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Integration of the Extranuclear and Nuclear Actions of Estrogen

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Abstract

Estrogen receptors (ERs) are localized to many sites within the cell, potentially contributing to overall estrogen action. In the nucleus, estrogen mainly modulates gene transcription, and the resulting protein products determine the cell biological actions of the sex steroid. In addition, a small pool of ERs localize to the plasma membrane and signal mainly though coupling, directly or indirectly, to G proteins. In response to steroid, signal transduction modulates both nontranscriptional and transcriptional events and impacts both the rapid and more prolonged actions of estrogen. Cross-talk from membrane-localized ERs to nuclear ERs can be mediated through growth factor receptor tyrosine kinases, such as epidermal growth factor receptor and IGF-I receptor. Growth factor receptors enact signal transduction to kinases such as ERK and phosphatidylinositol 3-kinase that phosphorylate and activate nuclear ERs, and this can also occur in the absence of sex steroid. A complex relationship between the membrane and nuclear effects of estrogen also involves membrane-initiated phosphorylation of coactivators, recruiting these proteins to the nuclear transcriptosome. Finally, large pools of cytoplasmic ERs exist, and some are localized to mitochondria. The integration of sex steroid effects at distinct cellular locations of its receptor leads to important cellular physiological outcomes and are manifest in both reproductive and nonreproductive organs.

Abbreviations

AP-1, Activator protein 1; E2, estradiol; EC, endothelial cell; ER, estrogen receptor; EGFR, epidermal growth factor receptor; eNOS, endothelial nitric oxide synthase; FKHRL1, Forkhead; GPR30, G protein-coupled receptor 30; GSK, glycogen synthase kinase; NLS, nuclear localization signal; PI3K, phosphatidylinositol 3-kinase; SERM, selective ER modulator

STEROID HORMONES HAVE traditionally been conceived to act exclusively through nuclear receptors (1). Regarding the estrogen receptor (ER), both nuclear and membrane/cytoplasmic pools of ERα and ERβ have been demonstrated (reviewed in Ref. 2), and the receptors have similar affinity for steroid ligand. Estrogen binding to nuclear receptors activates or represses gene transcription, resulting from the steroid-receptor complex binding to DNA at estrogen response elements in the promoters of target genes. A second, nonclassical mechanism involves the interaction of nuclear estradiol (E2)/ER with transcription factors, such as activator protein 1 (AP-1) or Sp-1, that in turn bind their cognate DNA elements (3). This leads to alteration of chromatin, histone unwinding, and interactions with components of the basal transcription machinery complex. In both scenarios, recruitment of coactivators (and displacement of core-pressors) to the sites of DNA binding modulates gene and subsequent protein expression.

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In addition, steroid actions occur at the cell surface, a function that is conserved from plants to humans (2,4,5). It is widely appreciated that membrane ER signaling through kinase cascades, calcium, and other second messengers impacts transcription (6,7). For instance, E2 stimulation of the cyclin D1 gene occurs through ERK or phosphatidylinositol 3-kinase (PI3K) activation, promoting G_1/S cell cycle progression in breast cancer cells expressing endogenous ERs (8). Many sex steroid effects reflect the integration of actions at several receptor pools. Work over the past decade has identified important mechanisms whereby membrane-initiated signaling through growth factor receptors or membrane ERs impacts the function of nuclear ERs, as one model. Integrative signaling by E2 from several sites in the cell results both in rapid and prolonged actions, and provides plasticity for the cell response to the sex steroid.

In this review, I highlight the recent developments in understanding the nature of the membrane ER, and the emerging field of integration of membrane and nuclear receptor signaling. Applications of these mechanisms to physiological and pathophysiological models of estrogen action are emphasized, and new concepts of receptor cross-talk for both genomic and nongenomic actions are identified.

