Biol* 2013;23:522–532.
- 59 Guo W, Giaccotti FG: Integrin signaling during tumor progression. *Nat Rev* 2004;5:816–826.
- 60 Giaccotti FG, Ruoslahti E: Integrin signaling. *Science* 1999;285:1028–1032.
- 61 Guo W, Pylayeva Y, Pepe A, et al: β₄ Integrin amplifies ErbB2 signaling to promote mammary tumorigenesis. *Cell* 2006;126:489–502.
- 62 Wu C: Heat shock transcription factors: structure and regulation. *Ann Rev Cell Dev Biol* 1995;11:441–469.
- 63 Bode AM, Dong Z: Mitogen-activated protein kinase activation in UV-induced signal transduction. *Sci STKE* 2003;167:RE2.
- 64 Neckers L, Lee YS: Cancer: the rules of attraction. *Nature* 2003;425:357–359.
- 65 Gutierrez MC, Detre S, Johnston S, et al: Molecular changes in tamoxifen-resistant breast cancer: relationship between estrogen receptor, HER-2, and p38 mitogen-activated protein kinase. *J Clin Oncol* 2005;23:2469–2476.
- 66 Larionov AA, Miller WR: Challenges in defining predictive markers for response to endocrine therapy in breast cancer. *Future Oncol* 2009;5:1415–1428.
- 67 Brodie A, Macedo L, Sabnis G: Aromatase resistance mechanisms in model systems in vivo. *J Steroid Biochem Molec Biol* 2010;118: 283–287.
- 68 Miller WR, Larionov A, Renshaw L, et al: Gene expression profiles differentiating between breast cancers clinically responsive or resistant to letrozole. *J Clin Oncol* 2009;27: 1382–1387.
- 69 Long BJ, Jelovac D, Handratta V, et al: Therapeutic strategies using the aromatase inhibitor letrozole and tamoxifen in a breast cancer model. *J Natl Cancer Inst* 2004;96:456–465.
- 70 Jelovac D, Macedo L, Golubeva OG, et al: Additive antitumor effect of aromatase inhibitor letrozole and antiestrogen fulvestrant in a postmenopausal breast cancer model. *Cancer Res* 2005;65:5439–5444.
- 71 Fedele P, Calvani N, Marino A, et al: Targeted agents to reverse resistance to endocrine therapy in metastatic breast cancer: where are we now and where are we going? *Crit Rev Oncol Hematol* 2012;84:243–251.
- 72 Cavazzoni A, Bonelli MA, Fumarola C, et al: Overcoming acquired resistance to letrozole by targeting the PI3K/AKT/mTOR pathway in breast cancer cell clones. *Cancer Lett* 2012; 323:77–87.
- 73 Barnett CM: Everolimus: targeted therapy on the horizon for the treatment of breast cancer. *Pharmacotherapy* 2012;32:383–396.
- 74 Massarweh S, Romond E, Black EP, et al: A phase II study of combined fulvestrant and everolimus in patients with metastatic estrogen receptor (ER)-positive breast cancer after aromatase inhibitor (AI) failure. *Breast Cancer Res Treat* 2014;143:325–332.
- 75 Greenburg G, Hay ED: Epithelial suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. *J Cell Biol* 1982;95:335–339.
- 76 Tse JC, Kalluri R: Mechanisms of metastasis: epithelial-mesenchymal transition and contribution of tumor microenvironment. *J Cell Biol* 2007;101:816–829.
- 77 De Craene B, Bex G: Regulatory network defining EMT during cancer initiation and progression. *Nat Rev Cancer* 2013;13:97–110.
- 78 Cano A, Perez-Moreno MA, Rodrigo I, et al: The transcription factor Snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* 2000;2:76–83.
- 79 Moreno-Bueno G, Cubillo E, Sarrío D, et al: Genetic profiling of epithelial cells expressing E-cadherin repressors reveals a distinct role for Snail, Slug, and E47 factors in epithelial-mesenchymal transition. *Cancer Res* 2006;66: 9543–9556.
- 80 Thiery JP, Sleeman JP: Complex networks orchestrate epithelial-mesenchymal transition. *Mol Cell Biol* 2006;26:131–142.
- 81 Sivakumar R, Koga H, Selvendiran K, et al: Autocrine loop for IGF-I receptor signaling in SLUG-mediated epithelial-mesenchymal transition. *Int J Oncol* 2009;34:392–338.
- 82 Evdokimova V, Tognon C, Ng T, et al: Translational activation of snail1 and other developmentally regulated transcription factors by YB-1 promotes an epithelial-mesenchymal transition. *Cancer Cell* 2009;15:402–415.
