

ER α , microRNAs, and the epithelial–mesenchymal transition in breast cancer

Irene K. Guttilla¹, Brian D. Adams² and Bruce A. White³

¹ Saint Joseph College, Department of Biology, 1678 Asylum Avenue, West Hartford, CT 06117, USA

² Yale University, Department of Genetics, 333 Cedar Street, New Haven, CT 06510, USA

³ University of Connecticut Health Center, Department of Cell Biology, 263 Farmington Avenue, Farmington, CT 06032, USA

The most common form of breast cancer, luminal A, is estrogen receptor α (ER α)-positive and epithelial, but nevertheless can metastasize. The process of epithelial–mesenchymal transition (EMT) is probably the first step in the metastasis of epithelial cancers. We discuss the characteristics of EMT, including factors that induce EMT, and the relationship of EMT to cancer stem cells (CSCs). Estrogen/ER α signaling maintains an epithelial phenotype and suppresses EMT. An overview of microRNAs in breast cancer is presented, including how microRNA biogenesis is altered in cancer and regulated by ER α . We also discuss the role of the miR-200 family in opposing EMT. Finally, we discuss specific microRNAs that target ER α and regulate EMT in breast cancer, and the role of these microRNAs in breast cancer progression.

Introduction

Breast cancer is second only to lung cancer as the cause of cancer-related deaths in women, and the vast majority of breast cancer-related deaths involve metastatic disease. Thus, an understanding of the molecular and cellular underpinnings of metastasis is required for accurate prognoses and for developing targeted treatments against metastatic cells. Currently, considerable attention is being directed towards EMT as the probable first step in the complex process of metastasis [1,2]. EMT is involved in normal embryogenesis and wound healing. In cancer, EMT is a multi-step and reversible process mediated by specific molecular cues that promote the loss of cell–cell junctions, cell–matrix adhesion, and reorganization of the cytoskeleton, resulting in loss of the apical polarity associated with epithelial cells and gain of mesenchymal characteristics such as spindle-shaped morphology and a high degree of motility [3–6] (Box 1). EMT also increases the CSC subpopulation within a tumor, thereby generating self-renewing cells that are both tumorigenic and resistant to chemo- and radiation therapies [3–6].

EMT in epithelial forms of breast cancer

The mammary epithelium is composed of two cell layers, luminal and basal. Luminal cells display standard

epithelial morphology, with junctional complexes and apical–basal polarity, and express luminal/epithelial markers such as E-cadherin. The basal layer of the mammary ductal epithelium is composed of myoepithelial cells. These differentiated cell types are probably progeny of one or more types of mammary stem cells which also reside within the epithelial layer [7,8]. The relationship between multipotent stem cells and more lineage-restricted progenitors and differentiated cells may be one of a unidirectional lineage, or a more complex one in which differentiated cells retain the capacity to become reprogrammed into stem cells [9].

Breast cancer presents as a heterogeneous disease, displaying a variety of histopathological features, genetic markers and diverse prognostic outcomes [10]. The majority of breast cancers arise from the luminal epithelium of small mammary ducts. Most newly diagnosed breast cancers are classified as the luminal-A subtype, which are typically low-grade, weakly proliferative and invasive, and confer a favorable prognosis. Although the cellular origin of luminal A tumors remains unresolved, these tumors are termed luminal because they display epithelial phenotypic markers, such as E-cadherin, and retain some degree of epithelial organization. Moreover, gene expression profiling studies indicate a high degree of similarity between luminal A bulk cancer cells and normal mammary luminal epithelia [10,11]. Many of the cells in luminal A breast cancers also express the nuclear hormone receptors ER α and progesterone receptor (PR), and luminal-associated transcription factors GATA3 and forkhead box protein A1 (FOXA1). Luminal A tumors appear to contain a relatively small subpopulation of breast CSCs, as defined by CD44^{high}/CD24^{low} expression or aldehyde dehydrogenase activity, or by the ability to form tumors in immunodeficient mice [10,11].

Despite an epithelial phenotype and indolent nature, untreated luminal A breast cancer can progress to metastatic disease. This progression probably involves the onset of EMT in a subpopulation of epithelial cancer cells, in which cells shed the constraints associated with epithelial tissue while gaining the motile and invasive capabilities of mesenchymal cancer cells. EMT may occur after oncogenesis in cells at the tumor margin (Figure 1) or at the time of cellular transformation in cooperation with other oncogenes [12].

Corresponding author: White, B.A. (bwhite@nso2.uchc.edu)

Box 1. Characteristics of epithelial–mesenchymal transition (EMT)

EMT involves expression of developmental transcription factors. EMT is induced by a plethora of factors within the tumor microenvironment and is influenced by genetic background. EMT-inducing signals converge on several transcription factors, including ZEB1, ZEB2, Snail, Slug, Twist, Goosecoid, E47 and LBX1. EMT can be experimentally induced in breast epithelia by ectopic expression of ZEB1, Snail, Twist or LBX1 [78–83]. In addition, Twist was demonstrated to be required for the metastasis of murine mammary cancer cells to the lung, indicating that EMT is a crucial first step in the process of metastasis [83].

EMT-related transcription factors act, in part, through transcriptional repression of multiple epithelial-related genes. The E-cadherin gene (*CDH1*) is a particularly well-studied target. Loss of E-cadherin is an early hallmark of EMT induction in cancers, and forced expression of E-cadherin in mesenchymal MDA-MB-231 breast cancer cells induced mesenchymal–epithelial transition (MET) [84]. Loss of E-cadherin facilitates the cytoskeletal rearrangement and establishment of back-to-front polarity that are associated with motility. Numerous other changes in gene expression, together with altered epigenetic controls, occur during the EMT program (e.g. upregulation of matrix metalloproteases, downregulation of junctional complex proteins, etc.), leading to a motile and invasive cancer cell [84].

EMT induces CSC properties of self-renewal, incomplete differentiation, the ability to form tumors, and resistance to anti-tumor therapies. Normal mammary epithelial stem cells were isolated from human mammary epithelial cells (HMECs) and from normal breast epithelial tissue from reduction mammoplasties, as determined by expression of CD44⁺/CD24/low surface markers. A direct relationship between EMT and ‘stemness’ was demonstrated by Mani et al. and Morel et al., who showed that induction of EMT by TGF- β or by ectopic expression of EMT-inducing transcription factors in HMECs and transformed mammary cells resulted in the generation of stem-like cells [85,86].

EMT is reversible. The epithelial versus mesenchymal state is remarkably plastic. Upon arrival at metastatic sites with new microenvironments, metastatic cells from luminal A tumors appear to revert back to a relatively sessile, epithelial phenotype to stably colonize a new tissue. Indeed, induced MET in murine 4T1 cells results in the formation of more distal metastases than in control 4T1 cells. Thus, MET appears to involve the expression of factors that were initially opposed to EMT [87]. In other words, successful metastasis may require both EMT and MET, a fact that clearly complicates the development of anti-metastatic agents by targeting EMT alone.

ER α supports the epithelial phenotype and opposes EMT

The hormone estradiol (E₂), acting through ER α , is required for the normal growth and development of the mammary tree [13,14]. ER α is also an important prognostic indicator in breast cancer [15]. A significant

fraction of cells within a luminal A tumor express ER α , and E₂/ER α signaling promotes and sustains proliferation in these cells. This expression of and dependence on ER α in luminal A cancers form the rationale for ‘hormonal’ therapies involving antiestrogens or CYP19/aromatase inhibitors.