MEMBRANE ER

Much evidence favors the idea that the membrane-localized receptor is the same protein as the nuclear receptor, transported to the plasma membrane by unclear mechanisms. This idea is based on immunohistochemistry of the endogenous membrane ER, using a panel of antibodies directed against multiple epitopes of nuclear ERs (9), loss of endogenous ER protein detection at the membrane in cells transfected with an antisense oligonucleotide to nuclear ERα (10), and the codetection of membrane and nuclear ERs after nuclear ER cDNA expression in ER null cells (11). Chambliss *et al*. (12) have identified endogenous ERα and ERβ receptors of 67 and 54 kDa, respectively, in the caveolae and cell membranes from endothelial cells (ECs). This was done using antibodies against the classical nuclear ERα and ERβ. More recent data indicate that cells from the DERKO mouse (ERα and ERβ deleted) fail to show endogenous membrane or nuclear ERα or ERβ, by Western blot (13), E2 binding, and rapid signaling. Definitive proof that endogenous membrane and nuclear ERs are the same protein requires isolation and sequencing of the two receptor pools.

Work from the laboratory of Bender and associates (14) has reported a truncated, 46-kDa receptor as the predominant ERα isoform at the plasma membrane of an immortalized human EC line. However, several other laboratories working with isolated ECs and/or aorta have not identified the 46-kDa receptor to be abundant, including at the cell membrane (12,13,15).

Several reports have surfaced describing endogenous membrane ERs in neurons with slightly altered receptor pharmacology (16,17). However, these receptors have not been isolated, and whether they exist in cells from DERKO mice is unknown at present. Non-ER or ER-indirectly mediated actions of E2 have also been proposed, often based on the lack of antagonism of E2 effects by ICI182780 (18). Perhaps this results from E2 facilitating the action of membrane-acting proteins (sex hormone-binding globulin) (19) or the allosteric modulation of receptors at the cell membrane (20). The full nature and relevance of these responses are unclear.

Work from several laboratories has affirmed the ability of endogenous or expressed ERs to activate G protein-related signaling at the plasma membrane (11,21,22). Membrane ERs possibly exist as a cytoplasmic pool tethered to the inner face of the plasma membrane bilayer through binding to proteins such as caveolin-1 (23), in conjunction with MNAR (24), or Shc and IGF-I (25). Alternatively, evidence exists for endogenous ERα within isolated caveolae vesicles shorn from the plasma membrane of ECs, as well as non-caveolar compartments of the membrane (12,23,26). Caveolae are typically spread throughout the cell membrane, and therefore ERs could habituate within the plasma membrane at times, like growth factor

receptors. It is likely that in a dynamic fashion, both models are correct, and endogenous membrane-localized ERs also spend time in non-clathrin-coated endosomes in the cytoplasm (27).

Whether ERs span the plasma membrane or contain an extracellular ligand binding region is controversial. The membrane-impermeant estrogen conjugate, E2-BSA, has been used by many laboratories to support E2 action at membrane ERs. However, it has been reported that this compound dissociates into BSA and E2 (28), requiring filtration and careful handling of this reagent. Furthermore, BSA is well known to be taken up into caveolae in the plasma membrane (29), and therefore E2-BSA can probably access ERs in this plasma membrane raft. Thus, this compound cannot be used as definitive proof that there is externalization of a segment of ERs beyond the plasma membrane. Another approach to this issue is to carry out functional studies or immunohistochemistry with antibodies to ER (9). The presumption is that antibodies do not gain access to the cell interior and therefore identify a functional extracellular domain of ERs. However, it is not clear that cells are impermeable to the antibody intruding into the plasma membrane, and thus this reagent may suffer from the same limitations as E2-BSA.

Several reports suggest that the ability of E2 to activate G proteins is mediated through an orphan G protein-coupled receptor, GPR30. However, the pharmacology of this interaction is atypical, as 17β-E2, equimolar 17α-E2, and even ICI 182780 trigger the GPR30-related response (30). One report indicated that this interaction with GPR30 required micromolar E2, perhaps indicating an allosteric modulation with questionable physiological significance (31). Furthermore, antisense oligonucleotides to GPR30 failed to prevent E2 signaling through ERK to proliferation in breast cancer (32). A recent report from Thomas *et al*. (33) identified GPR30 as a low-capacity receptor for E2, one capable of supporting a modest generation of cAMP. It was undetermined whether E2-GPR30 interactions contribute to the overall signaling by E2 at the plasma membrane or the downstream physiology effects in breast cancer cells.

