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MINIREVIEW

Bidirectional Signaling between the Estrogen Receptor and the Epidermal Growth Factor Receptor

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Interactions between the estrogen receptor (ER) and the epidermal growth factor receptor (EGFR) contribute to the biological effects of these binding protein families. EGFR stimulates DNA synthesis and gene transcription in the uterus, related in part to estrogen-independent activation of the nuclear ER. This results from signal transduction enacted by the plasma membrane tyrosine kinase growth factor receptor, leading to 1) phosphorylation and activation of the nuclear ER, and 2) phosphorylation of coregulator proteins. More recently, it has been shown that a pool of ERα resides in or associates with the plasma membrane as a cytoplasmic protein. These ERs utilize the membrane EGFR to rapidly signal through various kinase cascades that influence both transcriptional and nontranscriptional actions of estrogen in breast cancer cells. This is congruent with a general theme of receptor signaling, where membrane G protein-coupled receptors activate tyrosine kinase growth factor receptors (EGFR, IGF-I receptor) that subsequently signal to MAPKs and other pathways. Overall, the bidirectional cross-talk between EGFR and cellular pools of ER contributes to reproductive organ physiology and pathophysiology. (*Molecular Endocrinology* 17: 309–317, 2003)

MOST GROWTH FACTORS activate cell proliferation, differentiation, or survival programs through binding their attendant tyrosine kinase receptors, expressed in the plasma membrane (1–4). As a result, the receptors undergo dimerization and conformational changes that result in transphosphorylation at discrete tyrosine residues. This provides binding sites for signaling or linker/adapter molecules that contain Src-homology 2 domains, and the recruitment of additional signal molecules (5, 6). Such proteins include nonreceptor tyrosine kinases such as Src family members, or Grb and Sos family proteins. Signal cascades are then triggered, dependant upon the translocation, membrane association, and activation of tyrosine, serine/threonine, and lipid kinases, including ras, raf, protein kinase C, and phosphatidylinositol 3-kinase (PI3K). These kinases phosphorylate substrate proteins in the cytoplasm, altering target protein function. As an example, stimulation of PI3K results in AKT activation, which then phosphorylates a variety of proapoptotic proteins, including BAD, glycogen synthase kinase-3β, or Forkhead transcription factors (7, 8). This posttranslational modification sequesters/inactivates these proteins in cytoplasm, leading to cell survival.

Kinases also translocate to the nucleus, where they phosphorylate/activate and transcribe transcription factors that induce a variety of immediate early and late-arising genes. This important event underlies many of the biological effects of growth factor signaling. In fact, when nuclear localization of the ERK member of the MAPK family is prevented, cell proliferation often ceases (9). Important nuclear targets of ERK that are relevant to cell division include the transactivation of the cyclin D1 gene and the protooncogenes c-*fos* and c-*myc* (10–13). Therefore, the ability to signal from the membrane to both cytoplasmic and nuclear events is an essential feature of growth factor receptor function.

Steroid hormones have traditionally been conceived to act through the ligation of nuclear receptors (14). For estrogen, binding to estrogen receptor (ER)α or ERβ results in an active complex in the nucleus that binds DNA directly at estrogen response elements within the promoters of target genes. Alternatively, estradiol (E_2)/ER promotes transcription factor binding to DNA (15). Liganded ER forms complexes with coregulator proteins (16), and constituents of the basal

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Abbreviations: E_2, Estradiol; EGF, epidermal growth factor; EGFR, EGF receptor; ER, estrogen receptor; GPCR, G protein-coupled receptor; HB-EGF, heparin binding-EGF; IGF-IR, IGF-I receptor; KO, knockout; MMP, matrix metalloproteinase; PI3K, phosphatidylinositol 3-kinase; STAT, signal transducer and activator of transcription.

transcription machinery complex, leading to the modulation of RNA polymerase II activity, histone-induced chromatin unwinding, and transcription. However, it has become increasingly clear that estrogen (and other steroid hormones) also rapidly activates signaling in seconds to minutes, and this cannot be explained by any known function of nuclear receptors (17). Furthermore, ERs that lack a nuclear localization sequence (18) or truncated ERs that are targeted to the plasma membrane are fully capable of activating kinases and subsequent cell proliferation or survival (19, 20). A small pool of endogenous ERs that localize to the plasma membrane in various target cells can act similarly to classic growth factor receptors imbedded in the membrane. These ERs have been localized to caveolae raft domains isolated from the plasma membrane of target cells such as endothelial cells (19, 21). It is still unclear, however, whether these sex steroid receptors are integral membrane proteins and/or tether as cytoplasmic proteins to the cytoplasmic face of caveolae through binding to caveolin-1.