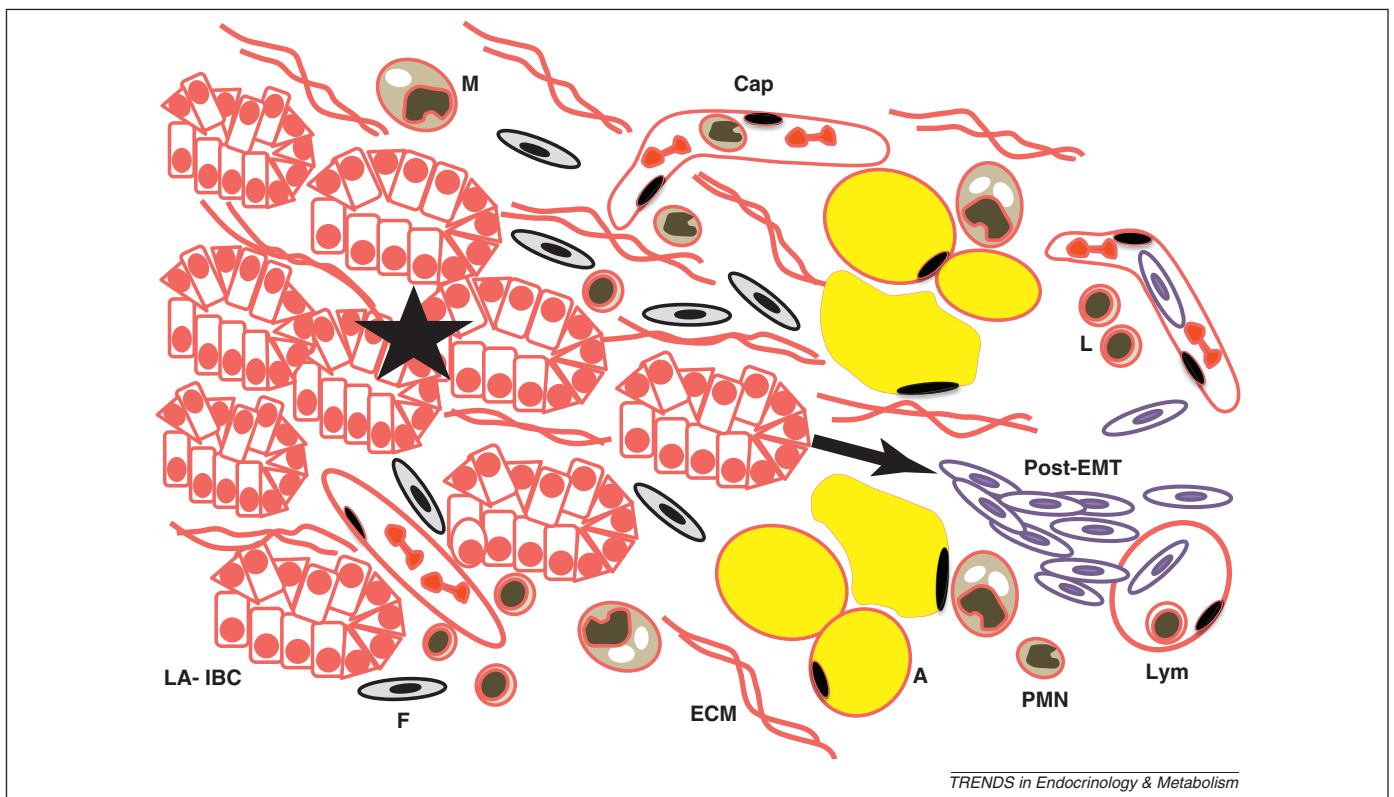


Figure 1. EMT in a luminal A invasive breast cancer. EMT takes place at the margin of luminal A invasive breast cancer (LA-IBC). At the central core of the tumor (star), tumor cells display some degree of epithelial organization, including cell–cell junctional complexes and basolateral polarity. A significant percentage of these cells express ER α , PR and luminal transcription factors (e.g. GATA3, FOXA1). With tumor growth and progression, cells move into and replace the stroma and adipose tissue of the breast. EMT can be induced by a plethora of signals that originate from numerous cell types [1–6], including stromal fibroblasts (F), polymorphonuclear leukocytes (PMN), macrophages (M), lymphocytes (L), and adipocytes (A). EMT can also be induced by extracellular matrix–integrin signaling or hypoxia. EMT (arrow) results in the emergence of fibroblast-like cancer cells (Post-EMT) with few intercellular junctions, downregulation of expression of ER α , PR and luminal transcription factors, and upregulation of mesenchymal proteins (vimentin, moesin) and EMT transcription factors (Snail, Slug, Zeb1, Zeb2, E47, LKB). Post-EMT breast cancer cells display increased motility and invasiveness, and may gain access to lymphatic vessels (Lym) or capillaries (Cap), termed ‘intravasation’ as one of the early steps in metastasis. These cells also express CSC markers (CD44, Bmi1, aldehyde dehydrogenase), attain the ability to self-renew, and are highly tumorigenic and resistant to chemo- and radiation therapy [1–6].

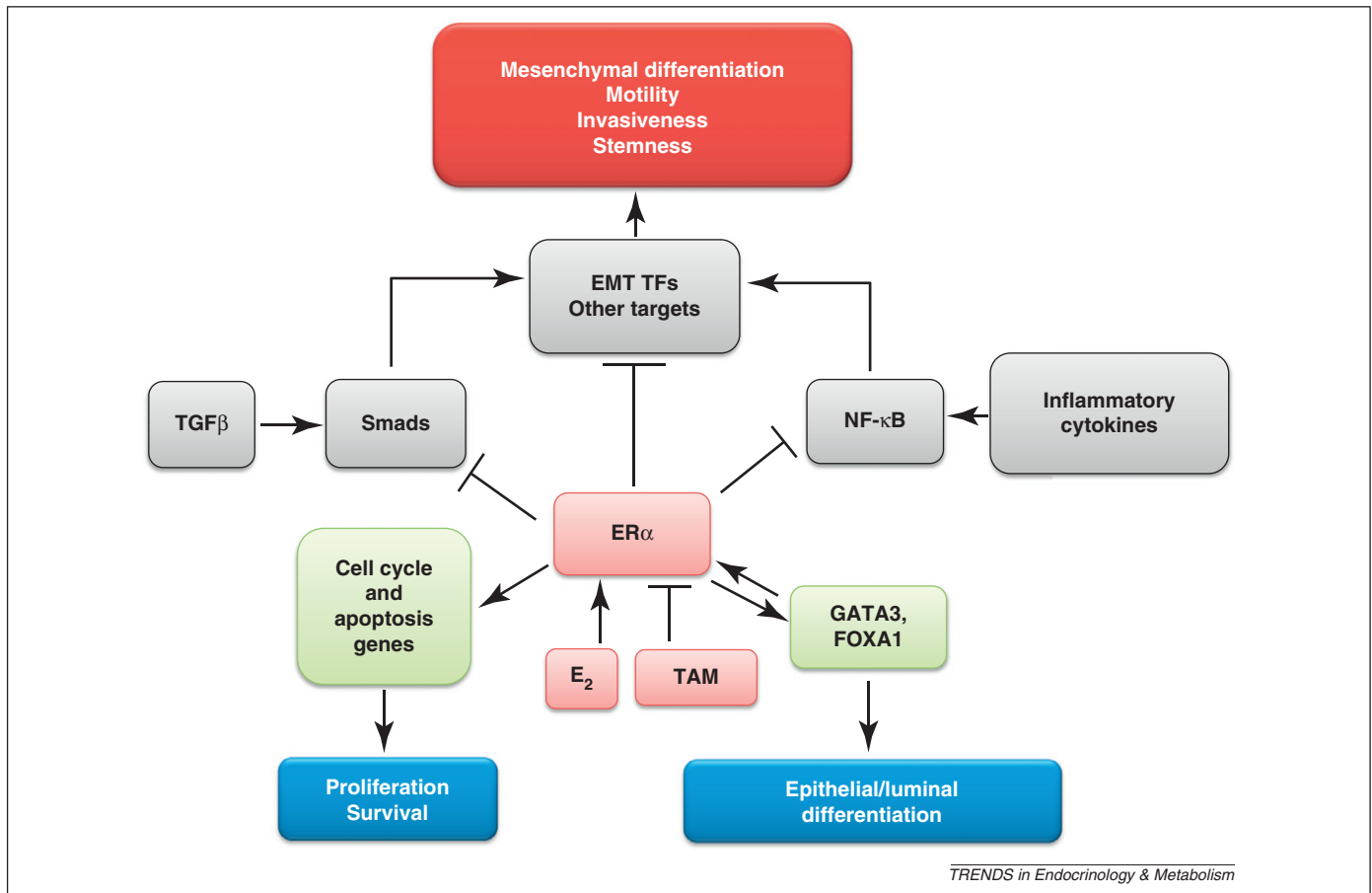


Figure 2. ER α signaling promotes proliferation and epithelial differentiation and opposes EMT. ER α activated by E₂ inhibits TGF- β signaling and cytokine signaling through Smad and NF- κ B, respectively, both of which promote EMT. ER α signaling also represses EMT-related transcription factors, as well as EMT-related microRNAs. This opposition to EMT is considered to be a major factor in why luminal A breast cancer is poorly metastatic and has a favorable prognosis. Conversely, ER α signaling increases cell cycle and anti-apoptotic gene expression, promoting proliferation and survival of ER α -positive breast cancer cells. Moreover, ER α signaling interacts with luminal-related transcription factors GATA3 and FOXA1 to promote an epithelial phenotype because GATA3 is a marker for luminal progenitor cell differentiation, and both GATA3 and FOXA1 are cofactors that modulate ER α signaling and activity. Together, these factors promote cell-cell adhesion, basolateral polarity and low motility, which are characteristic of epithelial tissues. An important issue not discussed in this review is the long-term effects of tamoxifen or other ‘hormonal’ therapies on the risk of a cancer cell losing its epithelial phenotype and undergoing EMT.