- 83 Bachelder RE, Yoon SO, Franci C, et al: Glycogen synthase kinase-3 is an endogenous inhibitor of Snail transcription: implications for the epithelial-mesenchymal transition. *J Cell Biol* 2005;168:29–33.
- 84 Xu Y, Lee SH, Kim HS, et al: Role of CK1 in GSK3β-mediated phosphorylation and degradation of snail. *Oncogene* 2010;29:3124–3133.

- 85 Grivnenkov SI, Greten FR, Karin M: Immunity, inflammation, and cancer. *Cell* 2010; 140:883–899.
- 86 Ge K, DuHadaway J, Du W, et al: Mechanism for elimination of a tumor suppressor: aberrant splicing of a brain-specific exon causes loss of function of Bin1 in melanoma. *Proc Natl Acad Sci USA* 1999;96:9689–9694.
- 87 Ye X, Tam WL, Shibue T, et al: Distinct EMT programs control normal mammary stem cells and tumour-initiating cells. *Nature* 2015;525:256–260.
- 88 Ye Y, Xiao Y, Wang W, et al: ER α signaling through slug regulates E-cadherin and EMT. *Oncogene* 2010;29:1451–1462.
- 89 Iseri OD, Kars MD, Arpacı F, et al: Drug resistant MCF-7 cells exhibit epithelial-mesenchymal transition gene expression pattern. *Biomed Pharmacother* 2011;65:40–45.
- 90 Eccles SA: The role of c-erbB-2/HER2/neu in breast cancer progression and metastasis. *J Mammary Gland Biol Neoplasia* 2001;6: 393–406.
- 91 Yu D, Hamada J, Zhand H, et al: Mechanisms of c-erbB2-2/neu oncogene-induced metastasis and repression of metastatic properties by adenovirus 5 E1A gene products. *Oncogene* 1992;7:2263–2270.
- 92 Jeong H, Ryu Y-J, An J, et al: Epithelial-mesenchymal transition in breast cancer correlates with high histological grade and triple-negative phenotype. *Histopathol* 2012;60: E87–E95.
- 93 Luqmani YA, Al Azmi A, Al Bader M, et al: Modification of gene expression induced by siRNA targeting of estrogen receptor in MCF-7 human breast cancer cells. *Int J Oncol* 2009;34:231–242.
- 94 Dhasarathy A, Kajita M, Wade PA: The transcription factor snail mediates epithelial to mesenchymal transition by repression of estrogen receptor- α . *Mol Endocrinol* 2007;21: 2907–2918.
- 95 Shih J-Y, Yang P-C: The EMT regulator slug and lung carcinogenesis. *Carcinogenesis* 2011;32:1299–1304.
- 96 Dixon MJ: Endocrine resistance in breast cancer. *New J Sci* 2014;2014:1–27.
- 97 Al Saleh S, Sharaf LH, Luqmani YA: Signaling pathways involved in endocrine resistance in breast cancer and associations with epithelial to mesenchymal transition. *Int J Oncol* 2011a;38:1197–1217.
- 98 Loh YN, Hedditch EL, Baker LA, et al: The Wnt signalling pathway is upregulated in an in vitro model of acquired tamoxifen resistant breast cancer. *BMC Cancer* 2013;13: 174–181.
- 99 Yoshida T, Ozawa Y, Kimura T, et al: Eribulin mesilate suppresses experimental metastasis of breast cancer cells by reversing phenotype from epithelial-mesenchymal transition (EMT) to mesenchymal-epithelial transition (MET) states. *Br J Cancer* 2014; 110:1497–1505.
- 100 Khan S, Shukla S, Sinha S, et al: Centchroman suppresses breast cancer metastasis by reversing epithelial-mesenchymal transition via downregulation of HER2/ERK1/2/MMP-9 signaling. *Intl J Biochem Cell Biol* 2014;58:1–16.
- 101 Bandyopadhyay A, Wang L, Agyin J, et al: Doxorubicin in combination with a small TGF β inhibitor: a potential novel therapy for metastatic breast cancer in mouse models. *PLoS One* 2010;5:1–13.
- 102 Klinge CM: miRNAs and estrogen action. *Trends Endocrinol Metab* 2012;23:223–233.
- 103 Li C, Feng Y, Coukos G, et al: Therapeutic microRNA strategies in human cancer. *AAPS J* 2009;11:747–757.
