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*Focus On*

# Role of plasma membrane ER protein in breast cancer

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**Abstract** The role of estradiol and the estrogen receptor (ER-α) in the etiology of breast cancer have long been appreciated. This understanding has been complicated by two discoveries in the 1990s: (1) a second estrogen receptor (ER-β) whose expression pattern and activity overlap with but are distinct from those of ER-α; and (2) a pool of ERs located at the plasma membrane. This plasma membrane-localized ER constitutes a distinct pool of receptors whose protein interactions, signaling mechanisms, and cellular functions are not the same as that of the cytoplasmic- and nuclear-localized ER and are not as well understood. Here, we will consider the structure and function of the membrane-localized ER protein. We will then discuss what is known about the role of the membrane ER in the development and its implications for breast cancer treatment.

**Keywords:** Breast cancer; Breast cancer susceptibility gene 1 (BRCA1); Epidermal growth factor receptor (EGFR); Estrogen receptor (ER-α); G protein; Insulin-like growth factor 1 receptor (IGF1R); Plasma membrane; Signaling

## Introduction

ER-α (ESRA), a member of the nuclear receptor superfamily, is a ligand-activated transcription factor that contains domains for DNA binding, transcriptional activation, and hormone (17β-estradiol, E2) binding. The full-length ER-α is a 595 amino acid, 66-kDa protein. ER-β (ESRB), which is encoded by a separate gene, was identified and characterized in 1996 [1,2]. The DNA-binding and ligand-binding domains of ER-β show a high degree of identity to ER-α; while the N-terminal activation function (AF-1), hinge, and F (C-terminal) domains are not conserved. A third receptor (ER-γ) was identified in teleost fish [3]; but a mammalian homolog has not been found. Early evidence of the existence of membrane-associated E2 receptors that can transduce rapid signaling events through G-protein activation has been reviewed elsewhere [4]. In the last few years, new data have emerged on the structure, function, and potential physiologic importance of membrane ERs.

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## Characterization of plasma membrane ER

While the presence of high-affinity cell-surface binding sites for E2 was known in the 1970s [5], structural characterization of these sites has only recently been achieved. Thus, expression of exogenous ER-α or ER-β in Chinese hamster ovary cells resulted in expression of ER in the nucleus and cell membrane [6]. The abundance of membrane ER was 2–3% of that of nuclear ER, but the dissociation constants ($K_d$s) were similar. Both membrane ERs were able to activate G proteins (Gαq and Gαs), generate cAMP (via Gαs), stimulate inositol triphosphate ($IP_3$) production and calcium influx (via Gαq), and induce extracellular signal-regulated kinase (ERK) signaling and cell proliferation [6]. About 5% of the endogenous ER-α localizes to the cell membrane in MCF-7, an E2-responsive breast cancer cell line, suggesting

a role for the membrane ER in breast cancer. Recently, a G protein-coupled receptor, GPR30, was found to localize to the endoplasmic reticulum, bind E2, and induce calcium mobilization and $IP_3$ synthesis in response to E2 [7].

The mechanism(s) by which ER-α localizes to the cell membrane is uncertain, since it does not contain a classical membrane localization signal. Serine-522 in the E-domain (ligand-binding domain) is required for membrane insertion, as is caveolin, a scaffold protein [8]. While full-length ER-α probably comprises most membrane ER, the E-domain is sufficient for insertion into the membrane. Membrane localization of ER-α also requires palmitoylation of cysteine-447. Mutation of this site blocked palmitoylation of ER-α, association with caveolin, membrane localization, rapid signaling, and proliferation in response to E2 [9]. Dimerization is required for membrane ER-α to mediate the rapid non-genomic actions of E2, but not for its insertion into the membrane [10].

Recent studies have identified an N-terminally truncated ER-α (46 kDa) in vascular endothelial cells that is generated by alternative splicing and recruited to the plasma membrane by palmitoylation [11,12]. ER46 transduces membrane-initiated E2 responses, including activation of eNOS (NOS3, endothelial nitric oxide synthetase), consistent with an earlier study showing that endogenous plasma membrane ER-α activates Gαi, leading to synthesis of nitric oxide [13]. Whether ER46 is expressed on the membranes of other cell types is unclear at present.