E₂/ER α signaling promotes the differentiation of mammary epithelia along a luminal/epithelial lineage, in part through transcriptional stimulation of luminal/epithelial-related transcription factors. ER α and GATA3 mutually stimulate each other [16]. GATA3 is required for luminal differentiation in normal breast epithelia [17], and GATA3 and ER α regulate many of the same genes [18] (Figure 2). Forced GATA3 expression in mesenchymal breast cancer cells induces mesenchymal–epithelial transition (MET), a process that, similar to EMT, is reversible, and inhibits tumor metastasis in mice [19]. FOXA1 is another ER α -interacting transcription factor that is required for the luminal lineage in mammary epithelia, and specifically promotes ductal growth in mice [20]. FOXA1 promotes accessibility of estrogen-response elements for ER α binding and stimulates ER α gene expression [21]. Conversely, E₂ appears to stimulate FOXA1 expression in breast cancer cells [21]. Importantly, ER α , FOXA1 and GATA3 are all favorable prognostic indicators in breast cancer [21].

ER α signaling promotes growth of the primary lesion (and therefore is mitogenic) but is able to keep the EMT process in check (and therefore is anti-metastatic) up to a point. E₂/ER α signaling antagonizes signaling pathways that lead to EMT. For example, transforming growth factor

beta (TGF- β) has been shown to induce EMT in human mammary epithelial cells, and overexpression of the EMT-inducing factor Snail in MCF-7 cells increased TGF- β signaling and invasiveness, and decreased adhesion and ER α expression [22]. Estrogen opposes TGF- β effects in MCF-7 cells. Ito *et al.* demonstrated that ER α forms a ternary complex with Smad2/3 and the Smad-selective E3 ubiquitin ligase Smurf, and promotes the proteosomal degradation of Smad proteins [23] (Figure 2).

The EMT pathway also involves the transcription factor NF- κ B as a downstream mediator of inflammatory cytokines [24]. Synthesis of the NF- κ B subunit RELB is inversely proportional to ER α expression in cell lines, as well as in breast cancer tissue [25,26]. RELB is required for maintenance of the mesenchymal phenotype in ER α -negative cells [26]. Moreover, stable overexpression of RELB in MCF-7 cells decreases E-cadherin expression, and increases levels of vimentin and fibronectin, and cell migration and invasion [26]. ER α -positive MCF-7 and T47D cells treated with the ER α inhibitor ICI 182,780 display increased RELB mRNA and protein levels and induced NF- κ B activity, whereas ectopic expression of ER α in the presence of E₂ decreases RELB expression in ER α -negative cell lines [26].

E₂/ER α signaling suppresses EMT-promoting transcription factors in breast cancer (Figure 2). For example, BRCA1 haploinsufficiency leads to an ER α -negative mesenchymal (Basal-like) form of breast cancer that is dependent on aberrant expression of Slug [27]. ER α indirectly represses the expression of Snail through the upregulation of metastasis-associated protein 3 (MTA3), which is a component of the Mi-2/NURD corepressor complex [28]. In repressing Snail transcription, MTA3 increases cell–cell adhesion and moderates invasiveness in breast cancer cells. Overexpression of Snail directly represses transcription of the ER α (*ESR1*) gene, as well as the E-cadherin (*CDH1*) gene, and ultimately induces EMT in MCF-7 cells [28].

More recently, Ye *et al.* [29] examined the effects of either overexpression of ER α in ER α -negative breast cancer cell lines (MDA-MB-468, MDA-MB-231) or ER α knockdown in ER α -positive cell lines (MCF-7, T47D) on Slug and Snail expression and resulting phenotypes. Forced expression of ER α represses Slug, increases E-cadherin, and induces cells to grow as adherent colonies with reduced invasiveness. By contrast, knockdown of ER α results in elevation of Slug expression, decreased E-cadherin and spindle-shaped invasive cells.

E₂ signaling regulates the activity of ER α and associated cofactors GATA3 and FOXA1 to promote an epithelial phenotype and suppress EMT and pro-metastatic progression in luminal-type breast cancers. Direct regulatory relationships have been established *in vitro*, such as increased invasion and onset of EMT in MCF-7 cells following overexpression of RELB or Snail due to repression of ER α and E-cadherin, and *in vivo* data correlate with these observations. Expression levels of ER α , GATA3, and FOXA1 are associated with a favorable prognosis in breast cancer patients. Conversely, high levels of RELB and Snail expression (as well as no/low expression of ER α , GATA3, and FOXA1) are associated with invasive basal-like and claudin-low breast cancer subtypes, which confer a less favorable prognosis.

MicroRNAs and EMT

Metastatic lesions of luminal A tumors frequently express ER α [30]. This may be because of the epithelial differenti-

ation of a luminal A CSC upon colonizing a metastatic site, or to the reversion of an EMT-generated ER α -negative metastatic cell back to an ER α -positive cell through MET [31]. In either case, the aberrant expression of genes that suppress ER α expression could result in the appearance of a stable population of ER α -negative cells. In fact, Creighton *et al.* reported recurrence of the ER α -negative mesenchymal claudin-low form of breast cancer in patients treated with aromatase inhibitor [32]. Thus, an understanding of how ER α is silenced and/or re-expressed could lead to more refined approaches to the treatment of metastatic luminal A cancer. We and others have examined whether microRNAs might target ER α as one mode of epigenetic silencing, and we propose that it might be useful to consider the relationship between ER α -targeting microRNAs and their effect on EMT.

MicroRNAs are short post-translational RNA regulators that bind to complementary sequences on mRNA, usually resulting in gene silencing through translational repression or degradation of the target mRNA [33]. Numerous studies have revealed that microRNAs are dysregulated in cancer versus normal tissue, and in non-invasive versus invasive forms [34,35]. Discordant microRNA expression between normal and tumor breast tissues and between ER α -positive and ER α -negative tumors is well established [36] (Table 1). In general, the levels of most mature (processed) microRNAs are lower in cancer versus normal tissue, and in ER α -negative versus ER α -positive breast cancer, and both Drosha and Dicer levels are subnormal in several cancers [37,38] (microRNA biogenesis is reviewed in [33]). Interestingly, E₂ induces Dicer expression in MCF-7 cells, suggesting that loss of ER α and estrogen signaling in breast cancers may contribute to the decreased expression of Dicer and consequently lower levels of microRNA expression [39]. Some evidence supports a role of microRNAs in dampening Dicer expression. Martello *et al.* [40] showed that miR-103/107 targets Dicer mRNA and inhibits Dicer expression, thereby promoting EMT in breast cancer cell lines and the metastatic spread of breast cancer cell lines in mice. These investigators also provided evidence that the miR-103/107-induced decrease in Dicer leads to compromised processing of pre-microRNAs belonging to the EMT-inhibitory miR-200 family (miR-200f; Box 2).

Table 1. Selected microRNAs and their role in breast cancer

microRNA	Expression levels in breast cancer	Action in breast tumors	Known targets	Refs
miR-200c	↓ BL; ↑ Lum A	Anti-EMT	ZEB1 and ZEB2 (repressors of E-cadherin); moesin and fibronectin (involved in cell migration); TrkB (anoikis resistance)	[47,76,77]
miR-206	↑ NC tumor; ↑ ER α - vs ER+	Pro-EMT, anti-proliferative in ER α ⁺ cell lines	Estrogen signaling molecules ER α , SRC-1, SRC-2, GATA-3	[36,48,56]
miR-221/222	↑ BL; ↓ Lum A	Pro-EMT, pro-proliferative in ER α ⁺ cell lines	ER α and cell cycle regulators p27 (Kip1), p57	[63,65,66]
miR-130a	↓ NC tumor	Anti-EMT	ER α and the oncogene c-MET in non-small cell lung cancer	[67,68]
miR-17/92	↑↓ NC tumor	Tumor suppressor or oncogene; pro-metastatic but affects proliferation depending on the microenvironment	ER α and SRC-3	[34,69–71]
miR-145	↓ NC tumor; ↓ BL; ↓ Lum A	Tumor suppressor	ER α and MUC-1 (associated with invasiveness)	[73–75]

NC tumor, nonclassified tumor; Lum A, luminal A; BL, basal-like; EMT, epithelial to mesenchymal transition.

