Review Article
Role of Estrogen Receptor Signaling in Breast Cancer Metastasis

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Metastatic breast cancer is a life-threatening stage of cancer and is the leading cause of death in advanced breast cancer patients. Estrogen signaling and the estrogen receptor (ER) are implicated in breast cancer progression, and the majority of the human breast cancers start out as estrogen dependent. Accumulating evidence suggests that ER signaling is complex, involving coregulatory proteins and extranuclear actions. ER-coregulatory proteins are tightly regulated under normal conditions with miss expression primarily reported in cancer. Deregulation of ER coregulators or ER extranuclear signaling has potential to promote metastasis in ER-positive breast cancer cells. This review summarizes the emerging role of ER signaling in promoting metastasis of breast cancer cells, discusses the molecular mechanisms by which ER signaling contributes to metastasis, and explores possible therapeutic targets to block ER-driven metastasis.

1. Introduction
The steroid hormone, estradiol, plays an important role in the progression of breast cancer, and a majority of the human breast cancers start out as estrogen dependent and express the estrogen receptor (ER). The biological effects of estrogen are mediated by its binding to one of the structurally and functionally distinct ERs (ERα and ERβ) [1]. Endocrine therapy using Tamoxifen, a selective estrogen receptor modulator [2], and aromatase inhibitors, which ablate peripheral estrogen synthesis, has been shown to substantially improve disease-free survival [3]. Endocrine therapy has also been shown to have a positive effect on the treatment of ER-positive breast cancer [4]. Despite these positive effects, initial or acquired resistance to endocrine therapies frequently occurs with tumors recurring as metastatic. Tumor metastasis comprises a series of discrete biological processes that moves tumor cells from the primary neoplasm to a distant location [5] and involves a multi-step cascade of coordinated cell adhesion and contractility as well as proteolytic remodeling of the extracellular matrix (ECM) [6, 7]. Even though substantial information is available on the process of metastasis, the molecular basis of breast cancer progression to metastasis and the role of ERα signaling in this process remain poorly understood. A few early studies suggested a negative effect of ERα signaling on motility and invasion of cells [8, 9], while several recent studies showed a positive effect of ER signaling on motility [10–14]. In this review, we summarized the emerging evidence for the role of ERα signaling in breast cancer progression to metastasis and discuss the possibility of targeting ERα signaling crosstalk with cytosolic kinases as a possible additional therapeutic target for treating/preventing ER-positive metastatic breast cancer.

2. ERα Signaling Mechanisms
ERα is the major ER subtype in the mammary epithelium and plays a critical role in mammary gland biology as well as in breast cancer progression [15, 16]. The ERα comprises an N-terminal AF1 domain, a DNA-binding domain, and a C-terminal ligand-binding region that contains an AF2 domain [17]. Upon the binding of estrogen to ERα, the ligand-activated ERα translocates to the nucleus, binds to the responsive element in the target gene promoter, and stimulates gene transcription (genomic/nuclear signaling) [18, 19]. Emerging evidence suggests that ER signaling is
Collectively, these emerging findings implicate the role of cancer metastasis. Specific gene expression and decreases metastasis [30]. Functional or transcriptional loss is disrupting intercellular contacts, an early step in metastatic cadherin is implicated with a critical role in metastasis by [31]. Loss of the epithelial adhesion molecule Eα−/− mice harboring the mouse mammary tumor virus-polyomavirus middle T (PyMT) transgene (Tg) revealed that AIB1 knock down significantly reduces lung metastasis but not mammary tumorigenesis. Compared with WT/PyMT mice, Tg SRC-1−/−/PyMT mice had invasation of mammary tumor cells. In addition, the frequency and extent of lung metastasis were drastically lower in the Tg mice than in the WT mice [29]. Another study using Tg SRC-1−/− mice reported that deficiency of SRC-1 coregulator increases MMTV-neu-mediated tumor latency and differentiation-specific gene expression and decreases metastasis [30]. Collectively, these emerging findings implicate the role of the ERα-coregulator-associated activities/functions in breast cancer metastasis.