The endogenous membrane receptor assembles as part of a large signalsome complex that includes G proteins, receptor tyrosine kinases (EGFR, IGF-I receptor), and nonreceptor tyrosine kinases, such as Src (34). In a confined space, undetermined dynamics of signal molecule configuration lead to discrete G protein subunit activation. For instance, E2/ER coimmunoprecipitates with and activates Gsα and Gqα in transfected or endogenous ER cell models (11,13), and Giα in ECs (21). Gα and Gβγ activation lead to downstream augmentation of kinase activities, such as ERK and PI3K in both breast cancer cells and ECs. This signaling impacts the cellular actions of E2 (21,34,35). In some instances, the association of ER with G proteins may be indirect. E2 signaling to endothelial nitric oxide synthase (eNOS) activation in ECs may involve the striatin protein, which binds to ER, Gαi, and caveolin-1 (36). This protein may help assemble and activate the eNOS-signaling cassette at the plasma membrane.

Recent work has defined motifs in the E domain of ERα that are critical to membrane localization and function (37). This includes E domain (ligand-binding domain) residues that are necessary for dimerization of the endogenous membrane ERα and ERβ (13). Mutation of these motifs prevents both receptor dimerization and signaling through ERK, PI3K, and cAMP: loss of the former signals prevents the cell survival action of E2 in breast cancer cells (13). In contrast, signaling to eNOS activation in ER-transfected COS cells may not require membrane ER dimerization (37). Another residue, S522 of ERα, is necessary for the physical association of this receptor with caveolin-1 protein, a protein the N-terminus scaffolding domain of which facilitates ER transport from the cytosol to the membrane (38). Interactions between the A/B domain of ERα and Shc may also contribute to membrane localization through unclear transport mechanisms (39).

Very recent studies have identified an important modification of ERα that facilitates both caveolin-1 binding and membrane localization. Marino and colleagues (40,41) have shown that cystine 447 of transfected human ERα is crucial to steroid-independent palmitoylation of the receptor. This E domain amino acid is necessary for ERα to physically associate with the caveolin-1 protein and localize at the membrane. Mutation of this single amino acid or inhibition of palmitoylation with 2-Bromopalmitate results in a significant decrease of expressed receptors at the plasma membrane, compared with wild-type ER expression. Furthermore, cystine 447-mutated ERα does not support proliferative signaling through ERK and PI3K (41). Palmitoylation as a possible mechanism for membrane ER localization was also suggested from previous studies in endothelial cells (14). A partial model for membrane localization involves palmitoylation of ERα to promote binding to caveolin-1 (which itself is palmitoylated). Subsequently, the membrane localization sequence of caveolin-1 (scaffolding domain) is crucial for ER transport to and localization at the membrane (38).

Other proteins may also facilitate the membrane localization of ERs, including MNAR, Shc and growth factor receptors, and striatin (21,22,36). Shc and striatin have been reported to interact with the A/B domain of ERα (25,36) whereas MNAR binds the E domain of the steroid receptor (24). Interestingly, A/B and C domain-deleted ERα localizes to the membrane and signals to ERK, in the same manner as to wild-type ERα (38,41). This is consistent with the idea that the E domain contains most of the information for both localization and function of ERα at the membrane (42). Very recently, elements within the nuclear localization sequence (NLS) of ERα (D domain) were reported as required for E2-induced ERK and PI3K and nitric oxide production through nitric oxide synthase (eNOS) activation in transfected COS cells (37). Although this may be specific to eNOS activation, Zhang *et al*. (43) previously reported that expression of an NLS-deleted ERα, targeted to the cell membrane, supported ERK activation by E2. Thus the extent of the role of the NLS in membrane ER function is not clear.