Box 2. The miR-200 family: opposition to EMT and stemness

MiR-200 family members (miR-200f) are expressed from the miR-200c/141 bicistronic gene on chromosome 12 and the miR-200b/200a/429 polycistronic gene on chromosome 1. Based on 5' seed sequences, these microRNAs form two functional groups, miR-141/200c and miR-200b/200a/429.

MiR-200f maintain E-cadherin expression through targeting ZEB1 and ZEB2, which are transcriptional repressors of E-cadherin, and through targeting Suz12, which contributes to polycomb-mediated repression of E-cadherin expression [47]. More recent studies have expanded the range of empirically proven mRNA targets of the miR-200 family. The Richer laboratory used forced expression of miR-200c to identify miR-200c-suppressed transcripts in ER α -negative mesenchymal breast cancer cell lines (BT549, MDA-MB-231) and mesenchymal type 2 endometrial cancer cell lines (Hec50, AN3CA), followed by empirical confirmation of predicted miR-200c sites in the 3'-UTR of moesin, fibronectin and TrkB [76]. Using functional assays, Howe *et al.* showed that, through targeting TrkB, miR-200c decreased migration via targeting of moesin and fibronectin, and diminished resistance to anoikis. MiR-200 family members target several components involved in Notch signaling, which is activated by Zeb1 and promotes EMT [77]. MiR-200 family members target the Notch ligand, Jagged 1 (Jag1), which induces EMT through upregulation of Slug in mammary epithelial cells. MiR-200 family members also

directly target the Notch transcriptional coactivator proteins Maml2 and Maml3. In eight separate basal-like breast cancer specimens, Zeb1 levels were relatively elevated, whereas miR-200c and miR-141 levels were low, and the converse was observed in ductal (luminal) breast cancer. Basal-like cancers displayed a positive correlation among Zeb1, Jag1 and activated Notch 1 and Notch 2 expression, specifically in the invasive front of these cancers.

Furthermore, miR-200f target the stem cell factors Bmi-1, Sox2 and Klf4. Shimono *et al.* [88] reported that members of the miR-200 family are downregulated in human and mouse mammary stem cells, as well as in human breast CSCs. Lentiviral-mediated overexpression of miR-200c in murine mammary cancer cells suppressed colony formation, and this phenotype was rescued by co-expression of a BMI1 transgene devoid of the 3'-UTR. Overexpression of miR-200c also severely stunted the outgrowth of murine mammary epithelial cells *in vivo* and significantly reduced tumor-initiating ability in human breast CSCs in NOD/SCID (non-obese diabetic/severe combined immunodeficiency) mice. These findings indicate that miR-200c drives differentiation at the expense of stemness and self-renewal in breast epithelial cells. By contrast, miR-200c induces pluripotency in fibroblasts [89], indicating the importance of cellular context. MiR-200c may also promote metastatic colonization [87].

Cochrane *et al.* [37] demonstrated that miRs-221, -222 and -29a, which are elevated in ER α -negative breast cancer cell lines, target Dicer. Upregulation of miR-200c (Box 2) increased Dicer in two ER α -negative cell lines. Although the majority of microRNAs are decreased after neoplastic transformation, some microRNAs clearly show an increase [34]. A major unanswered question is how some microRNAs appear to maintain elevated levels in the face of reduced Dicer activity in breast cancer: are they so overexpressed at the transcriptional level that they effectively compete for what little Dicer is available? Or do these microRNAs simply bypass Dicer during maturation [41]? It is also important to consider the significance of Dicer activity in maintaining an epithelial phenotype and/or opposing EMT.

The expression of the microRNA effector Argonaute proteins (AGOs) is also altered in cancer. The *AGO1* gene is frequently deleted in several cancers, including breast cancer [42]. Both AGO1 and AGO2 were reported to be elevated in ER α -negative versus ER α -positive breast cancer [43]. We showed that enforced expression of AGO2 reduced E-cadherin expression and enhanced motility in epithelial breast cancer cell lines [44]. Additional work should examine whether increased AGO2 expression induces a complete EMT in breast cancer.

Specific microRNAs have been demonstrated to promote aspects of EMT and metastasis. One of the seminal studies on this subject was that by Ma *et al.* [45], who showed that Twist (but not Snail) induced expression of miR-10b. MiR-10b targets and represses homeobox D10 (*HOXD10*) expression, thereby relieving transcriptional inhibition of the prometastatic Ras homolog gene family member C (*RHOC*). More recently, several other 'metastamiRs' have been characterized [46].

Conversely, several microRNAs have been characterized as suppressors of metastasis [46]. In particular, the miR-200 family (Box 2; Table 1) has been shown to promote epithelial differentiation and resist EMT [47]. For example, members of the miR-200 family directly target the mRNAs encoding the zinc finger E-box-binding homeobox proteins ZEB1 and

ZEB2, and are, in turn, transcriptionally repressed by ZEBs [47]. The role of microRNAs that target ER α is discussed below, and a major unanswered question is whether these microRNAs ultimately alter miR-200f levels.

ER α -targeting microRNAs that may regulate EMT

As discussed above, ER α opposes EMT, and microRNAs either promote or oppose EMT (Table 1). These findings beg the question as to whether microRNAs contribute to the regulation of EMT through their ability to target ER α mRNA. The probability of microRNA-mediated regulation of the human ER α mRNA is supported by the long (>4000 nt) 3' untranslated region (3'-UTR) of ER α mRNA, and by findings that specific microRNAs are differentially expressed between ER α -positive and ER α -negative breast cancers [35].

miR-206

An *in silico* search for microRNAs predicted to target ER α mRNA identified miR-206 [48] as a potential modulator of ER α mRNA levels. Ectopic miR-206 expression repressed endogenous ER α mRNA and protein levels in ER α -positive MCF-7 cells and T47D cells, and two predicted miR-206 target sites in ER α mRNA 3'-UTR were confirmed as functional sites. These studies provide the first demonstration of the regulation of a mammalian steroid hormone receptor by a specific microRNA. Several groups have confirmed these findings, as well as the finding of elevated miR-206 in ER α -negative tumors [49,50]. MiR-206 also directly targets the mRNAs encoding the nuclear receptor co-activator proteins steroid receptor co-activator-1 (SRC-1) and SRC-3, as well as GATA-3 [51]. These findings reveal that miR-206 represses estrogen signaling through the coherent targeting of multiple components within the ER α signaling pathway (Figure 3).

In a double-negative feedback loop, miR-206 expression is suppressed by E₂/ER α -signaling [48,52] such that robust ER α expression in luminal A cancers would maintain low levels of miR-206 (Figure 3). In this context, it is worth

