Estrogen Receptor: Current Understanding of Its Activation and Modulation

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Abstract
Breast cancer development and progression are directly related to the effects of the female hormone estrogen. The nuclear receptor for estrogen (ER) functions as a transcription factor controlling estrogen-regulated genes. Receptor conformation on ligand binding, its interaction with various coregulators, and response elements in the promoter region of target genes all contribute to the net estrogenic effects in a cell. ER is an important diagnostic and therapeutic target in breast cancer. Various polypeptide growth factors and their membrane receptors also contribute to breast cancer development and progression. Pathways mediating cell survival, cell proliferation, and response to stress not only generate signals through various protein kinase pathways to enhance cell survival and proliferation, but these pathways also interact with ERs. Kinases in the growth factor cascade can phosphorylate and activate ER, and ER in turn activates and augments signaling through the growth factor pathways. Signaling through the growth factor pathways may contribute to hormonal resistance states by ligand-independent activation of ER. Targeting growth factor pathways, in addition to ER, is a developing strategy that hypothetically may represent optimal therapy by preventing the development of resistance to endocrine therapy.

Introduction
The development and progression of breast cancer have long been known to be directly related to the effects of the female hormone estrogen. The nuclear receptor for estrogen (ER) has been shown to play a role in breast cancer development and progression. ER is an important diagnostic and therapeutic target in breast cancer. Various polypeptide growth factors and their membrane receptors also contribute to breast cancer development and progression. Pathways mediating cell survival, cell proliferation, and response to stress not only generate signals through various protein kinase pathways to enhance cell survival and proliferation, but these pathways also interact with ERs. Kinases in the growth factor cascade can phosphorylate and activate ER, and ER in turn activates and augments signaling through the growth factor pathways. Signaling through the growth factor pathways may contribute to hormonal resistance states by ligand-independent activation of ER. Targeting growth factor pathways, in addition to ER, is a developing strategy that hypothetically may represent optimal therapy by preventing the development of resistance to endocrine therapy.

ER Structure and Function
Two ERs have now been identified, ERα and ERβ (2). Although ERβ is expressed in human breast cancer, its normal function and potential role in cancer progression have not yet been clearly defined. ERα, on the other hand, has been studied in great detail, and it serves as a clinically useful predictive marker and treatment target. The ER proteins contain several important functional domains (Fig. 1). The hormone-binding domain in region E of the ER also contains an estrogen-inducible transcription-activating function called AF-2 (3–9). A second, constitutively active transcription-activating function (AF-1) is located in the A/B region of the receptor. The DNA-binding domain and the hinge region reside between the two transcription-activating functions, AF-1 and AF-2. Several mutant and variant ERs have also been identified; and one of these, K303R ERα, may be very important in clinical breast cancer (10).

In the absence of ligand, ER exists as monomers bound by heat shock proteins (11, 12). Ligand binding activates the receptor, dissociates the heat shock proteins, and alters receptor conformation in a specific way. Estrogen also causes phosphorylation of ER in several distinct serine/threonine residues (13). These activated receptors then homodimerize, complex with a...
variety of coregulatory molecules, and the complex then binds to an ER element in the promoter region of target genes to alter gene transcription (11, 12). The coregulatory molecules, many of which have now been identified and studied, modulate this classical pathway for ER transcriptional activation (2, 14-24).

Some function as coactivators to amplify ER-mediated gene transcription by interaction with the basal transcription machinery and by altering chromatin structure via their histone acetylase activity. Others function as corepressors to inhibit this function by preventing chromatin unwinding via their deacetylase activity. The corepressor proteins may be important for those genes, the transcription of which is normally blocked by estrogen, and they may also be important for the antiestrogenic activities of certain ligands.

Various ligands modify the receptor in different ways. Drugs now known as SERMs, such as tamoxifen, bind ER, dissociate heat shock proteins, and induce receptor dimerization and binding to ER elements on target genes (2). However, the conformation of the receptor is different when bound by tamoxifen, and tamoxifen-bound receptor associates with a different set of coregulatory molecules. Tamoxifen has long been known to exert both agonist and antagonist effects, depending somewhat on the species, tissue, or gene. Tamoxifen is predominately an agonist in bone or in endometrium, whereas it is an antagonist, at least on genes important for cell proliferation and survival, in the breast. The agonist/antagonist profile of tamoxifen and other SERMs may in part be related to the particular milieu of coactivators and corepressors in a cell. Different response elements in the promoter of target genes may also contribute to the agonist/antagonist properties of these drugs. Experimental manipulation of coactivators and corepressors can greatly affect ER activity from tamoxifen-bound receptor (14, 23, 24). An abundance of coactivators leads to greater agonist activity, whereas a reduction in coactivators or an increase in corepressors enhances tamoxifen’s antagonist activity. These studies have led to the hypothesis, not yet proved, that altered expression of coactivators and corepressors over time may contribute to acquired endocrine resistance.