## Signaling from membrane ER

One signaling mechanism of membrane located G protein-coupled ERs involves cross-activation of growth factor receptor signaling pathways, including those of the epidermal growth factor receptor (EGFR) and the insulin-like growth factor 1 receptor (IGF1R) [14–16]. In a recent study, E2-stimulated ERK activation in breast cancer and vascular endothelial cells required both ER-α and EGFR activation, which was mediated by the rapid release of the heparin-binding EGF-like growth factor (HBEGF) in MCF-7 cells [17]. Other events in this pathway were identified, including its dependence upon several G proteins (Gαq, Gαi, and Gβγ) and the role of SRC-mediated activation of several matrix metalloproteinases (MMP2 and MMP9) in E2-induced HBEGF release and activation of several protein kinases (ERK, c-Akt, and p38β MAP kinase (SAPK2B)) [17].

Shc is a ubiquitous signaling adapter protein containing Src homology 2 and 3 ($SH_2$ and $SH_3$) domains. In MCF-7 cells, E2 causes the rapid association of Shc with ER-α and their translocation to the plasma membrane. This process involves E2-induced phosphorylation of IGF1R and formation of a ternary protein complex of ER-α, Shc, and IGF1R at the membrane [18,19]. The E2-induced association of ER-α with IGF1R and membrane localization of ER-α required Shc; and all three components were necessary for E2-induced ERK phosphorylation. The ability of membrane ER-α to transduce EGFR and IGF1R signaling (leading to ERK activation and cell proliferation) has other implications. Thus, EGF and IGF1 can activate unliganded ER-α via ERK-mediated phosphorylation of serine-118 on the AF-1 domain [19,20], although other kinases may mediate the E2-induced phosphorylation of serine-118 [21]. c-Akt, another target of growth factor signaling, phosphorylates ER-α on serine-167 of AF-1; and this event may contribute to Tamoxifen resistance [22]. Coregulator proteins are also phosphorylation targets. Thus, E2 rapidly induces phosphorylation of the coactivator AIB1 (amplified in breast cancer 1) in an ER-dependent manner [23]. The p160 family coactivator GRIP1 is phosphorylated by ERK at serine-736, an event that is required for growth factor induction of GRIP1 coactivator function [24]. These findings suggest that growth factor signaling initiated by membrane ER may activate the nuclear ER-α via phosphorylation of nuclear ER-α or its coactivators [25], allowing the membrane ER to regulate both transcriptional and non-genomic actions of E2.

## Role of membrane ER in breast cancer

Previous studies revealed that the tumor suppressor protein encoded by the breast cancer susceptibility gene 1 (BRCA1) interacts directly with ER-α and inhibits its transcriptional activity and estrogen-responsive gene expression [26–29]. In ER-α positive breast cancer cells (MCF-7 and ZR-75-1), E2 caused activation of ERK and cell proliferation that was blocked by exogenous BRCA1 or inhibition of ERK signaling [30]. BRCA1 also inhibited EGF-induced ERK activity and cell proliferation that was mediated, in part, through the mitogen-activated kinase phosphatase 1 (MKP1). These findings suggest that the ability of BRCA1 to inhibit E2-stimulated breast cancer cell proliferation is due, in part, to inhibition of membrane ER-α signaling.

A role for the membrane ER in breast cancer response to hormonal manipulation has been postulated. Thus, it was suggested that membrane ER-α contributes to estrogen hypersensitivity in women who relapse following oophorectomy [31]. These patients often respond to aromatase inhibitors, which block peripheral conversion of androgens to estrogen. In the setting of long-term E2 deprivation, ER-α is up-regulated, as are growth factor pathways

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that mediate the rapid non-genomic effects of E2 (including those involving ERK, phosphatidylinositol-3-kinase (PI3K), mammalian target of rapamycin (MTOR), and several signaling adaptor proteins (Shc, Grb2, and Sos)) [31]. These are some of the same components that mediate membrane ER-α signaling, suggesting that the membrane ER contributes to the E2 hypersensitivity. A similar mechanism may mediate relapse in patients treated with Tamoxifen. In this respect, it has been reported that resistance to Tamoxifen-induced apoptosis is mediated by HER2/Neu and the plasma membrane ER [32].

Just as membrane ERs can mediate cardioprotective and neuroprotective effects of E2, they may mediate survival of breast cancer cells. Thus, E2-blocked chemotherapy and radiation-induced apoptosis in ER-positive breast cancer cells through stimulation of ERK and inhibition of c-Jun N-terminal kinase (JNK) activity by the membrane ER-α [33]. Expression of the metastasis-associated gene 1 (MTA1) is associated with aggressive breast cancer. Interestingly, a short form of MTA1 (MTA1s) sequesters ER-α in the cytoplasm and represses E2-induced transcriptional activity, while promoting its non-genomic responses [34]. These findings suggest that cell membrane (or at least non-nuclear) ER-α signaling may promote the malignant phenotype of breast cancer.