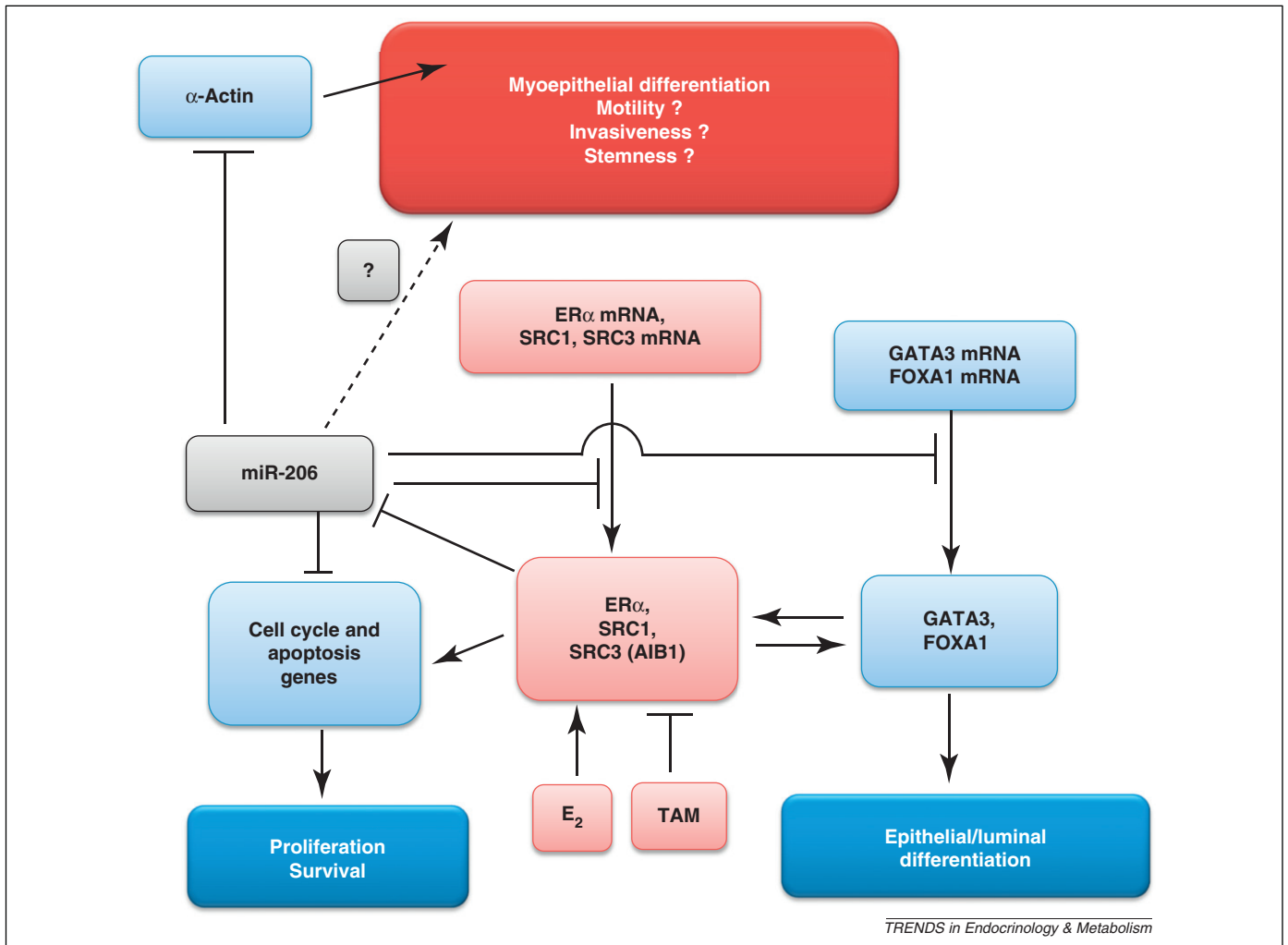


Figure 3. MiR-206 coordinately targets mRNAs encoding ER α , SRC1, SRC3 and GATA3. MiR-206 also targets proliferative genes and, overall, inhibits proliferation. MiR-206 also inhibits ER α and GATA3 (and is predicted to target FOXA1), thereby undermining the stability of an epithelial phenotype. MiR-206 promotes myoepithelial differentiation, increases IL6 and may promote some features of EMT. Dotted line, indirect effect.

noting that processing of the primary transcript of miR-206 (pri-miR-206) by the microprocessor complex was lowered in p72 helicase^{-/-} mouse embryos [53] and that ER α binds to p72 and inhibits the activity of the p68/p72 complex [54]. This raises the questions of whether pri-miR-206 processing is similarly dependent of p72 helicase activity in human breast cancer cells and whether ER α signaling inhibits miR-206 expression through p72.

Mir-206 has been most studied in the context of myogenic induction [55], and in MCF-7 cells miR-206 was shown to repress non-muscle β -actin expression [48]. These findings raise the question of whether miR-206 plays a role in normal mammary epithelia by directing progenitor cells along a myoepithelial lineage. In this context, it is noteworthy that miR-206 decreases expression of the DNA polymerase A1 subunit and the oncogenic receptor c-MET, and increases the tumor suppressor forkhead box O3 (FOXO3) [52]. Overall, this leads to inhibition of cell proliferation [51,52,56], suggesting that miR-206 might repress the proliferation of ER α -positive breast cancer by promoting myoepithelial differentiation, together with silencing of ER α (Figure 3). In fact, miR-206 has been used for 'differentiation therapy' *in vivo* to block growth of rhabdomyosarcomas in mice through the induction of adult

myogenic differentiation [57]. Thus, in some contexts (but clearly not all), ectopic expression of miR-206 might drive slow proliferation and induce myoepithelial differentiation in mesenchymal breast cancers. A major unanswered question is whether miR-206 is expressed in CSCs, or whether enforced miR-206 expression would drive the CSC population to differentiate.

Epidermal growth factor (EGF) stimulates miR-206 expression [51], and miR-206 is elevated in some ER α -negative, highly aggressive forms of breast cancer [36,56]. Because basal-like cancers frequently overexpress EGF receptor (EGFR/HER1) [58], EGF signaling in some mesenchymal basal-like breast cancers might contribute to ER α silencing, in part through miR-206, thereby maintaining a mesenchymal phenotype of cells. It is also noteworthy that ectopic expression of miR-206 in MCF-7 cells enhances IL-6 expression [51]. IL-6 has been shown to induce EMT through STAT signaling and to maintain its own levels through stable autocrine positive feedback loops involving NF- κ B or Notch 3 in mammary epithelial cells and breast cancer [59–62]. Thus, in some microenvironments, miR-206 may promote EMT (i.e. exert prometastatic actions) but repress proliferation (i.e. exert an anti-proliferative function).

miR-221/222

miR-221 and miR-222 (denoted miR-221/222) are expressed from a bicistronic gene on human chromosome X. MiR-221/222 expression is upregulated in several cancers [34] (Table 1). Zhao *et al.* reported that miR-221/222 are also enriched in ER α -negative versus ER α -positive breast cancer and breast cancer cell lines [63]. This study also demonstrated that miR-221 and miR-222 each directly targeted ER α mRNA at a conserved site. Several studies have now shown that miR-221/222 inhibit the translation of ER α mRNA and are involved in anti-estrogen resistance [52,64,65]. As described for miR-206, miR-221/222 expression is repressed by ER α signaling in a double-negative feedback loop (Figure 4). Utilizing miR-221/222 gene promoter-luciferase constructs and chromatin immunoprecipitation, DiLeva *et al.* demonstrated that ER α directly represses the miR-221/222 gene promoter by recruiting the corepressors NCoR and SMRT [52].

In contrast to miR-206, miR-221/222 increase proliferation of ER α -positive breast cancer cell lines [52], with early studies showing that p27 and p57 are miR-221/222 targets. More recent studies by Di Leva *et al.* [52] and Rao *et al.* [65] have extensively examined miR-221/222-regulated genes in ER α -positive MCF-7 breast cancer cells. These genes fall into several KEGG pathways involved in growth factor, cytokine and morphogen signaling, cell-cell junctions and the actin cytoskeleton, and p53 signaling. Rao *et al.* [65]

examined the regulation of β -catenin as part of the canonical Wnt signaling pathway, and showed that miR-221/222 upregulated β -catenin expression and increased the nuclear fraction of β -catenin. Increased Wnt/ β -catenin signaling probably contributes to the observed effects of miR-221/222 on proliferation (Figure 4). Stinson *et al.* [58] recently reported that miR-221/222 promote EMT in mammary MCF-10A cells. MiR-221/222 decrease E-cadherin through targeting the 3'-UTR of the GATA family-related TRPS1, which represses ZEB2 expression. Thus, the targeting of ER α , miR-200f elevated growth factor signaling and enhanced expression of ZEB2 are all consistent with a role of miR-221/222 in promoting EMT (Figure 4). Furthermore, miR-221/222 directly target the microRNA effector Dicer, and expression of Dicer is repressed in ER α -negative breast cancers, which are associated with a poor prognosis [39].