Steroidal antiestrogens, such as ICI 182,780 (fulvestrant, Faslodex), also bind the ER (25, 26). However, this class of agents has an entirely different effect. These drugs inhibit ER dimerization and binding to DNA. Furthermore, they antagonize both the AF-1 and AF-2 transcription-activating function of ER, whereas the SERMs only inhibit AF-2. Finally, the steroidal antiestrogens induce ER degradation and ER loss from the cell. Because ER is the therapeutic target, these drugs theoretically offer the optimal approach to endocrine therapy by eliminating the cellular target.

ER transcriptional activity thus is governed by the particular ensemble of ligand, receptor subtype, receptor phosphorylation, the milieu of coregulatory proteins, and the promoter sequences in specific genes. This complexity of ER structure and function may offer new diagnostic and treatment strategies. Just as ER is necessary for response to endocrine therapy, it is possible that the levels of coactivators and corepressors are just as important, and studies are under way to measure these factors in clinical samples. Blockade of coactivator function or drugs mimicking corepressor function might even offer new treatment approaches. Finally, it is theoretically possible to develop new SERMs that modulate the receptor in specific ways, thereby achieving many of the desirable estrogen agonist activities in specific tissues while avoiding undesirable estrogenic effects in others.

**Mutant ERs**

Given the importance of the ER in breast cancer development and progression, it is surprising that mutant ERs, especially those that could provide a selective growth advantage, have not been frequently identified in clinical breast cancer specimens. Several ER variants/mutants have been reported, some of which function as dominant negatives, others that are constitutively active, and others that alter the agonist/antagonist activity of SERMs (1). Such mutants have not been found to be clinically important in human breast cancers, rendering them almost a curiosity valuable only for research into the structure and function of ER. However, a recently identified mutant ERa, called K303R, has now been observed commonly in human breast cancers, and it demonstrates a unique functional difference compared with wild-type receptor (10).

The K303R ERa mutation was first identified in premalignant breast lesions that were carefully microdissected, avoiding contamination of wild-type receptor from normal elements (10). The mutation results in a lysine (K) to arginine (R) change at an important acetylation site in the hinge domain of the ER (27). The receptor demonstrates normal estrogen-binding affinity, but the cells expressing the mutation are 100- to 200-fold more sensitive to estrogen. The explanation for the hypersensitivity to estrogen is not yet clear, but these data suggest that even the low estrogen levels in postmenopausal women could maximally activate this receptor. Although more studies are needed, preliminary data suggest that K303R ERa is present in 35% of typical and atypical ductal hyperplasias of the breast and in more than one-half of invasive cancers (10, 28). Furthermore, the mutation appears to be found much more frequently in patients with more advanced, node-positive breast cancer, suggesting that it could play a role in tumor progression, a question that is now being intensively investigated.
Cross-Talk between ER and Growth Factor Receptor Pathways

The PI3K/Akt (PKB) Pathway. The PI3K pathway mediates cell survival and proliferation signals coming from a variety of growth factors, including insulin, the IGFs, and members of the EGF family (Ref. 29; Fig. 2). PI3K is activated by the binding of growth factors to their respective membrane receptors. PTEN is a tumor suppressor gene that inhibits PI3K. In addition, PDK1/2 is downstream of PI3K and it activates Akt/PKB, and thereby inactivates several apoptosis mediators including FKHR, Bad, GSK-3 and caspases.

The ER is also known to modulate this pathway at several levels. For instance, estrogen up-regulates IGF-1 receptor and other signaling molecules including IRS-1 (31). In addition, recent data suggest that ER can directly interact with PI3K in the cell membrane to activate it (32). The PI3K/AKT pathway can also modulate the ER. Akt phosphorylates ER at serine 167, thereby enhancing transcriptional activation by the ER (33). Interestingly, recent data demonstrate that FKHR, which is inactivated by Akt, can interact with the ER and repress its transcription-activating function (34). Loss of FKHR function would, therefore, have important effects in breast ductal epithelium by enhancing cell survival and augmenting ER-mediated events. The cross-talk between the ER and the PI3K pathways may explain previous observations demonstrating additive or synergistic effects on cultured breast cancer cells treated with estrogen and IGFs.

The MAPK (Erk1/2) Pathway. This MAPK pathway consists of a protein kinase cascade that links growth factor signals with activation of transcription factors in the nucleus (Ref. 35; Fig. 3). Again, among several growth factors, the IGF and the EGF families activate the tyrosine kinase activity of their respective receptors, which then activate Ras. Ras then activates other signaling intermediates, eventuating in the phosphorylation and activation of MAPK/Erk 1/2. Erk 1/2 then activate by phosphorylation other kinases, such as P90RSK and MSK, and also activate transcription factors such as Myc and Elk 1. These pathways activate cell proliferation and have transforming capabilities.