Could nuclear ER also rapidly up-regulate kinase activity? As mentioned, NLS-deficient ERs have been reported to either affect or not affect rapid signaling by E2 (37,43). When the E domain of ERα or the full-length receptor is targeted to the nucleus, no rapid activation of ERK is detected (8). Instead, when either construct is targeted exclusively to the plasma membrane, multiple rapid signals are generated in response to E2 (44). Thus, the preponderance of available evidence suggests that the membrane localization of ERs is necessary for kinase activation, consistent with the localization and signaling by growth factor and typical G protein-coupled receptors.

MEMBRANE ER CROSS-TALK TO THE NUCLEUS

Impact of Membrane/Cytoplasmic Signaling on Nuclear ERs

Signaling from the membrane ER to the nucleus potentially impacts various functions of the sex steroid (Fig. 1). In breast cancer, the endogenous membrane ER cross-talks to the *trans* activation of the EGF, ErbB2, and IGF-I receptors (30,45,46). This results in downstream signaling to ERK MAPK and PI3 /AKT kinases. Important nonreceptor tyrosine kinases such as Src are also involved as intermediary steps (34). ERK and PI3K phosphorylate discrete residues of the endogenous nuclear ERs, up-regulating its transcriptional activity or stability (47,48). In response to EGF or IGF-I, the nuclear ER can be activated in steroid ligand-independent fashion (47). As an example, kinase-induced phosphorylation of nuclear ERα on serine 305 enhances cyclin D1 transcription in breast cancer (49). Activation from the membrane of PI3/AKT kinases can repress the downstream inhibitory actions of kinases. PI3K/AKT phosphorylation of glycogen synthase kinase (GSK)-3β (50) disinhibits the repressive effect of GSK-3β on ERα serine 118 phosphorylation. This results in enhanced transcriptional action of the nuclear ER.

Membrane signaling also impacts the phosphorylation and recruitment of coactivator proteins. This augments the unique participation of cyclin D1 (51), or more traditional steroid receptor coactivator proteins, such as glucocorticoid receptor-interacting protein 1 (52), steroid receptor coactivator 1, or cAMP response element-binding protein (CREB)-binding protein (53). One can envisage a carefully controlled modulation of nuclear ER-induced transcription, depending upon which signaling pathway(s) are activated by E2 in a given cell context. It is likely that discrete signaling pathways also (negatively) regulate the access of corepressor proteins to promoters of target genes, although this mechanism is not well studied. As a corollary to this, phosphorylation of coactivators at discrete motifs may be inhibitory as well.

Receptor phosphorylation also impacts selective ER modulator (SERM) effects. Tamoxifen has recently been reported to convert from an antagonist to an agonist in breast cancer, depending upon Ser 305 phosphorylation (54). This site can be phosphorylated by both protein kinase A (54) and p21-activated kinase 1 (49). Circulating or locally produced E2 or growth factors acting at the membrane could potentially stimulate this nuclear ER modification via protein kinase A (55) and contribute to tamoxifen resistance in women (56).

Membrane ER Signaling to Nuclear ER-Independent Functions

Activation of target gene transcription also occurs through membrane signaling, independently of nuclear ERs. ERK activation up-regulates AP-1 mediated genes (*e.g.* c-*fos*) (3,52,57). This results in part from serum response factor/elk-1 stimulation by E2, and in part by recruitment of nuclear ERs and coactivators to AP-1 sites on gene promoters. Similarly, PI3K activation by E2-induced signaling from the endogenous membrane ER rapidly up-regulates hundreds of genes in a target cell (58). One such E2-induced gene, cyclooxygenase-2, is regulated by signaling to nuclear factor-κB, potentially contributing to an important cell biological outcome of tumor angiogenesis (59).

The loss of inhibition produced by tumor suppressor gene mutation often underlies carcinogenesis (60). Such a role for membrane signaling can be proposed in steroid hormone-responsive cancers. PI3K/AKT inhibition of GSK-3β function allows β-catenin to translocate to the nucleus. β-Catenin cooperates with TCF/Lef transcription factors to up-regulate proliferation genes, such as cyclin D1 (61), contributing to carcinogenesis. PI3K/AKT activation also leads to decreased Forkhead (FKHRL1) transcription factor function, the inhibition of which results in cell survival (60). Thus, membrane signaling to the nucleus through these and other, undiscovered pathways contributes to estrogen-induced cell proliferation and survival, essential features of tumor biology. It has been estimated that more than 500 kinases are encoded within the human genome (62). The ability of membrane ERs and/or growth factor receptor tyrosine kinases to signal through multiple kinases to the nucleus undoubtedly impacts all aspects of cellular function.