An important principle in the signaling field is that growth factor receptors cross-talk to each other. This includes heterodimerization between receptor family members, exemplified by the four members of the epidermal growth factor (EGF) receptor (EGFR) family (22). Additionally, signaling from one receptor activates cytoplasmic nonreceptor kinases (*e.g.* -Src) that positively or negatively modulate the activity of adjacent receptors (23). In this respect, EGFRs expressed on a population of cells may spread signal transduction enacted by a variety of unrelated growth factor receptors on adjacent cells (24).

Emerging data suggest cross-talk may exist between plasma membrane steroid receptors. Progesterone can stimulate ERK signaling via the utilization of ER (25), and estrogen or androgen can promiscuously stimulate signaling to ERK (and cell survival) through either sex steroid receptor (20). Furthermore, both membrane growth factor and steroid receptors interweave their actions with those of nuclear steroid receptors, thereby impacting cell biology. An example is that nuclear receptors transcribe genes, the protein products of which are acutely altered in function by phosphorylation, resulting from membrane receptor signaling.

In this overview, I will describe the current state of cross-talk between ERs and EGFRs. Work in this area has established a requirement of nuclear ER for some EGFR [and perhaps IGF-I receptor (IGF-IR)] actions. Recent findings suggest the important role of EGFR (or similar receptors) for estrogen signaling from the membrane in breast cancer. Bidirectional signaling between these essential cellular factors augments the actions of the individual steroid and growth protein.

SIGNALING FROM EGFRs TO NUCLEAR ERs

EGF binds to one or more members of the EGFR family that enact signaling cascades to the nucleus and cytoplasm, resulting in cell biological actions (22, 26). This pathway is indirectly used by E_2. In reproductive organs, E_2 induces the EGFR and stimulates growth and rapid proteolytic activity in the uterus (27). Subsequent investigations established that this sex steroid stimulates the synthesis of EGF in this reproductive organ (28). Up-regulation of EGF probably explains the strong proliferative effect of E_2 on uterine epithelium, an action that is prevented by EGF antibody (29). Increased synthesis of EGF resulting from E_2 action extended the earlier observation that E_2 induces EGF secretion from breast cancer cells (30) and implicates this interaction in the growth of hormonally responsive cancer. In EFGR knockout (KO) mice, the stromal compartment, but not the epithelial response to E_2, is severely limited in both the uterus and vagina (31).

A novel model of ER and EGFR interaction is derived from the observation that EGFR signaling depends upon an ER-mediated function but in an estrogen-independent fashion (32). Studies from Ignar-Trowbridge *et al.* (33) showed that EGF induction of DNA and lipid synthesis in the uterus could be prevented by ICI 164,384, an ER antagonist. More recent studies suggest that the effects of ER antagonists could be mediated through recruiting corepressors, thereby inhibiting growth factor-induced ER transcriptional effects (34). Continued work from the laboratories of DiAugustine and Korach (35) showed that EGF-induced DNA synthesis and transcription were absent in uteri from ERα KO (ERKO) mice. These results clearly show dependency on ER for EGF action in reproductive organs.