The MAPK pathway also interacts with the ER at several levels. Again, ER can increase expression of both growth factors themselves and their receptors to amplify signals generated through this pathway, whereas at the same time Erks can phosphorylate the ER at serine 118 to turn on receptor transactivation (36, 37). Erks also phosphorylate P90RSK, which can then also phosphorylate ER (38).

Stress Response Pathways. The SAPK/JNK pathway and the p38 MAPKs are activated by distinct extracellular stimuli including inflammatory cytokines and various cellular stresses such as UV light, osmotic stress, ischemia, ionizing radiation, heat shock, oxidative stress, and receptor systems of the tumor necrosis factor (TNF) family (Fig. 4; Refs. 39–41). The phosphorylation cascade coming from these pathways activate a variety of transcription factors including ATF-2, c-JUN, Elk-1, and MSK-1, which can then activate CREB. The ER can also activate gene expression through nonclassical means by its direct interaction with c-JUN on AP-1 complexes, which mediate a variety of proliferation, developmental, and even apoptotic signals (42). In turn, both JNK and p38 MAPK can phosphorylate ER or its coregulatory proteins, presumably augmenting ER-mediated gene transcription (43, 44).

Thus, there is considerable cross-talk between ER pathways and pathways mediating a variety of other important cellular events. Experimental and clinical data also suggest that this cross-talk could be important for resistance to specific endocrine therapies. Breast tumors that overexpress members of the EGF receptor family may be less responsive to antiestrogen therapy such as tamoxifen. Blockade of these receptor pathways restores growth inhibition by the antiestrogen (45). It is intriguing to speculate that phosphorylation of ER, mediated by growth factor signaling, explains in part de novo, and perhaps, acquired tamoxifen resistance, and that simultaneous treatment with inhibitors of these growth factor pathways and tamoxifen may overcome or prevent the development of this form of resistance. Clinical trials are now underway to determine whether simultaneous inhibition of a growth factor pathway combined with tamoxifen or other endocrine therapies represents a therapeutic advantage. It is also likely that some of the many proteins known to modulate ER activity may eventually prove to be...
the presence of estrogen, looking for differences? I was struck by your statement that the corepressor N-CoR may have nothing to do with ER regulation, especially in light of the report that cells selected for resistance to tamoxifen have low levels of N-CoR. Can you expand a little more why you said that you are not that excited about the N-CoR reduction?

Dr. Osborne: No, no one has done that. It's one of the problems that we haven't had reagents to study receptor phosphorylation sites on ER in the presence of tamoxifen and then in the absence of ligand, and then what it binds to when you've phosphorylated it through growth factors?

Dr. Osborne: No, we haven't. It's a good experiment to do. Our HER2-overexpressing MCF-7 tumors are unaffected by tamoxifen but are affected by estrogen withdrawal in research that we published with Chris Benz several years ago (C. C. Benz et al., Breast Cancer Res. Treat., 24: 85–95, 1993). So we hypothesized that HER2-overexpressing tumor could be treated by estrogen withdrawal or by fulvestrant, which gets rid of the receptor entirely. In our mouse model, we found that treatment with estrogen withdrawal or with fulvestrant totally stopped tumor growth. In fact, it's very dramatic, but it's also very short lived, and the tumors start to regrow within a few months. We're exploring this further, at least with fulvestrant, to see if maybe some fulvestrant-bound estrogen receptors do make it to the estrogen response element. Then even fulvestrant would act as an agonist. I think we need to get rid of all those receptors, and the dose of fulvestrant is not quite right.

Dr. Arteaga: Is the degradation of ER that is mediated by fulvestrant dose-dependent? Does it occur in every ER+ tumor cell?

Dr. Osborne: There are very little data. What is needed is a study measuring the ER in tumors at the time of resistance to fulvestrant. In our mouse model, the receptor doesn't totally go away. It goes from 300 fmol down to about 10–15 fmol.

Dr. Steven Come: In the significant proportion of breast cancer patients who are clinically ER−, has there been any manipulation of pathways that's ever actually increased or re-stored estrogen sensitivity? Is there any way to get the toothpaste back in the tube in that group of patients?

Dr. Osborne: Not via growth factors, but the mechanism of loss of estrogen receptor is a really important question: 99% of atypical hyperplasias are ER+, but only 70% of ductal carcinomas in situ, the next stage. How is the estrogen receptor lost there?

Dr. Anthony Howell: I think those cells in that pathway never had an estrogen receptor in the first place, that there's a pathway which doesn't go through ADH but straight to grade 3 ductal carcinoma in situ.

References

Stress, GFs, Inflammation

\[ \text{Fig. 4 Stress kinase pathways. GF, growth factor.} \]
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Cancer Res 2001;7:4338s-4342s.

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