It was recently demonstrated that prolonged mammosphere culture of MCF-7 breast cancer cells resulted in an EMT, and when these cells were returned to adherent culture, a novel cell line (termed MCF-7_M) with a mesenchymal phenotype was generated [66]. Mammosphere culture is commonly used to enrich stem-like cells in various populations, including cell lines and tumor samples. MCF-7_M cells are significantly more proliferative, motile and drug-resistant in *in vitro* assays, and more invasive in SCID (severe combined immunodeficiency) mice compared to the

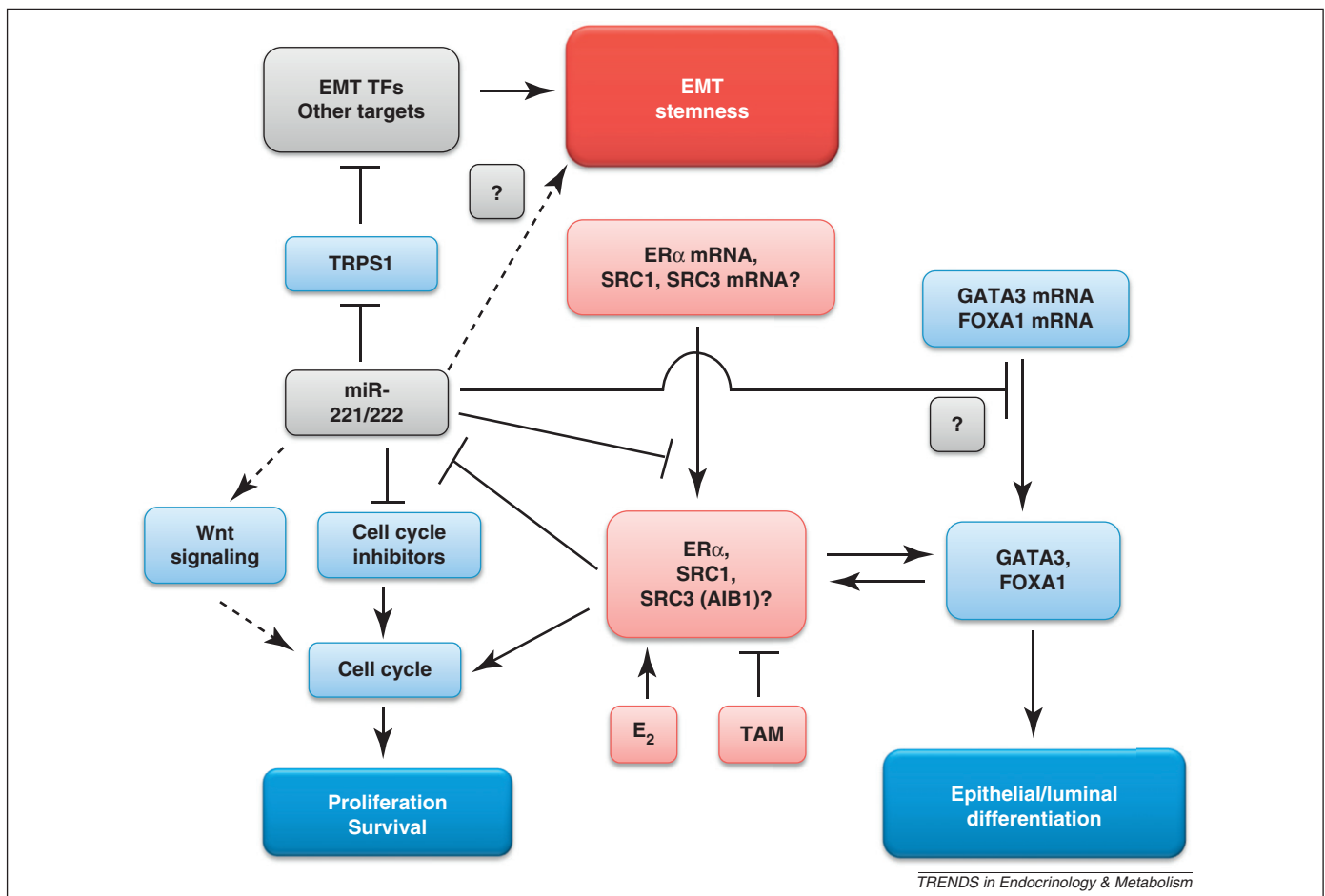


Figure 4. MiR-221/221 regulation of ER α and breast cancer behavior. MiR-221/222 target ER α mRNA, while promoting proliferation through ER α -independent pathways (e.g. Wnt signaling). MiR-221/222 also promote EMT by indirectly increasing Zeb2 (and other EMT-related transcription factors?). It has not been established whether miR-221/222 target other luminal-related transcription factors (e.g. GATA3, FOXA1). Dotted line, indirect effect.

parental MCF-7 cells. Moreover, the majority of MCF-7_M cells reverted to a stem cell-associated CD44^{high}/CD24^{low} phenotype. Mammosphere culture of MCF-7 cells caused marked downregulation of miR-200c, -203 and -205. In addition, upregulation of miR-221 and miR-222 was observed with later passages, and the levels of these microRNAs remained high in the MCF-7_M cell line, as well as in tumors induced by MCF-7_M cells. Repression of miR-221 expression restored ER α protein expression in MCF-7_M cells [66]. Further study is now required to examine the exact relationship between miR-221/222, the anti-EMT miR-200f and EMT-related transcription factors operating via either ER α -dependent or ER α -independent pathways.

miR-130a

In a screen of microRNA-induced suppression of a luciferase reporter with the entire ER α mRNA 3'-UTR, miR-130a induced about 65% repression [67]. In non-small-cell lung cancer 549 cells, miR-130a targets the oncogene c-MET represses miR-221/222 levels via suppression of AP-1 signaling, and suppresses motility [68] (Table 1). Thus, miR-130a is a potentially important microRNA in breast cancer that may repress ER α expression but also suppress EMT. This class of microRNA might play a role in MET, particularly in ER α -negative forms of breast cancer.

miR-17/92

The miR-17/92 cluster on chromosome 13 encodes miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1 and miR-92-1. These microRNAs have been characterized as both oncogenes and tumor suppressors [34,35]. Conflicting reports have reported lower [69] and higher [70] levels of miR-17/92 in breast cancer. MiR-17/92 members (and members of the paralog miR-106a/363 cluster) directly target the mRNAs for ER α and the co-activator SRC3 [71] (Table 1). The miR-17/92 gene is stimulated by both c-myc [71] and cyclin D [69], both of which are downstream targets of ER α , and both c-myc and cyclin D mRNAs are targeted by members of miR-17/92. A role for miR-17/92 in EMT is indicated by findings that the metastatic-promoting Rho kinase increases c-myc levels in breast cancer cell lines, and this in turn increases miR-17/92 [72]. Clearly, more study is required to connect the complex feedback loops between miR-17/92 and ER α , c-myc and cyclin D (as well as E2F1; further references are given in [72]) and oncogenesis in breast tissue, and whether miR-17/92 targets factors that oppose EMT or metastasis.

miR-145

miR-145 is a tumor-suppressor microRNA that is under-expressed in numerous cancers, including breast cancer [73] (Table 1). The miR-145 gene is located on chromosome 5, near a fragile site (5q31.1) and is deleted in about 10% of invasive breast cancers [73]. miR-145 transcription and processing are enhanced by activated p53 [73]. Moreover, miR-145 activated p53 signaling in MCF-7 cells, and p53 stimulated miR-145 expression in a pro-apoptotic positive feedback loop [74]. MiR-145 also represses endogenous ER α expression in MCF-7 cells through interaction of two miR-145 target sites within the coding region of ER α mRNA [74]. Thus, miR-145 appears to act, in part,

as a tumor suppressor in breast cancer through its ability to reduce ER α signaling while increasing p53 expression and activity.

With respect to EMT and metastasis, miR-145 targets mucin 1 (MUC1), which is associated with invasiveness [73]. Gotte *et al.* [75] recently demonstrated that overexpression of miR-145 in several breast cancer cell lines directly targeted the cell adhesion protein JAM-A and changed the expression of several other motility-related proteins, causing a significant reduction in cell motility and invasiveness in both ER α -positive MCF-7 cells and ER α -negative MDA-MB-231 cells. Thus, miR-145 targets several proteins required for motility and invasiveness, even though it represses ER α expression and, thus, ER α -mediated inhibition of EMT. It is noteworthy that miR-145 directly targets mRNAs encoding stemness-related genes and promotes the differentiation of several stem/precursor cell types. It is worth examining whether miR-145 promotes epithelial differentiation and whether it directly antagonizes EMT factors, as ER α does.

Concluding remarks

We have discussed some of the findings related to ER α , microRNAs and EMT in the context of how the most commonly diagnosed form of invasive breast cancer, ER α -positive luminal A, might progress to metastatic disease. We have described ER α -targeting microRNAs that are pro-metastatic/anti-proliferative (miR-206), pro-metastatic/pro-proliferative (miR-221/222), and anti-proliferative/anti-metastatic (miR-130a, miR-145). MiR-17/92 appears to be prometastatic, but is involved in multiple feedback loops that may cause the expression and effects on proliferation of miR-17/92 to be highly dependent on the microenvironment and the genetic and epigenetic background.