The presence of ER-α and ER-β in plasma membrane caveolae from cultured lung carcinoma cells was described recently; and ER-α immunostaining was detected at the cell membrane in archival human breast and lung tumor samples, based on confocal microscopy [35]. Consistent with a plasma membrane ER-α in lung cancer cells, E2 induced ERK signaling and lung cancer cell proliferation that was blocked by Faslodex (ICI 182,780), a pure anti-estrogen [35]. A recent study compared the properties of stably integrated wild-type nuclear ER-α vs. a modified membrane-targeted ER-α in originally ER-negative MDA-MB-231 breast cancer cells. Unlike the nuclear ER-α, the membrane receptor expression was not decreased by E2 or Faslodex; and the ability to regulate ERK activity, c-Akt activity, and cell proliferation differed between the nuclear vs. membrane receptors [36]. Interestingly, an inverse correlation between EGFR and ER-α levels in human breast cancers has been described [37,38]. This correlation may be mediated through an E2-sensitive negative regulatory element within first intron of the EGFR gene. It has been postulated that increased

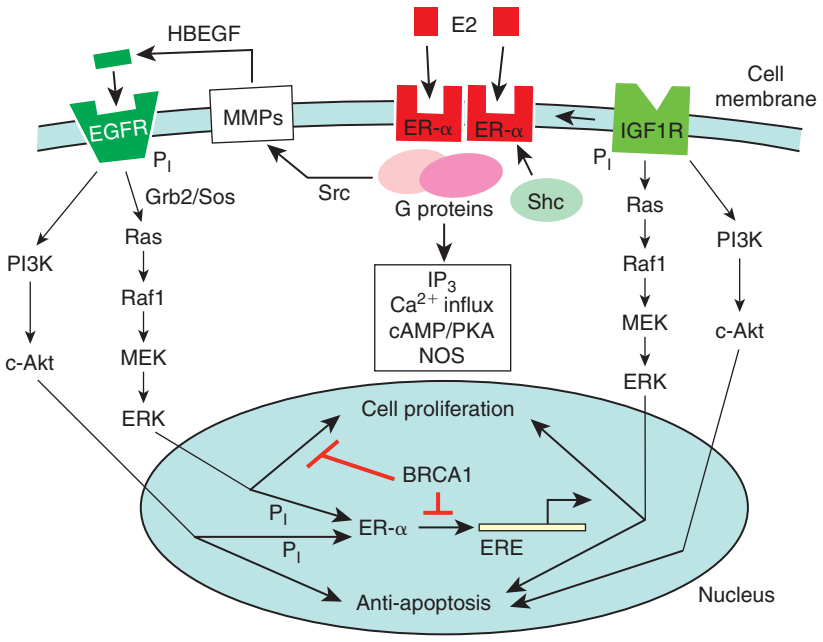


**Figure 1.**
*Schematic illustration of signaling pathways for cell membrane-localized ER-α in breast cancer cells. The ligated plasma membrane ER-α (shown here as a homodimer) signals through several pathways, involving IGF1R, G proteins, and EGFR. Activation of growth factor signaling pathways through several kinases (including c-Akt, ERK, and others (e.g. JNK)) lead to the following consequences: (1) stimulation of cell proliferation (which is modulated by BRCA1 and MKP1); (2) inhibition of apoptosis; and (3) stimulation of transcription by the nuclear ER-α. Activation of nuclear ER-α is mediated by phosphorylation of nuclear receptor coactivators (e.g. AIB1, GRIP1, and CBP (CREB-binding protein)) or, more directly, by phosphorylation of ER-α itself. Abbreviations: ERE: estrogen response element; MEK: MAPK/ERK kinase; $P_i$: inorganic phosphate; PKA: protein kinase A. Other abbreviations, see text.*

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EGFR expression may contribute to the aggressive behavior and poor prognosis of ER-negative breast cancers.

## Perspectives

Figure 1 shows a model for cross-talk between plasma membrane ER-$\alpha$, EGFR, IGF1R, and nuclear ER-$\alpha$ that may occur in human breast cancer cells and result in stimulation of proliferation and inhibition of apoptosis. The studies described herein suggest potential roles for the cell membrane ER-$\alpha$ in the development and progression of breast cancer, including a role in relapse following hormonal therapy. Studies of membrane ER structure and function are hindered by the low abundance of ER protein localized at the membrane and the difficulty in isolating effects due to membrane vs. nuclear ER pools in the same cell. The development of more specific reagents to investigate the cell membrane ER *in vitro* and *in vivo* and to identify functions of other intracellular receptor pools (e.g. mitochondrial and endoplasmic reticulum) should better position us to: (1) understand the physiologic roles of these receptors; and (2) selectively target extranuclear ER for cancer treatment.

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