4. ERα Extranuclear Actions and Metastasis

Emerging evidence suggests that the ERα participates in extranuclear signaling [39]. ERα activation, by E2, induces key features of motile cells including rapid cytoskeletal reorganization and the development of specialized structures including fillopodia and ruffles [37]. To establish the role of E2-mediated extranuclear actions, researchers developed E2-Dendrimers (EDCs), which are nanoparticles coated with estrogen. These EDCs uniquely localize in the membrane and cytoplasm, preferably activating ERα extranuclear signaling. Using these EDCs, researchers have demonstrated that ERα extranuclear pathways have distinct biological outcomes [40]. Our laboratory using EDCs provided further evidence that ERα extranuclear signaling has the potential to contribute to the breast cancer cell motility (Figure 1) [10]. ERα extranuclear signaling promotes stimulation of the Src kinase, mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K), and protein kinase C pathways in the cytosol (10, 11). Recent studies identified PELP1 as one of the components of the ERα signalosome in the cytoplasm, and estrogen-mediated extranuclear signaling promotes cytoskeleton reorganization via ER-Src-PELP1-Pi3K-ILK1 pathway [10]. Many of the kinases activated by ERα extra-nuclear signaling are implicated in breast cancer metastasis. For example, ERK and protein kinase B (AKT) phosphorylation play important roles in breast cancer cell migration [14], and Src and ILK1 kinases play critical roles in invasion and metastasis of breast cancer cells [41, 42].

In addition to ERα interactions with cytosolic kinases, few other mechanisms by which the ERα activates extranuclear signaling have been reported. Membrane-bound ERα has been reported to be associated with growth factor receptors such as IGF-1R, EGFR, and HER2; such interactions play a role in cytoskeleton reorganization [43]. Dysregulation of HER2 in breast cancer cells enhances the expression of an isoform of MTA1 (MTA1s), which promotes the cytoplasmic sequestration of ERα leading to constitutive activation of MAPK. These study findings implicate the regulation of the cellular localization of ERα by MTA1s as a mechanism for enhancing ERα extranuclear actions by nuclear exclusion [44]. Recent studies also found that the ERα was methylated via posttranslational modifications, and methylated ERα was predominantly present in the cytoplasm, suggesting that deregulation of arginine methylases may have consequences in activation of ERα extranuclear actions [45]. Collectively, these emerging results suggest that ER extranuclear signaling has the potential to promote breast cancer cell migration and metastasis.

3. ERα Genomic Actions and Metastasis

Within the last decade, research has provided substantial data to suggest that alteration in cellular concentration or genetic dysfunction of coregulators can contribute to a pathologic outcome by modulating ER genomic actions and have potential to drive cancer cell proliferation and metastasis [31]. Loss of the epithelial adhesion molecule E-cadherin is implicated with a critical role in metastasis by disrupting intercellular contacts, an early step in metastatic dissemination [32]. Functional or transcriptional loss is commonly associated with an invasive and poorly differentiated phenotype [33]. Deregulation of ER-coregulator signaling can lead to aberrant expression of Snail, resulting in the loss of expression of E-cadherin and invasive growth. For example, MTA1, a commonly deregulated coregulator in breast cancer, promotes transcriptional repression of ER, leading to metastatic progression [34]. The ERα coregulator (AIB1) amplified in breast cancer has been shown to promote breast cancer metastasis by activation of PEA3-mediated matrix metalloproteinase 2 (MMP2) and MMP9 expression [35]. SRC-1, another ER coregulator, has also been shown to promote breast cancer invasiveness and metastasis by coactivating PEA3-mediated Twist expression [36]. Recent studies have found deregulation of the ER coregulator PELP1 in invasive and metastatic breast tumors [37, 38]. Recent studies using PELP1 overexpression and knockdown demonstrated that PELP1 plays an important role in ERα-positive metastasis [10]. Collectively, these studies indicate that ERα and ER coregulators modulate expression of genes involved in metastasis.
5. ERα Regulation of Metastasis

