

Breast Cancer Endocrine Resistance: How Growth Factor Signaling and Estrogen Receptor Coregulators Modulate Response¹

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Abstract

Endocrine therapy, and especially tamoxifen, is the most important systemic treatment of estrogen receptor (ER)-positive breast cancer at all stages. A serious obstacle, however, is intrinsic or acquired resistance to these therapies, which in the case of selective ER modulators, such as tamoxifen, involves some imbalance of their agonist *versus* antagonist actions. Recent data suggest that levels of both ER coregulatory proteins and extra and intracellular signaling from growth factor-related pathways may be important in adjusting this mixed agonist/antagonist activity of selective ER modulators in resistant breast tumors. We suggest that ER coregulators' mediation of growth factor and other cellular signaling to the ER pathway is an important feature in endocrine response and resistance in breast cancer. Indeed, we find that failure of the antitumor activity of tamoxifen in patients with breast cancer is actually determined by both the levels of and the interaction between the ER coactivator amplified in breast cancer-1 (AIB1) and the epidermal growth factor-related protein HER. Thus, the interactions of these diverse elements are essential considerations in defining new predictive and therapeutic tools.

Introduction

Breast cancer development and progression are influenced by steroid hormones, particularly estrogen, via their interaction with specific target cell receptors. The idea that therapeutic antagonists to estrogen action could also prevent or treat breast cancer was first suggested in the 1930s (1), long before either the target ER³ or antiestrogen drugs were identified. At present, estrogen antagonist therapy is the most effective treatment for

women with ER-positive breast cancer. Unfortunately, many patients present with primary (*de novo*) resistance to endocrine therapy, despite high tumor levels of ER, and all patients with advanced disease eventually acquire resistance to therapy. The potential mechanisms for either intrinsic or acquired endocrine resistance are still poorly comprehended, but they clearly include ER-coregulatory proteins and cross-talk between the ER pathway and other GF and kinase networks. Identifying the factors and pathways responsible for this resistance, and defining ways to overcome it, are therefore important diagnostic and therapeutic challenges in current breast cancer research.

Tamoxifen Treatment and Resistance in Breast Cancer

Current endocrine therapies of breast cancer are based mainly on targeting the ER signaling pathway by either: (a) reducing levels of estrogen; (b) antagonizing ER function with antiestrogens such as tamoxifen; or (c) down-regulating ER levels with pure antiestrogens such as fulvestrant (Faslodex).

Tamoxifen, a nonsteroidal SERM, is the most frequently prescribed drug for the treatment of all stages of breast cancer (2), and it is now also used in prevention for women at high risk of developing breast cancer (3). The widespread use of tamoxifen over the last 20 years is probably one of the major reasons for the observed decrease in breast cancer mortality in the past decade. Aromatase inhibitors (estrogen depletion) have recently been proven superior to tamoxifen (4). However, a longer follow-up is required before a final benefit:risk assessment can be made to justify ousting tamoxifen in the adjuvant setting for postmenopausal women. If so, tamoxifen will remain a useful therapeutic option in advanced disease.

Tamoxifen is thought to inhibit breast cancer growth mainly through competitive blocking of the ER, thereby inhibiting estrogen-induced growth. In the adjuvant setting, tamoxifen therapy results in a 40–50% reduction in the annual odds of recurrence and leads to prolonged disease-free and overall survival (2). In addition, tamoxifen has also been shown to induce clinical benefit in more than half of patients with metastatic disease who have ER-positive tumors (5). However, although tamoxifen is initially effective in many patients, and in general is very well tolerated, a major obstacle to its use is tumor resistance. Almost 50% of breast cancers, despite the presence of ER, fail to respond to tamoxifen; furthermore, even patients who initially respond eventually acquire tamoxifen resistance, leading to tumor progression and death. In general, acquired resistance to tamoxifen is not attributable to loss of or alteration in the ER, and resistant tumors often respond to second-line endocrine therapy (5, 6). Experimental and clinical evidence suggests that in many scenarios, tamoxifen resistance of breast cancers may be attributable to the ability of the tumor cells, either *de novo* at the beginning of the treatment or in the acquired setting after prolonged treatment, to be stimulated

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³ The abbreviations used are: ER, estrogen receptor; GF, growth factor; AIB1, amplified in breast cancer-1; EGFR, epidermal growth factor receptor; IGF, insulin-like growth factor; MAPK, mitogen-activated protein kinase; NCoR, nuclear receptor corepressor; NCoA, nuclear receptor coactivator; SRC, steroid receptor coactivator; DFS, disease-free survival; SERM, selective estrogen receptor modulator.