NONTRANSCRIPTIONAL ACTIONS OF ER

Signaling from membrane ERs also induces the posttranslational modification of many existing proteins. This includes the phosphorylation and regulation of enzymes, such as kinases or phosphatases, that impact cell physiology. These enzymes act both within and outside the nucleus. As an example, E2 rapidly down-regulates MAPK phosphatase 1 activity, leading to the up-regulation of ERK activity in breast cancer cells within 10 min (8). However, E2 also cooperates with the BRCA1 tumor suppressor protein (63) to modulate MAPK phosphatase 1 production, stemming from nuclear action. The overall regulation impacts the proliferation and survival of the cancer and represents an important integration of E2 action, occurring in both membrane/cytoplasm and nucleus. Loss of restraint of kinase signaling underlies the interaction between BRCA1 and ER that may contribute to breast cancer when BRCA1 is mutated (8,63).

Signaling by membrane ERs to PI3K reveals additional integrated functions in breast cancer. PI3K activation causes the phosphorylation of BAD, which is then sequestered by 14–3-3 proteins in the cytoplasm (64). Sequestration dissociates phosphorylated BAD from binding the antiapoptotic proteins, Bcl2 and Bcl-xl, which allows these proteins to maintain mitochondrial membrane integrity and prevent the release of cytochrome *c* and subsequent cell death. In some instances, downstream signaling by E2 to additional kinase substrates (mammalian target of rapamycin for PI3K/Akt or p70S6 kinase for AKT or ERK) is required, and these target signals expand the potential repertoire of E2 actions. For instance, the p70S6 kinase modulates protein translation and is important for sensing and responding to the nutrient status of the organism (65). The localization and duration of kinase signaling may importantly contribute to E2 actions in these respects. For instance, the ability of estrogen to rescue osteocytes or induce cell death in osteoclasts is dictated, in part, by the duration of ERK activation, and whether this kinase signals predominantly in cytoplasm or nucleus (66,67).

E2 also promotes the motility of cells through signaling via p38 (35) and perhaps PI3K pathways (68). The latter signal activates the small GTP proteins Rac and Cdc42, which in conjunction with Rho kinase, modulate target cell migration (69). Cell motility is ultimately driven by the formation of lamellipodia and extension of filipodia, and estrogen promotes this (25). Motility depends on nucleated actin-related processes, the synthesis of capping proteins, and association of ARP 2/3 and Ena/VASP regulatory proteins with actin-barbed ends (70). These proteins are potential substrates for posttranslational modification by E2 signaling.

ERs IN CYTOPLASM

The ability of E2 to act at receptor pools throughout the cell brings into focus the issue of cytoplasmic ERs that are not proximal to the cell membrane or nucleus. One model is that this pool of receptors is in the process of translocating to other sites in the cell. Dynamic imaging using techniques such as fluorescence resonance energy transfer will be needed to determine whether this occurs. However, mitochondria have been identified to respond to estrogenic compounds, both in whole cell or isolated organelle preparations (71,72). Saturation binding of sex steroid to putative ER in the mitochondria has been proposed for many years, but often these studies identified a low-affinity receptor, requiring micromolar E2 for the activation of a mitochondrial metabolic process. More recent studies have identified high-affinity E2 binding proteins (73,74) that, by partial peptide degradation and MALDI-TOF mass spectrometry, are identical to nuclear ERs (74). Currently, the functions of this pool of ERs are unknown.

CELL PHYSIOLOGY OUTCOMES OF RAPID SIGNALING BY E2

Understanding how estrogen action at the membrane contributes to the overall cellular functions of the steroids has been facilitated by the development of reagents that act in a relatively specific fashion at the membrane ER. One such compound, estren, activates signal transduction to a variety of kinases. This signaling by estren or E2 subsequently activates transcription factors to rescue HeLa or bone cells from apoptotic cell death (44,76,77). Cell survival contributes to the ability of this weakly ER-binding steroid to prevent osteoporosis *in vivo*, yet estren does not stimulate breast or uterine proliferation (77). This compound apparently does not have the ability to activate genes through nuclear ERs.