- 104 Manavalan TT, Teng Y, Appana SN, et al: Differential expression of microRNA expression in tamoxifen-sensitive MCF-7 versus tamoxifen-resistant LY2 human breast cancer cells. *Cancer Lett* 2011;313:26–43.
- 105 Yin JQ, Zhao RC, Morris KV: Profiling microRNA expression with microarrays. *Trends Biotechnol* 2008;26:70–76.
- 106 Hatley ME, Patrick DM, Garcia MR, et al: Modulation of K-Ras-dependent lung tumorigenesis by microRNA-21. *Cancer Cell* 2010;18:282–293.
- 107 Ryan BM, Robles AI, Harris CC: Genetic variation in microRNA networks: the implications for cancer research. *Nat Rev Cancer* 2010;10:389–402.
- 108 Kosaka N, Iguchi H, Ochiya T: Circulating microRNA in body fluid: a new potential biomarker for cancer diagnosis and prognosis. *J Cancer Sci* 2010;101:2087–2092.
- 109 Inui M, Martello G, Piccolo S: MicroRNA control of signal transduction. *Nat Rev Mol Cell Biol* 2010;11:252–263.
- 110 Cho WC: MicroRNAs as therapeutic targets and their potential applications in cancer therapy. *Expert Opin Ther Targets* 2012;16: 747–759.
- 111 Iorio MV, Casalini P, Piovan C, et al: Breast cancer and microRNAs. *Ther Impact Breast* 2011;20:S63–S70.
- 112 Lyng MB, Laenkholm AV, Sokilde R, et al: Global microRNA expression profiling of high-risk ER+ breast cancers from patients receiving adjuvant tamoxifen mono-therapy: a DBCG study. *PLoS One* 2012;7:e36170.
- 113 Luqmani YA, Khajah MA: MicroRNA in breast cancer gene regulators and targets for novel therapies; in Gunduz M (ed): *A Concise Review of Molecular Pathology of Breast Cancer*. Rijeka, InTech, 2015.
- 114 Ma L, Young J, Prabhala H, et al: miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. *Nat Cell Biol* 2010;12:247–256.
- 115 Di Leva G, Gasparini P, Piovan C, et al: MicroRNA cluster 221–222 and estrogen receptor alpha interactions in breast cancer. *J Natl Cancer Inst* 2010;102:706–721.
- 116 Zhao JJ, Lin J, Yang H, et al: MicroRNA-221/222 negatively regulates estrogen receptor alpha and is associated with tamoxifen resistance in breast cancer. *J Biol Chem* 2008;283:31079–31086.
- 117 Rao X, di Leva G, Li M, et al: MicroRNA-221/222 confers breast cancer fulvestrant resistance by regulating multiple signaling pathways. *Oncogene* 2011;30:1082–1097.
- 118 Wei Y, Lai X, Yu S, et al: Exosomal miR-221/222 enhances tamoxifen resistance in recipient ER-positive breast cancer cells. *Breast Cancer Res Treat* 2014;47:423–231.
- 119 Pandey DP, Picard D: miR-22 inhibits estrogen signaling by directly targeting the estrogen receptor alpha mRNA. *Mol Cell Biol* 2009;29:3783–3790.
- 120 Tavazoie SF, Alarcon C, Oskarsson T, et al: Endogenous human microRNAs that suppress breast cancer metastasis. *Nature* 2008; 451:147–152.
- 121 Liang YJ, Wang QY, Zhou CX, et al: MiR-124 targets Slug to regulate epithelial-mesenchymal transition and metastasis of breast cancer. *Carcinogenesis* 2013;34:713–722.
- 122 Li QQ, Chen ZQ, Cao XX, et al: Involvement of NF-kappaB/miR-448 regulatory feedback loop in chemotherapy-induced epithelial-mesenchymal transition of breast cancer cells. *Cell Death Differ* 2011;18:16–26.
- 123 Li L, Luo J, Wang B, et al: MicroRNA-124 targets flotillin-1 to regulate proliferation and migration in breast cancer. *Mol Cancer* 2013;12:1476–4598.
- 124 Lv XB, Jiao Y, Qing Y, et al: miR-124 suppresses multiple steps of breast cancer metastasis by targeting a cohort of pro-metastatic genes in vitro. *Chin J Cancer* 2011;30: 821–830.
- 125 Ling DJ, Chen ZS, Zhang YD, et al: MicroRNA-145 inhibits lung cancer metastasis. *Mol Med Rep* 2015;11:3108–3114.
- 126 Xu N, Papaginnakopoulos T, Pan G, et al: MicroRNA-145 regulates OCT4, SOX2, and KLF4 and represses pluripotency in human embryonic stem cells. *Cell* 2009;137:647–658.