Metastases spawned by malignant tumors that have acquired increased invasiveness are responsible for almost all breast-cancer-related morbidity and mortality. The majority of ERα-positive cells retain their ERα and respond positively to initial endocrine therapy for the treatment of advanced metastatic disease. Several recent studies have detected the presence of ERα expression in metastatic tumors [46–48]. A correlation between ERα-positive tumors and the development of bone metastasis has been observed clinically [49, 50]. Many metastatic tumors retain ERα. If primary tumors are ERα positive, greater than 80% of the lymph node metastases, and 65–70% of distant metastases retain ERα [46, 47]. A clinical correlation has also been reported between ERα-positive tumors and the development of bone metastasis [49, 50]. ERα signaling has also been shown to enhance lung metastasis [51]. In addition, ERα-mediated signaling has enhanced lung metastasis by promoting host-compartment response [51]. These emerging findings suggest that ERα signaling plays a role in metastasis.

6. ERβ Regulation of Cell Migration and Metastasis

ERβ, similar to ERα, also functions as a transcription factor that mediates different physiological responses to estrogen signaling. However, the physiological consequences of ERβ-mediated transcriptional regulation are distinct from those of ERα [1]. A number of recent studies suggest that an increase in ERβ expression decreases cell proliferation and that ERβ has antiproliferative (tumor suppressor) functions [52–54]. Reduced expression of ERβ was reported in invasive breast cancer [55], and ERβ expression is associated with less invasive and proliferating tumors [56]. Downregulation of ERβ is shown to promote epithelial-to-mesenchymal transition (EMT) in prostate cancer cells [57]. A recent study using breast cancer model cells provided evidence that ERβ expression was associated with less cell migration. Mechanistic studies indicated that ERβ affects integrin expression and clustering and consequently modulates adhesion and migration of breast cancer cells [58]. Collectively, the emerging evidence in various model cells (including ovary and prostrate) suggests that ERβ signaling may promote antimigratory and anti-invasive responses; however, future studies using breast models are needed to further validate these findings.

7. Estrogen Regulation of EMT

EMT constitutes the loss of hallmark structures and physiological properties associated with the epithelia and the gain of new properties, including migratory and invasive growth patterns [59]. Loss of E-cadherin is a key initial step in the transdifferentiation of epithelial cells to a mesenchymal phenotype, which occurs when tumor epithelial cells invade the surrounding tissues [60]. Evolving evidence suggests that estrogen signaling can influence EMT and ERα signaling.
crosstalk with several EMT regulators such as Snail and Slug. ERα directly binds to and regulates the promoter of metastasis tumor antigen (MTA) 3 that suppresses Snail, a gene implicated in EMT transition [61]. ERα downregulates Slug transcription by the formation of a corepressor complex involving HDAC1 (histone deacetylase 1) and N-CoR (nuclear receptor co-repressor) [62]. Estrogen promotes down-regulation of E-cadherin via transcriptional regulation by recruitment of corepressors such as scaffold attachment factor B [63]. Estrogen plays an important role in cytoskeletal rearrangements mediated by delocalization of E-cadherin [64]. Furthermore, a recent study found that E2 promotes reversible EMT-like transition as well as collective motility in ERα-positive cells [65]. Estrogen-regulated EMT is complex and is dependent on temporal expression patterns of MTA family members, cell-adhesion-essential regulators, and ER coregulators [66]. ERα signaling negatively regulates EMT by modulating MTA3 expression and thus promotes differentiation [61]. Collectively, these findings implicate that estrogen-mediated EMT depends on the cellular repertoire of ERα coregulators and EMT regulators and that their cross talk has potential to differentially affect breast cancer progression, leading to metastasis via EMT changes.

8. Tumor Microenvironment Regulation of ER Signaling

The metastasis signaling cascade is orchestrated through the activation of biochemical pathways that involve the tumor microenvironment. Stromal cells (fibroblasts, inflammatory cells, and endovascular cells) play important roles to create a supportive environment for tumor cell growth [67, 68]. Chemokines produced by stromal cells have potential to influence ERα-positive breast cancer progression to metastasis. The chemokine CXCL12/SDF-1 and its G-protein-coupled receptor CXCR4-mediated signaling pathways play important roles in the migration and invasion of breast cancer cells. Some evidence suggests that HER2-mediated breast tumor metastasis may involve HER2 and CXCR4 signaling pathway cross talk [69]. CXCR4 overexpression correlated with worse prognosis in patients and constitutive activation of CXCR4 in poorly metastatic ER-positive MCF7 cells led to enhanced tumor growth and metastasis. The results from this study showed that enhanced CXCR4 signaling is sufficient to drive ERα-positive breast cancers to a metastatic and endocrine-therapy-resistant phenotype via increases in MAPK signaling [70].