How does EGFR utilize ER for biological actions? Insight resulted from the observations that several peptide growth factor receptors signal to the phosphorylation and activation of the nuclear ER (36, 37). This includes EGF and was originally attributed to the ability of growth factor receptor-activated MAPK (ERK) to phosphorylate serine 118 in the A/B domain of the nuclear ERα. Serine 118 phosphorylation results in an increased ER-related transactivation of genes that are up-regulated by EGFR. Work by Ignar-Trowbridge *et al.* (38) showed that EGFR ligation induces the transcriptional up-regulation of an estrogen response element reporter construct, in ER-dependent fashion. This group also demonstrated that EGFR-to-ER cross-talk requires the A/B domain of ERα (39). Subsequent studies implicated several kinases that phosphorylate additional residues within ERα, resulting in the increased transcriptional activity of the nuclear receptor (40–44). Thus, impact of the growth factor receptor-ER interaction depends upon the signaling milieu within a particular cell that differentially phosphorylates numerous residues in the nuclear ER.

Another mechanism through which EGFR-induced signaling modulates ER transcriptional activity is via coregulator protein phosphorylation. As an example, EGF-induced ERK phosphorylates serine 736 of glucocorticoid receptor interacting protein 1. This increases the activity of this nuclear receptor nonspecific coactivator protein (45). EGF-triggered Src and Jnk activation may have a similar function for the cAMP response element-binding protein (46). Other coactivator proteins that are important and specific to ER function could be similarly activated or recruited through signaling-induced posttranslational modifications. Interestingly, growth factor receptors signal to cyclin D1 production, as part of promoting G_1/S phase cell cycling (47). Cyclin D1 activates ER transcriptional function (48) and interacts with the coactivator proteins, steroid receptor coactivator 1 and cAMP response element-binding protein/p300, as an additional mechanism to amplify nuclear ER action (49, 50).

It is conceivable that EGFR signaling also inhibits the activity/function of corepressor proteins on targeted promoters, and that other EGFR family members could also cross-talk to the nuclear ER. In breast cancer and other estrogen target cells, EGFR family members often heterodimerize, and ligands [heparin binding-EGF (HB-EGF), TGFα, or EGF] can be somewhat promiscuous in their binding. Such considerations may be relevant to the interactions of the erb2/Neu oncogene and ER in early breast cancer. In this respect, breast tumor formation in mouse mammary tumor virus-erb2/neu mice is delayed on an ERKO background (51). A summary of mechanisms of EGFR signaling through ER is seen in Fig. 1.

Finally, it has recently been reported that the EGFR translocates to the nucleus, where it can bind to AT-rich DNA sequences and modulate the transcription of the cyclin D1 gene (52). Modulation of this controversial event by EGF occurs 48 h after ligation (53), and any interactions with the nuclear ER would be expected to impact the more chronic effects of the growth factor receptor.

In parallel to the interaction between the EGF system and ER, there is abundant evidence indicating cross-talk between the IGF-I system and ER. IGF-I binding activates its receptor, leading to PI3K/AKT activation, increased ERα synthesis, and augmented ERα transcriptional activity. This probably results from the phosphorylation of several serine residues in the AF-1 region (43) and EGFR accomplishes a similar action. Similarly to EGF, IGF-I activates parameters of