One challenge is the accurate identification of microRNAs that significantly contribute to a single pathway (such as EMT) within breast tumors *in situ*. MicroRNAs have potentially hundreds of targets, and *in vivo* studies will be required to establish physiologically important targets in the context of breast cancer, and to develop effective treatment of breast cancers involving manipulation of microRNA expression levels and identification of off-target effects. In addition, because EMT is a reversible process, it will be imperative to define factors in the tumor microenvironment that contribute to the onset of EMT and/or maintenance of the epithelial phenotype. Understanding the interaction between microRNA regulatory pathways and ER α establishes a foundation on which this knowledge can be built.

References

- 1 Chaffer, C.L. and Weinberg, R.A. (2011) A perspective on cancer cell metastasis. *Science* 331, 1559–1564
- 2 Valastyan, S. and Weinberg, R.A. (2011) Tumor metastasis: molecular insights and evolving paradigms. *Cell* 147, 275–292
- 3 Creighton, C.J. *et al.* (2010) Epithelial–mesenchymal transition (EMT) in tumor-initiating cells and its clinical implications in breast cancer. *J. Mammary Gland Biol. Neoplasia* 15, 253–260
- 4 Kalluri, R. and Weinberg, R.A. (2009) The basics of epithelial–mesenchymal transition. *J. Clin. Invest.* 119, 1420–1428

- 5 Singh, A. and Settleman, J. (2010) EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. *Oncogene* 29, 4741–4751
- 6 May, C.D. *et al.* (2011) Epithelial–mesenchymal transition and cancer stem cells: a dangerously dynamic duo in breast cancer progression. *Breast Cancer Res.* 13, 202
- 7 Van Keymeulen, A. *et al.* (2011) Distinct stem cells contribute to mammary gland development and maintenance. *Nature* 479, 189–193
- 8 Visvader, J.E. (2009) Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. *Genes Dev.* 23, 2563–2577
- 9 Chaffer, C.L. *et al.* (2011) Normal and neoplastic nonstem cells can spontaneously convert to a stem-like state. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7950–7955
- 10 Peppercorn, J. *et al.* (2008) Molecular subtypes in breast cancer evaluation and management: divide and conquer. *Cancer Invest.* 26, 1–10
- 11 Sims, A.H. *et al.* (2007) Origins of breast cancer subtypes and therapeutic implications. *Nat. Clin. Pract. Oncol.* 4, 516–525
- 12 Ansieau, S. *et al.* (2008) Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. *Cancer Cell* 14, 79–89
- 13 Brisken, C. and O'Malley, B. (2010) Hormone action in the mammary gland. *Cold Spring Harb. Perspect. Biol.* 2, a003178
- 14 Stingl, J. (2011) Estrogen and progesterone in normal mammary gland development and in cancer. *Horm. Cancer* 2, 85–90
- 15 Parl, F.F. *et al.* (1984) Prognostic significance of estrogen receptor status in breast cancer in relation to tumor stage, axillary node metastasis, and histopathologic grading. *Cancer* 54, 2237–2242
- 16 Eeckhoutte, J. *et al.* (2007) Positive cross-regulatory loop ties GATA-3 to estrogen receptor alpha expression in breast cancer. *Cancer Res.* 67, 6477–6483
- 17 Kourou-Mehr, H. *et al.* (2008) GATA-3 and the regulation of the mammary luminal cell fate. *Curr. Opin. Cell Biol.* 20, 164–170
- 18 Wilson, B.J. and Giguere, V. (2008) Meta-analysis of human cancer microarrays reveals GATA3 is integral to the estrogen receptor alpha pathway. *Mol. Cancer* 7, 49
- 19 Yan, W. *et al.* (2010) GATA3 inhibits breast cancer metastasis through the reversal of epithelial–mesenchymal transition. *J. Biol. Chem.* 285, 14042–14051
- 20 Bernardo, G.M. *et al.* (2010) FOXA1 is an essential determinant of ERalpha expression and mammary ductal morphogenesis. *Development* 137, 2045–2054
- 21 Nakshatri, H. and Badve, S. (2009) FOXA1 in breast cancer. *Expert Rev. Mol. Med.* 11, e8
- 22 Taylor, M.A. *et al.* (2010) The pathophysiology of epithelial–mesenchymal transition induced by transforming growth factor-beta in normal and malignant mammary epithelial cells. *J. Mammary Gland Biol. Neoplasia* 15, 169–190
- 23 Ito, I. *et al.* (2010) Estrogen inhibits transforming growth factor beta signaling by promoting Smad2/3 degradation. *J. Biol. Chem.* 285, 14747–14755
- 24 Shen, H.M. and Tergaonkar, V. (2009) NFkappaB signaling in carcinogenesis and as a potential molecular target for cancer therapy. *Apoptosis* 14, 348–363
- 25 Wang, X. *et al.* (2007) Oestrogen signalling inhibits invasive phenotype by repressing RelB and its target BCL2. *Nat. Cell Biol.* 9, 470–478
- 26 Wang, X. *et al.* (2009) RelB NF-kappaB represses estrogen receptor alpha expression via induction of the zinc finger protein Blimp1. *Mol. Cell Biol.* 29, 3832–3844
- 27 Proia, T.A. *et al.* (2011) Genetic predisposition directs breast cancer phenotype by dictating progenitor cell fate. *Cell Stem Cell* 8, 149–163
- 28 Dhasarathy, A. *et al.* (2007) The transcription factor snail mediates epithelial to mesenchymal transitions by repression of estrogen receptor-alpha. *Mol. Endocrinol.* 21, 2907–2918
- 29 Ye, Y. *et al.* (2010) ERalpha signaling through slug regulates E-cadherin and EMT. *Oncogene* 29, 1451–1462
- 30 Puzstai, L. *et al.* (2010) Estrogen and HER-2 receptor discordance between primary breast cancer and metastasis. *Oncologist* 15, 1164–1168
- 31 Hugo, H. *et al.* (2007) Epithelial–mesenchymal and mesenchymal–epithelial transitions in carcinoma progression. *J. Cell. Physiol.* 213, 374–383
- 32 Creighton, C.J. *et al.* (2009) Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. *Proc. Natl. Acad. Sci. U.S.A.* 106, 13820–13825
- 33 Cochrane, D.R. *et al.* (2011) Steroid receptors and microRNAs: relationships revealed. *Steroids* 76, 1–10
- 34 Garofalo, M. and Croce, C.M. (2011) MicroRNAs: master regulators as potential therapeutics in cancer. *Annu. Rev. Pharmacol. Toxicol.* 51, 25–43
- 35 Adams, B.D. *et al.* (2008) Involvement of microRNAs in breast cancer. *Semin. Reprod. Med.* 26, 522–536
- 36 Iorio, M.V. *et al.* (2005) MicroRNA gene expression deregulation in human breast cancer. *Cancer Res.* 65, 7065–7070
- 37 Cochrane, D.R. *et al.* (2010) MicroRNAs link estrogen receptor alpha status and Dicer levels in breast cancer. *Horm. Cancer* 1, 306–319
- 38 Krol, J. *et al.* (2010) The widespread regulation of microRNA biogenesis, function and decay. *Nat. Rev. Genet.* 11, 597–610
- 39 Bhat-Nakshatri, P. *et al.* (2009) Estradiol-regulated microRNAs control estradiol response in breast cancer cells. *Nucleic Acids Res.* 37, 4850–4861
- 40 Martello, G. *et al.* (2010) A MicroRNA targeting dicer for metastasis control. *Cell* 141, 1195–1207
- 41 Winter, J. *et al.* (2009) Many roads to maturity: microRNA biogenesis pathways and their regulation. *Nat. Cell Biol.* 11, 228–234
- 42 Davis-Dusenbery, B.N. and Hata, A. (2010) Mechanisms of control of microRNA biogenesis. *J. Biochem.* 148, 381–392
- 43 Cheng, C. *et al.* (2009) mRNA expression profiles show differential regulatory effects of microRNAs between estrogen receptor-positive and estrogen receptor-negative breast cancer. *Genome Biol.* 10, R90
- 44 Adams, B.D. *et al.* (2009) Argonaute-2 expression is regulated by epidermal growth factor receptor and mitogen-activated protein kinase signaling and correlates with a transformed phenotype in breast cancer cells. *Endocrinology* 150, 14–23
- 45 Ma, L. *et al.* (2007) Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. *Nature* 449, 682–688
- 46 Wang, L. and Wang, J. (2011) MicroRNA-mediated breast cancer metastasis: from primary site to distant organs. *Oncogene* DOI: 10.1038/onc.2011.444
- 47 Brabletz, S. and Brabletz, T. (2010) The ZEB/miR-200 feedback loop – a motor of cellular plasticity in development and cancer? *EMBO Rep.* 11, 670–677
- 48 Adams, B.D. *et al.* (2007) The micro-ribonucleic acid (miRNA) miR-206 targets the human estrogen receptor-alpha (ERalpha) and represses ERalpha messenger RNA and protein expression in breast cancer cell lines. *Mol. Endocrinol.* 21, 1132–1147
- 49 Yoshimoto, N. *et al.* (2011) Distinct expressions of microRNAs that directly target estrogen receptor alpha in human breast cancer. *Breast Cancer Res. Treat.* 130, 331–339
- 50 O'Day, E. and Lal, A. (2010) MicroRNAs and their target gene networks in breast cancer. *Breast Cancer Res.* 12, 201
- 51 Adams, B.D. *et al.* (2009) The role of miR-206 in the epidermal growth factor (EGF) induced repression of estrogen receptor-alpha (ERalpha) signaling and a luminal phenotype in MCF-7 breast cancer cells. *Mol. Endocrinol.* 23, 1215–1230
- 52 Di Leva, G. *et al.* (2010) MicroRNA cluster 221–222 and estrogen receptor alpha interactions in breast cancer. *J. Natl. Cancer Inst.* 102, 706–721
- 53 Fukuda, T. *et al.* (2007) DEAD-box RNA helicase subunits of the Drosha complex are required for processing of rRNA and a subset of microRNAs. *Nat. Cell Biol.* 9, 604–611
- 54 Yamagata, K. *et al.* (2009) Maturation of microRNA is hormonally regulated by a nuclear receptor. *Mol. Cell* 36, 340–347
- 55 Williams, A.H. *et al.* (2009) MicroRNA control of muscle development and disease. *Curr. Opin. Cell Biol.* 21, 461–469
- 56 Kondo, N. *et al.* (2008) miR-206 Expression is down-regulated in estrogen receptor alpha-positive human breast cancer. *Cancer Res.* 68, 5004–5008
- 57 Taulli, R. *et al.* (2009) The muscle-specific microRNA miR-206 blocks human rhabdomyosarcoma growth in xenotransplanted mice by promoting myogenic differentiation. *J. Clin. Invest.* 119, 2366–2378
- 58 Stinson, S. *et al.* (2011) TRPS1 targeting by miR-221/222 promotes the epithelial-to-mesenchymal transition in breast cancer. *Sci. Signal.* 4, ra41