The intratumoral levels of estrogens and growth factors are regulated by the tumor-stromal interactions in the tumor microenvironment [71]. Cross talk between the tumor and stromal cells promote expression of aromatase, a key enzyme in E2 biosynthesis, resulting in intra-tumoral estrogen production in postmenopausal breast tumors [72]. Tumor-stromal cross talk regulates aromatase gene expression via the production of various factors such as COX2, tumor necrosis factor-α, interleukin-6, and interleukin-11 [71]. Tumor-stromal interactions also contribute to the expression of growth factors such as EGF and IGF-1, which activate the ERα through growth factor receptor cross talk, leading to ERα-positive breast cancer progression [73].

9. ER Signaling Components as Potential Biomarkers for Predicting Metastasis

ERα status is routinely used in the clinic for treatment selection; however, additional markers are urgently needed to predict metastasis. Considering the evolving significance of ERα coregulators (SRC family members such as SRC-3/AIB1) in mammary tumor invasion and metastasis [74], SRC-3 status could be used as a diagnostic biomarker. Similarly, expression of the ER coregulator PELP1 is deregulated in metastatic breast tumors [37], and PELP1 protein expression is an independent prognostic predictor of breast cancer-specific survival and disease-free survival [38]. Since PELP1 plays a critical role in estrogen-mediated extranuclear signaling, these findings suggest that PELP1 could be used as a potential biomarker for predicting ER-driven metastasis. Several studies using various Src kinase inhibitors and dominant-negative mutants demonstrated that inhibiting c-Src activity decreased the metastatic potential of breast cancer cells [75]. Given the role of Src kinase in ER signaling, phospho-c-Src is an attractive biomarker for predicting breast cancer metastasis in conjunction with other prognostic factors. Few recent preclinical studies using Src inhibitors confirmed the downstream target of Phos-Src and -FAK could be possible diagnostic markers [76]. Because AKT signaling is implicated in invasive ductal carcinoma of the breast and implicated in ERα-mediated extranuclear actions leading migration/invasion, Phospho AKT (pAKT) status could be a potential biomarker in the prediction of therapeutic response in invasive ductal carcinoma of the breast [74]. Even though these emerging findings suggest ERα-signaling molecules as potential biomarkers, additional studies using a large set of human tumor samples are needed to clearly establish them as prognostic markers.

10. Therapeutic Targeting of ERα Signaling for Blocking Metastasis

The emerging significance of the ERα in the metastatic cascade indicates novel possibilities for therapeutic targeting of specific ERα signaling components that mediate migration, invasion, and EMT. A large portion of metastases retain their ERα when the primary tumors are ERα positive. Several recent studies detected the presence of ERα and aromatase expression in metastatic tumors [46–48]. We envision that the therapies targeting ER signaling axis leading to metastasis are more suitable for early stage patients who have tumors that are amenable to biopsy and IHC analysis. Potential markers of ERα signaling that are implicated in metastasis (including kinases such as Src, AKT, and PI3K and coregulators such as PELP1, AIB1, and SRC-1) could be used in addition to traditional ERα status to identify this subset of patients.

Aromatase is recognized as a potent target in endocrine therapy for the treatment of postmenopausal breast cancers
Because some metastases retain their ERα signaling, screening of patients with advanced breast cancer for expression of ERα, ER-coregulators, and aromatase may provide a rationale for the development of customized treatment of a subset of patients with ERα-positive and aromatase-positive cancer. These patients could be treated with an aromatase inhibitor (Letrozole) that ablates peripheral estrogen synthesis and ERα degraders/signaling blockers for their ERα-positive metastatic tumors.

Because ERα and ERβ have different physiological functions and have ligand-binding properties that differ enough to be selective in their ligand binding, opportunities now exist for testing of novel ER subtype-specific, selective ER modulators [77]. Several synthetic or novel natural compounds derived from plant materials have the potential to function as ERβ agonists [54, 78], and these compounds may have utility in augmenting ERβ tumor suppressive functions.