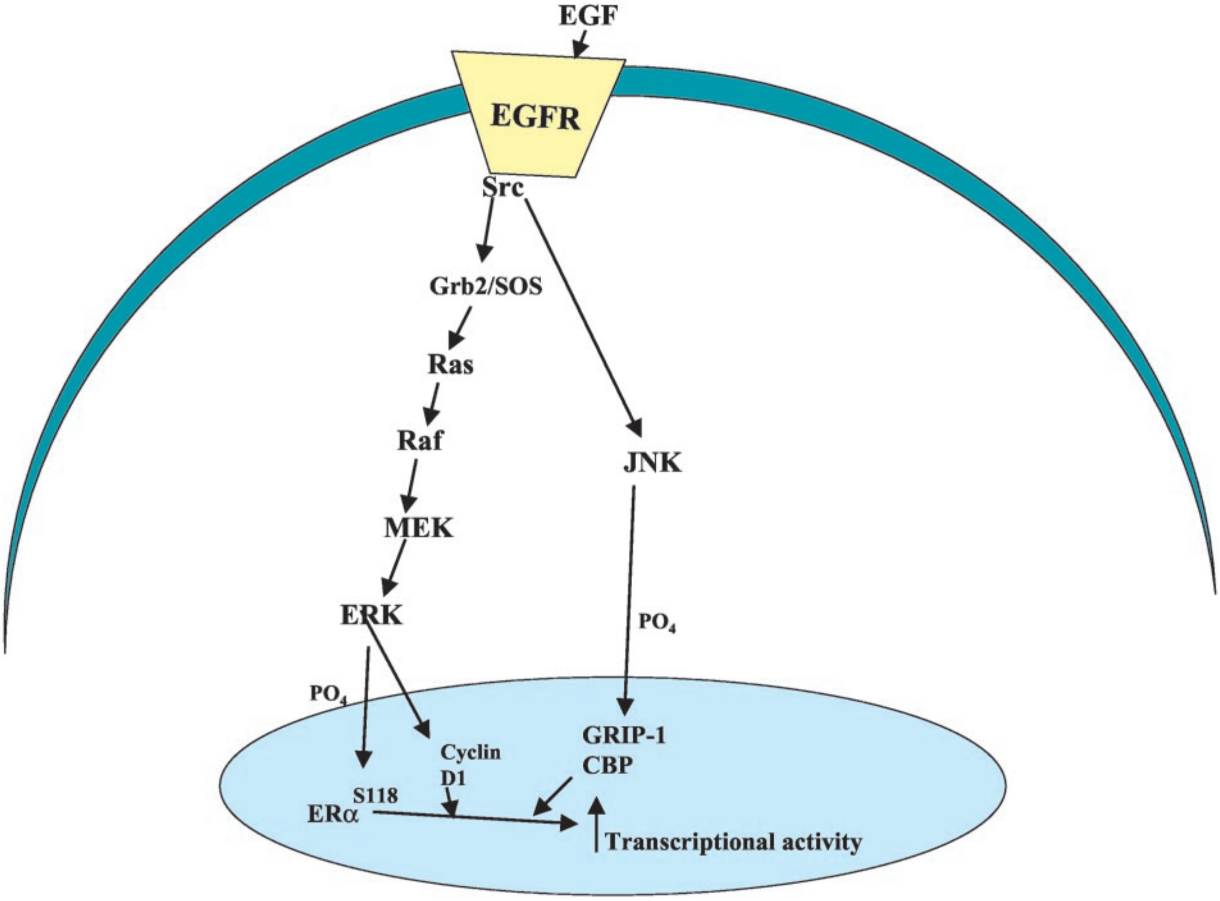

Fig. 1. EGFR Activation of ER or Coregulator Proteins via Signaling through MAPK Cascades
GRIP-1, Glucocorticoid receptor-interacting protein; CBP, cAMP response element-binding protein.

uterine cell proliferation *in vivo*, and this is dependent on ERα (54). Interestingly, in both the uterus and in breast cancer models, IGF-I signaling to ERK and PI3K/AKT is unaffected by ERα loss or antagonism (54, 55). When ERα is reexpressed in breast cancer cells that have lost ER through repetitive culturing, both E_2 and IGF-I resume their growth-inducing function (56). E_2 stimulates many proteins in the IGF-I system, including IRS proteins, IGF-IR, and IGF-binding proteins (57, 58), and ERα binds and phosphorylates the IGF-1R and enhances signaling through the growth factor receptor (59). In breast cancer, IGF-I and E_2 cooperate to promote G_1/S cell cycle progression (60, 61), and in the uterus of the IGF-I KO mouse, E_2-induced growth is absent (62). Thus, there appears to be an important cooperation and cross-talk between these two systems as well.

SIGNALING FROM ER THROUGH EGFR

The realization that E_2 has rapid effects in cells led to the characterization of the many generated signals. E_2 stimulates calcium channel opening and calcium influx or mobilization within seconds of binding receptors expressed in target tissues (63, 64). E_2 rapidly generates cAMP (65), phospholipase C, and inositol phosphate (66, 67). This results from G protein activation, and these early signals are transmitted to the rapid stimulation of protein kinase C, protein kinase A, MAPK, and PI3K (68). Functional and immunohistological identification of endogenous membrane ER (69, 70) led to the characterization of these receptors after expression of the cDNAs for classical ERα and ERβ in Chinese hamster ovary cells (71). These latter studies indicated that membrane ER physically associate with and activate various G protein α-subunits, including Gαs and Gαq. G protein activation explains how ER generates cAMP (Gαs function) or inositol 1,4,5-triphosphate and calcium (Gαq function), as examples. Subsequent work showed that endogenous ERα activates Gαi, leading to the generation of nitric oxide in endothelial cells (72).