- 127 Cui S-Y, Wang R, Chen L-B: MicroRNA-145: a potent tumor suppressor that regulates multiple cellular pathways. *J Cell Mol Med* 2014;18:1913–1926.
- 128 Huang TH, Wu F, Loeb GB, et al: Up-regulation of miR-21 by HER2/neu signaling promotes cell invasion. *J Biol Chem* 2009; 284:18515–18524.
- 129 le Sage C, Nagel R, Egan DA, et al: Regulation of the p27(Kip1) tumor suppressor by miR-221 and miR-222 promotes cancer cell proliferation. *EMBO J* 2007;26:3699–3708.
- 130 Falkenberg N, Anastosov N, Rappl K, et al: MIR-221/-222 differentiate prognostic groups in advanced breast cancers and influence cell invasion. *Br J Cancer* 2013;109: 2714–2723.
- 131 Li W, Zang W, Liu P, et al: MicroRNA-124 inhibits cellular proliferation and invasion by targeting Ets-1 in breast cancer. *Tumour Biol* 2014;35:10897–10904.
- 132 Kureishy N, Sapountzi V, Prag S, et al: Fascins and their roles in cell structure and function. *Bioessays* 2002;24:350–361.

- 133 McSherry EA, Brennan K, Hudson L, et al: Breast cancer cells migration is regulated through junctional adhesion molecule-A-mediated activation of Rap1 GTPase. *Breast Cancer Res* 2011;13:1–14.
- 134 Sachdeva M, Mo YY: MicroRNA-145 suppresses cell invasion and metastasis by directly targeting mucin 1. *Cancer Res* 2010;70:378–387.
- 135 Foekens JA, Sieuwerts AM, Smid M, et al: Four miRNAs associated with aggressiveness of lymph node-negative, estrogen receptor-positive human breast cancer. *Proc Natl Acad Sci USA* 2008;105:13021–13026.
- 136 Camps C, Buffa FM, Colella S, et al: hsa-miR-210 is induced by hypoxia and is an independent prognostic factor in breast cancer. *Clin Cancer Res* 2008;14:1340–1348.
- 137 Rothe F, Ignatiadis M, Chaboteaux C, et al: Global microRNA expression profiling identifies MiR-210 associated with tumor proliferation, invasion and poor clinical outcome in breast cancer. *PLoS One* 2011;6:e20980.
- 138 Buffa FM, Camps C, Winchester L, et al: microRNA-associated progression pathways and potential therapeutic targets identified by integrated mRNA and microRNA expression profiling in breast cancer. *Cancer Res* 2011;71:5635–5645.
- 139 Radojicic J, Zaravinos A, Vrekoussis T, et al: MicroRNA expression analysis in triple-negative (ER, PR and Her2/neu) breast cancer. *Cell Cycle* 2011;10:507–517.
- 140 Qian B, Katsaros D, Lu L, et al: High miR-21 expression in breast cancer associated with poor disease-free survival in early stage disease and high TGF-beta1. *Breast Cancer Res Treat* 2009;117:131–140.
- 141 Gong C, Yao Y, Wang Y, et al: Up-regulation of miR-21 mediates resistance to trastuzumab therapy for breast cancer. *J Biol Chem* 2011;286:19127–19137.
- 142 Valastyan S, Reinhardt F, Benaich N, et al: A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. *Cell* 2009;137:1032–1046.
- 143 Kota J, Chivukula RR, O'Donnell KA, et al: Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. *Cell* 2009;137:1005–1017.
- 144 Krutzfeldt J, Rajewsky N, Braich R, et al: Silencing of microRNAs in vivo with 'antagomirs'. *Nature* 2005;438:685–689.
- 145 Elmen J, Lindow M, Silahatoglu A, et al: Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. *Nucleic Acids Res* 2008;36:1153–1162.
- 146 Esau CC: Inhibition of microRNA with antisense oligonucleotides. *Methods* 2008;44:55–60.
- 147 Si ML, Zhu S, Wu H, et al: miR-21-mediated tumor growth. *Oncogene* 2007;26:2799–2803.
- 148 Yan LX, Huang XF, Shao Q, et al: MicroRNA miR-21 over-expression in human breast cancer is associated with advanced clinical stage, lymph node metastasis and patient poor prognosis. *RNA* 2008;14:2348–2360.
- 149 Lanford RE, Hildebrandt-Eriksen ES, Petri A, et al: Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science* 2010;327:198–201.