From this work, it has been proposed that estrogenic compounds that only act at the cell membrane might not stimulate the proliferation of cells, and thus not promote breast or uterine neoplasia. This concept requires rigorous demonstration, because signaling through kinase activation strongly promotes breast cancer cell proliferation *in vitro* and *in vivo* (78,79). Developing agonists or antagonists that act strictly at membrane ERs should allow better

understanding of the contributions of this receptor pool to overall E2 action and provide possible targets for therapeutic intervention.

Analogous to this, the development of newer generations of SERMS should consider whether their actions result from binding membrane and/or nuclear ERs. It has been shown that raloxifene activates the same kinase signaling pathways as E2, to trigger nitric oxide production in ECs (80,81). This leads to enhanced cardiac perfusion and contractility of the ischemic heart *in vivo* (82). This is consistent with the ability of E2 to prevent ischemia-reperfusion injury in mice, via PI3K signaling from the membrane receptor (83).

Estrogenic compounds also prevent neuronal cell death, induced by cerebrovascular ischemia *in vivo*. Several groups have shown that E2 rescues rodents from neuronal apoptosis, in part through ERα up-regulation of cell survival genes (84–87). This may be related, in part, to signaling though PI3K (88,89). E2 also prevents alcohol-induced neuronal injury by modifying protein kinase C activity (90). These results suggest that membrane ER signaling contributes to the overall neuroprotective effects of the sex steroid. In this regard, rapidly signaling ERαs are localized to the neurites, and not the nuclei of neurons (91). These *in vivo* data have resulted in the development of estrogen compounds that protect neurons against insult-induced cell death but bind poorly to ERα or ERβ (87). ER-independent neuroprotective effects of estrogenic compounds during stroke may be related to the prevention of reactive oxygen species formation (92). In other work, estrogen rapidly signals to CREB phosphorylation and ERK activation in neurons. This is mediated by the classical ER (α or β) expressed in a specific brain region, and was shown using ER knockout mice (93).

Estrogen action certainly impacts ovarian follicle development, and this may reflect integrated functions of membrane and nuclear ERs. An important family of transcription factors in follicle development are the Forkhead proteins. *In vivo* and *in vitro* rodent models indicate that the transcription and actions of Foxo1, FKHRL1, and AFX are regulated by growth factors and estrogen and depend on kinase modulation (94). For instance, its is well recognized that PI3K/AKT phosphorylates and thus sequesters Forkhead proteins in the cytoplasm (50), thus limiting transcriptional activity. This is a pathway that E2 activates in virtually all ER-producing cell types investigated. Activation of this pathway in ovarian cancer by the sex steroid leads to increased telomerase gene expression and activity, thus contributing to tumor propagation (95). E2 activates p21-activated kinase in breast cancer cells, also leading to FKHR phosphorylation/sequestration (96). In breast cancer, hypersensitivity of estrogen-deprived cells corresponds to increased ERK activation and resulting Elk-1 transcription factor activation (97). Additional *in vivo* models utilizing physiological concentrations of estrogen will be necessary to define the pathophysiological roles of sex steroid signaling from the membrane to tumor promotion.

Tamoxifen resistance in breast cancer may be related to enhanced cross-talk between the membrane ER and EGFR family member receptors, most notably ErbB2 (HER-2). When ErbB2 is experimentally overexpressed, tamoxifen activates both ER and ErbB2 to signal downstream through ERK and PI3K to AIB1 phosphorylation (98). Tamoxifen acts as an agonist in this situation, promoting coactivator recruitment as a result of kinase signaling. EGFR tyrosine kinase inhibition reconverts tamoxifen to an antagonist and disassembles the positive transcription complex on the promoter of a target gene. An emerging therapeutic approach in this malignancy is estrogen purging, in which raloxifene-resistant cancer cells undergo E2-induced apoptosis (99). Thus, integrative signaling from the membrane through kinase-induced transcription, or posttranscriptional effects on protein function, contributes to the tumor-promoting actions of estrogen *in vivo* and may contribute to SERM resistance.