An important finding described by Ullrich and colleagues (73) indicates that several G protein-coupled receptors (GPCRs) signal to ERK via the transactivation of the EGFR. Later studies from other laboratories confirmed and extended these observations to many GPCRs and provided additional details underlying this cross-talk. Identification of the membrane ER as a receptor capable of activating G proteins (71, 72) invoked the possibility that this receptor signaled through cross-talk/activation of the membrane EGFR. Filardo *et al.* (74, 75) showed that estrogen rapidly acts in breast cancer cells to stimulate the transactivation of EGFR, leading to cAMP and ERK up-regulation. This occurs through a linked path, first described for other GPCRs by Ullrich and colleagues (76). E_2 induces mainly unknown proximal signaling to cause the activation of undefined matrix metalloproteinases (MMPs). Increased MMP function leads to the liberation of HB-EGF, which then binds and activates the EGFR. However, Filardo *et al*. (74) reported that 17β-E_2, 17α-E_2, or the ER antagonist, ICI 182780, were equivalent in activating EGFR and ERK. EGFR transactivation was proposed to occur independently of any ER and resulted from an undetermined effect of E_2 to activate the orphan GPCR, GPR 30 (77). More recent studies from Razandi *et al.* (78) demonstrated that E_2 requires an ER to signal to EGFR in breast cancer and is consistent with most studies that show an ER is necessary for rapid signaling by E_2 at the membrane (19, 42, 66, 79–81). Razandi *et al.* (78) also found that E_2/ER triggers a Gαq, Gαi, and G$\beta\gamma$-dependent activation of MMP-2 and -9, mediated through Src activation. By antisense studies, MMP-2 and MMP-9 were shown to be necessary for E_2-induced HB-EGF cleavage and liberation, the transactivation of EGFR, and downstream signaling to ERK and PI3K in breast cancer cells, and p38 MAPK in endothelial cells. It is possible that GPR30 may complex with and mediate membrane ER cross-talk to EGFR. However, recent studies from Ahola *et al.* (82) have called this idea into question. These investigators found that antisense inhibition of endogenous GPR-30 had no effect on E_2 signaling to cell proliferation in MCF-7 cells. Thus, this definitive approach suggests that GPR30 is not required and supports previous studies that ER 1) directly associates with and activates G proteins, and 2) this leads to downstream signaling (71, 72). The molecules involved in the ER-to-EGFR cross-talk are shown in Fig. 2.

The full extent of membrane-initiated signaling by E_2/ER and its dependence on EGFR remains to be defined, and the *in vivo* significance is incompletely understood. However, it was demonstrated more than 10 yr ago, that EGF antibody prevents E_2-induced vaginal and uterine growth (29), implying that cross-talk from ER to the EGFR at the membrane may be physiologically important. Recent studies concerning the role of E_2/ER signaling at the membrane support this idea. Simoncini *et al.* (83) showed that in endothelial cells, ERα directly associates with the membrane-tethered p85 subunit of PI3K. E_2 rapidly activates this kinase, leading to the generation of nitric oxide, and the rescue of rats from ischemia-reperfusion injury of their muscle. It is known that EGFR and PI3K associate (84), and so it is possible that a multiprotein complex exists between ER/PI3K/EGFR and endothelial nitric oxide synthase molecules, perhaps scaffolded onto caveolin-1 at the membrane (19, 21, 85). Similarly, Migliaccio *et al.* (79) showed that ER and Src form a complex. The interaction between ER and Src may be mediated by a newly described adapter protein, modulator of nongenomic activity of estrogen receptor (86). Src activation by E_2 leads to a kinase cascade resulting in ERK activation and DNA synthesis in cancer cells (79). Interestingly, EGFR and Src associate, and both molecules also form complexes