- 59 Sullivan, N.J. *et al.* (2009) Interleukin-6 induces an epithelial-mesenchymal transition phenotype in human breast cancer cells. *Oncogene* 28, 2940–2947
- 60 Iliopoulos, D. *et al.* (2011) Inducible formation of breast cancer stem cells and their dynamic equilibrium with non-stem cancer cells via IL6 secretion. *Proc. Natl. Acad. Sci. U.S.A.* 108, 1397–1402
- 61 Iliopoulos, D. *et al.* (2009) An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell* 139, 693–706
- 62 Sansone, P. *et al.* (2007) IL-6 triggers malignant features in mammospheres from human ductal breast carcinoma and normal mammary gland. *J. Clin. Invest.* 117, 3988–4002
- 63 Zhao, J.J. *et al.* (2008) MicroRNA-221/222 negatively regulates estrogen receptor alpha and is associated with tamoxifen resistance in breast cancer. *J. Biol. Chem.* 283, 31079–31086
- 64 Miller, T.E. *et al.* (2008) MicroRNA-221/222 confers tamoxifen resistance in breast cancer by targeting p27Kip1. *J. Biol. Chem.* 283, 29897–29903
- 65 Rao, X. *et al.* (2011) MicroRNA-221/222 confers breast cancer fulvestrant resistance by regulating multiple signaling pathways. *Oncogene* 30, 1082–1097
- 66 Guttilla, I.K. *et al.* (2011) Prolonged mammosphere culture of MCF-7 cells induces an EMT and repression of the estrogen receptor by microRNAs. *Breast Cancer Res. Treat.* DOI: 10.1007/s10549-011-1534-y
- 67 Xiong, J. *et al.* (2010) An estrogen receptor alpha suppressor, microRNA-22, is downregulated in estrogen receptor alpha-positive human breast cancer cell lines and clinical samples. *FEBS J.* 277, 1684–1694
- 68 Acunzo, M. *et al.* (2011) miR-130a targets MET and induces TRAIL-sensitivity in NSCLC by downregulating miR-221 and 222. *Oncogene* DOI: 10.1038/onc.2011.260
- 69 Yu, Z. *et al.* (2008) A cyclin D1/microRNA 17/20 regulatory feedback loop in control of breast cancer cell proliferation. *J. Cell Biol.* 182, 509–517
- 70 Volinia, S. *et al.* (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. *Proc. Natl. Acad. Sci. U.S.A.* 103, 2257–2261
- 71 Castellano, L. *et al.* (2009) The estrogen receptor-alpha-induced microRNA signature regulates itself and its transcriptional response. *Proc. Natl. Acad. Sci. U.S.A.* 106, 15732–15737
- 72 Liu, S. *et al.* (2009) Inhibition of rho-associated kinase signaling prevents breast cancer metastasis to human bone. *Cancer Res.* 69, 8742–8751
- 73 Sachdeva, M. and Mo, Y.Y. (2010) miR-145-mediated suppression of cell growth, invasion and metastasis. *Am. J. Transl. Res.* 2, 170–180
- 74 Spizzo, R. *et al.* (2010) miR-145 participates with TP53 in a death-promoting regulatory loop and targets estrogen receptor-alpha in human breast cancer cells. *Cell Death Differ.* 17, 246–254
- 75 Gotte, M. *et al.* (2010) miR-145-dependent targeting of junctional adhesion molecule A and modulation of fascin expression are associated with reduced breast cancer cell motility and invasiveness. *Oncogene* 29, 6569–6580
- 76 Howe, E.N. *et al.* (2011) Targets of miR-200c mediate suppression of cell motility and anoikis resistance. *Breast Cancer Res.* 13, R45
- 77 Brabletz, S. *et al.* (2011) The ZEB1/miR-200 feedback loop controls Notch signalling in cancer cells. *EMBO J.* 30, 770–782
- 78 Yu, M. *et al.* (2009) A developmentally regulated inducer of EMT, LBX1, contributes to breast cancer progression. *Genes Dev.* 23, 1737–1742
- 79 Moody, S.E. *et al.* (2005) The transcriptional repressor Snail promotes mammary tumor recurrence. *Cancer Cell* 8, 197–209
- 80 Sanchez-Tillo, E. *et al.* (2010) ZEB1 represses E-cadherin and induces an EMT by recruiting the SWI/SNF chromatin-remodeling protein BRG1. *Oncogene* 29, 3490–3500
- 81 Cheng, G.Z. *et al.* (2007) Twist transcriptionally up-regulates AKT2 in breast cancer cells leading to increased migration, invasion, and resistance to paclitaxel. *Cancer Res.* 67, 1979–1987
- 82 Yang, M.H. *et al.* (2010) Bmi1 is essential in Twist1-induced epithelial-mesenchymal transition. *Nat. Cell Biol.* 12, 982–992
- 83 Yang, J. *et al.* (2004) Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 117, 927–939
- 84 Chao, Y.L. *et al.* (2010) Breast carcinoma cells re-express E-cadherin during mesenchymal to epithelial reverting transition. *Mol. Cancer* 9, 179
- 85 Mani, S.A. *et al.* (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 133, 704–715
- 86 Morel, A.P. *et al.* (2008) Generation of breast cancer stem cells through epithelial-mesenchymal transition. *PLoS ONE* 3, e2888
- 87 Dykxhoorn, D.M. *et al.* (2009) miR-200 enhances mouse breast cancer cell colonization to form distant metastases. *PLoS ONE* 4, e7181
- 88 Shimono, Y. *et al.* (2009) Downregulation of miRNA-200c links breast cancer stem cells with normal stem cells. *Cell* 138, 592–603
- 89 Miyoshi, N. *et al.* (2011) Reprogramming of mouse and human cells to pluripotency using mature microRNAs. *Cell Stem Cell* 8, 633–638