PERSPECTIVE

A complex interaction between ERs in various cellular locations affords the opportunity for exquisite control of E2 function. The cell context-specific environment impacts the integration of rapid signaling by E2 from the membrane, and subsequent nuclear transcription. This leads to different signal cascades or transcribed genes in response to the same steroid hormone, and a different cell biological outcome. In part, this is dictated by the different physiology and functions of diverse cell types.

Nevertheless, many cells divide, attempt to survive, migrate, and differentiate. These common functions allow us the opportunity to deduce cellular programs that are shared between steroid hormone-responsive cells. Such programs provide potential interventional targets to enhance or inhibit estrogen functions. Elucidating the details of these programs could provide a more informed approach to disorders resulting from reproductive organ dysfunction, the prevention of osteoporosis after the menopause, or sex steroid-responsive cancers. Estrogen-related reagents that target a particular receptor pool may be efficacious in these respects (25,76), but we will have to better understand the integrative nature of hormonal action to avoid undesirable consequences of this approach.

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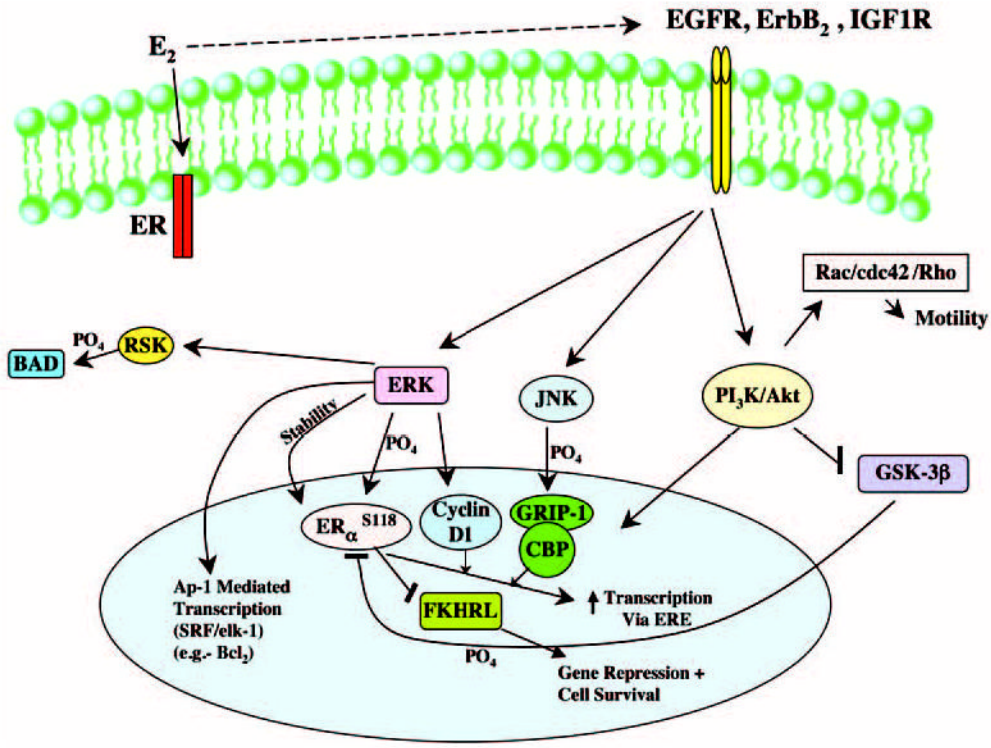

Fig. 1.

Integrative Signaling by Estrogen through Receptors (ER) at the Membrane and Nucleus in Breast Cancer IGF1R, IGF-I receptor; JNK, c-Jun N-terminal kinase; RSK, ribosomal S6 kinase; ERE, estrogen response element; CBP, cAMP response element binding protein (CREB)-binding protein; SRF, serum response factor.