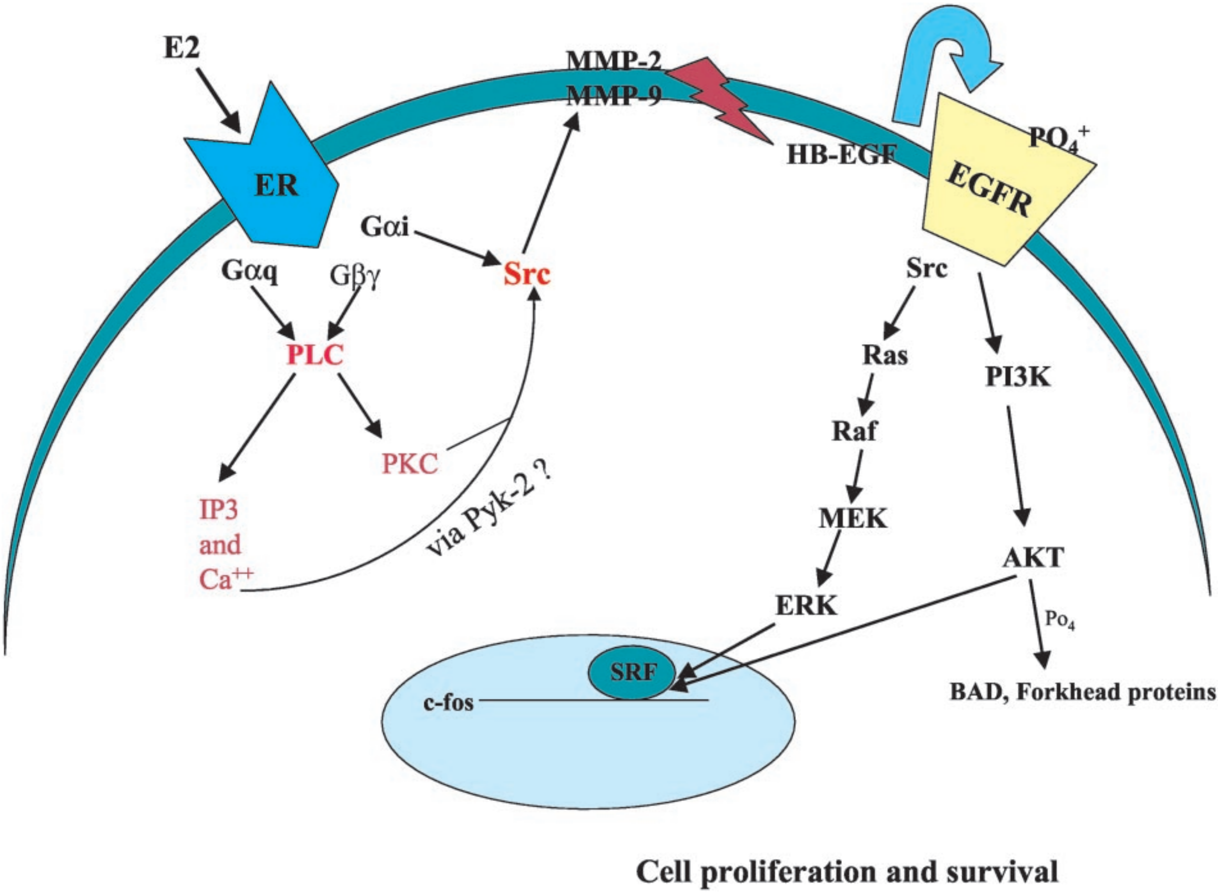

Fig. 2. Membrane ER Cross-Talk to EGFR Leads to Downstream Signaling and Changes in Cell Biology of Breast Cancer