If ERβ can hamper the regulation of ERα and inhibit the proliferation as well as affect the crosstalk with growth factors and their receptors, testing of ERβ agonist in combination with other endocrine therapies will provide a novel means to target ERα-driven metastasis. Recent studies found a therapeutic efficacy using ERβ agonists in combination with aromatase inhibitors, and this strategy may be useful in treating aromatase-inhibitor-(AI-) resistant metastatic breast cancer [79].

ERα-positive metastasis has been associated with chemo-kine signaling through SDF-1–CXCR4. Therefore, CXCR4 signaling is a rational therapeutic target for the treatment of ER-positive advanced breast carcinomas [70]. Integrin-linked kinase (ILK) is a nodal molecule in many molecular pathways that are implicated in cancer metastasis. Recent evidence suggests that ER extranuclear signaling utilizes the ILK axis [10]; therefore, ILK inhibitors such as QLT-0267 could be used to curb motility of breast cancer cells [80]. Since arginine methylation is implicated in ERα extranuclear signaling, blocking arginine methylases could be a possible therapeutic target. Compounds such as guanidine-arginine-nitrosated peptides or the thioglycolic amide RM65 may be useful to block this pathway [81, 82]. SRC3/AIB1 is frequently amplified or overexpressed in human breast cancer and is implicated in breast cancer progression to advanced ERα-positive tumors. Mechanistic studies showed AIB1 overexpression activates the mammalian target of rapamycin (mTOR), and activation of mTOR pathway is critical for AIB1-driven tumorigenesis [83]. Recent studies suggest that mTOR inhibition and ER-targeted endocrine therapy may improve the outcome of the subset of patients with ERα-positive breast cancers overexpressing AIB1 [84].

Emerging evidence suggests that Src participates in ERα extranuclear actions and its wide deregulation in breast tumors suggests that it could be a potential candidate for treating ERα-positive metastasis [85]. The fact that Src can mediate interactions between the ERα and growth-factor-signaling pathways is of particular importance because cross talk between these pathways is implicated in activation of ERα extranuclear signaling leading to cell migration and invasion [10]. Further, the ability of the Src axis to promote local estrogen synthesis via aromatase activation has potential to form an autocrine loop of ERα signaling leading to tumor cell proliferation and metastasis [86]. Thus, blocking the Src axis could block ERα signaling at multiple fronts and thus reducing the ability of the ERα to promote metastasis. Recent studies found that inhibition of the Src family tyrosine kinases using inhibitors such as dasatinib can block ERα-mediated extranuclear actions leading to cell migration and invasion [10]. Therefore, it is tempting to speculate that combination of hormonal therapy with dasatinib, an orally available inhibitor of Src family tyrosine kinases that is currently approved for clinical trials to treat solid tumors [87–89], may be useful in curbing breast cancer metastases.

**11. Conclusions/Significance**

The most deadly aspect of breast cancer is its ability to spread or metastasize. Recent mechanistic studies have increased our understanding and highlight a role of estrogen-induced rapid ERα extranuclear signaling in facilitating the metastatic process. This signaling pathway thus provides new targets for therapeutic intervention. During progression from tumorigenesis to invasion, tumor cells trigger signals that activate ERα-extranuclear-signaling pathways, leading to enhanced cell migratory functions and metastasis, thus ER extranuclear signaling represents an important target for metastatic control of ERα-positive tumors (Figure 2). Since multiple signaling pathways in addition to estrogen are involved in activating ERs, combination therapies using both endocrine and nonendocrine agents that block different pathways may have better therapeutic effects and
may delay the development of estrogen-driven metastasis. Future studies identifying the molecular mechanisms of ERα signaling contributing to ERα-driven metastasis as well as examining the prognostic/diagnostic significance of ERα signaling components using a larger sample size of tumors is warranted. Further, elucidation of the pathologic roles of ERα extranuclear signaling in metastasis will have important implications for development of novel breast cancer therapeutics and in the development of the next generation of selective ER modulators.

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