with caveolin-1 (87). Src or EGFR phosphorylates caveolin-1 at the important tyrosine 14, and this leads to the down-regulation of signaling (88). The ability of E_2/ER at the membrane to signal to ERK (via the demonstrated EGFR transactivation) has additional importance for cell biology. Song *et al.* (89) recently demonstrated that ERα lacking a nuclear localization signal and targeted to the plasma membrane activates ERK and cell proliferation in Chinese hamster ovary cells. Also, the survival of breast cancer cells that are subjected to radiation or taxol chemotherapy is enhanced by E_2, partially through ERK activation (90). In aggressive breast cancer, a truncated MTA1 protein was recently found to be highly expressed (91). This protein sequesters ER away from the nucleus and strongly reduces E_2-activated transcription, yet promotes increased ERK signaling and aggressive behavior of the tumor. In neurons subjected to several inducers of apoptosis, E_2 protects these cells through ERK activation (92). The actions of E_2 mediated by this MAPK occur through both protein phosphorylation (90) and gene transcription (93, 94). Most recently, bone loss *in vivo* was prevented by a compound (4-estren-3α, 17β-diol) that has no direct transcriptional activities but activates ERK signaling (95). Therefore, it is probable that the cross-talk from membrane ER through EGFR to downstream kinase activation is biologically important.

The precise structural aspects of the membrane ER that are required for G protein activation are unclear at present but appear to mainly reside in the E domain. This conclusion is based upon the observations that targeting the E domain alone to the plasma membrane allows E_2 activation of ERK (19) and rescues bone cells from an apoptotic cell death (20). Similarly, sending the E domain to the plasma membrane of ER-negative, breast cancer cells results in E_2-induced, Src-dependent matrix metalloproteinase activation, HB-EGF liberation, and EGFR transactivation (78). Thus, the membrane E domain alone can recapitulate the key elements of the pathway from ER to EGFR. These findings are supported by the earlier observation that Src complexes with (and is activated by) E_2/ER, and that tyrosine 537 within the E domain is an essential structural component (96). This may be important for specific signaling pathways, however. Bjornstrom and Sjoberg (97) have recently examined the E_2 rapid activation of signal transducer and activator of transcription (STAT) transcription factor-induced β-casein promoter activation. STAT activation requires both ERK and PI3K, induced by E_2/ER. These authors report that mutating tyrosine 541 of the mouse ERα (equivalent of human ERα tyrosine 537) has no effect on E_2 induction of the STAT-β-casein pathway. Also, Song *et al.* (89) recently showed that the Src homology 2-domain containing adapter protein, Shc, complexes with ERα through the AF-1 domain, and suggested that this interaction may underlie E_2-induced ERK. However, we recently found that expression of only the membrane-targeted E domain (19), or A/B domain-deleted ERα (unpublished observations) 1) fully binds steroid at the membrane, and 2) comparably activates ERK, compared with expressed wild-type ERα. Thus, current data support a unique and complete role for the E domain in effecting signal transduction initiated at the membrane.

PERSPECTIVE

The bidirectional cross-talk between ER and the growth factor receptors EGFR and IGF-IR indicates a potent method of augmenting E_2 or growth factor action. In a particular cell type and situation, there may be a predominant contribution from one of these pathways, essential to the cell biology of breast cancer, for instance. Tamoxifen is effective in preventing the reoccurrence of ER-positive breast cancer, in part because it inhibits aspects of E_2 and EGFR signaling. In ER-negative breast cancer, there is possibly less restraint on EGFR signaling to cell proliferation or survival in the absence of ER antagonism, thereby contributing to a more aggressive phenotype. Interestingly, in human breast cancer, ER and EGFR concentrations are inversely correlated (98, 99), and ER appears to repress the EGFR gene through a first intron sequence (98). Increased EGFRs in ER-negative breast cancer may also contribute to the more active growth and invasive behavior of these tumors.

The interactions of ER and EGFR impact both the transcriptional and nontranscriptional effects of steroid hormones and protein growth factors, but these are not mutually exclusive actions. Membrane E_2/ER activates PI3K signaling via EGFR (78). As shown by DNA microarray, PI3K activation by E_2 leads to the up-regulation of 250 genes after just 40 min of exposure of endothelial cells to sex steroid (100). Thus, ER-EGFR cross-talk at the membrane enacts multiple signaling pathways that likely have a profound impact on the transcriptional effects of E_2. It is certainly possible that manipulating the specific pathways that result from the bidirectional signaling will yield therapeutic interventions for human disorders that result from excessive growth factor and steroid hormone action.

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I regret that many fine contributions to this scientific area could not be recognized due to space limitations.

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