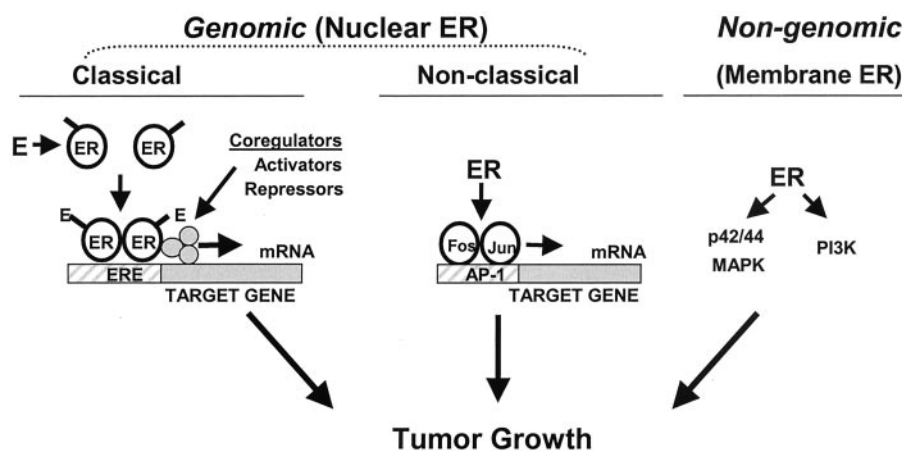


Fig. 1 ER function: on binding of its estrogen ligand (*E*), nuclear ER activates transcription (genomic action) either by direct DNA binding to its own response elements in targeted gene promoters (classical mode) or by tethering to other transcription factors, such as the Fos/Jun activating protein-1 (AP-1) complex (nonclassical mode). Membrane ER activity (nongenomic action), through direct interaction with different signaling intermediates at the vicinity of the membrane, can lead to rapid induction of key GF-dependent kinases, such as p42/44 MAPK and phosphoinositide 3-kinase.

rather than inhibited by the drug (7–11). The clinical implication is that tamoxifen treatment should be avoided or stopped as soon as resistance develops, because tamoxifen not only does not provide the desired protective antitumor signals but may instead stimulate the tumor.

We have extensively studied an *in vivo* xenograft model of tamoxifen-stimulated tamoxifen resistance, using ER-positive MCF-7 human breast cancer cells growing in nude mice (7). Tamoxifen treatment initially suppresses tumor growth for several months, but growth eventually resumes as the tumors become stimulated by the drug (7). In these tumors, however, tamoxifen is still able to partially antagonize the more potent growth-promoting activity of estrogen when the drugs are administered together (8), whereas the pure steroidal antiestrogen fulvestrant, which induces ER degradation and depletion from the cell, can fully inhibit cell growth (12). These findings argue that the resistant/stimulated growth of these tumors is mediated through the ER, but the ER activity has been modulated so that tamoxifen's antagonistic activity has switched to an agonist effect.

Molecular Basis for the Mixed Agonist/Antagonist Activity of SERMs

Estrogens exert profound effects on growth and differentiation of many reproductive tissues, as well as on other tissues, including bone, liver, the cardiovascular system, and brain (13). SERMs are synthetic agents that act as either ER agonists or ER antagonists, depending on the tissue (14). In fact, tamoxifen was the first example of such a SERM, exhibiting estrogen antagonist activity in the breast concurrently with an estrogen agonist activity in the endometrium and bone. Recent laboratory research has provided exciting new information on how estrogen and its receptor activate growth of breast cancer cells (Fig. 1) and how SERMs like tamoxifen modify ER activity (Fig. 2).

ER Subtypes α and β . With the recent discovery of ER β (15), estrogen, like many other steroid and nuclear hormones, is now known to signal through more than one form of receptor. The ERs are members of a large superfamily of nuclear receptors (16, 17) that includes receptors for other steroid hormones, as well as nonsteroid hormones such as thyroid hor-

mones and retinoids. On binding of their respective ligands, these receptors function as transcription factors to modulate the transcription of target genes critical to different biological processes. ERs mediate most biological effects of estrogens on cell and tumor growth (18).

Estrogen diffuses into cells and binds to the ER proteins. This binding triggers receptor dimerization as well as a specific conformational change in the receptor (17). This change facilitates binding of coregulatory proteins that modify and activate its transcriptional activity on specific consensus ER response elements in the promoter regions of target genes (classical action, Fig. 1). ERs possess two major transcriptional activation domains residing in their NH₂ and the COOH termini, which harbor, respectively, the constitutively active, ligand-independent AF-1 and the ligand-dependent AF-2 functions (recently reviewed in Ref. 19). The agonist function of SERMs may come from the constitutively active AF1 domain, and modulation of this activity has been suggested to be important for tamoxifen resistance (20).

ERs can also regulate gene expression without directly interacting with DNA, via tethering to other transcription factors such as activating protein-1 (21), SP-1 (22), and others (Refs. 23 and 24; nonclassical action, Fig. 1). Importantly, tamoxifen has been found to have a specific reverse pharmacology on some of these sites (25). Estrogens, and maybe some SERMs, also clearly have some nongenomic actions (Fig. 1), which are manifested in the rapid induction of some intermediate kinase signaling and are probably mediated by the receptors' activity in the vicinity of the cytoplasmic membrane (26–28). However, the biological significance of this mode of action is not clear at present, and it does seem that the majority of the cellular effects of estrogens in the breast are mediated through ER functioning as a transcription factor.

ER β and its splicing isoforms are widely expressed in both normal and malignant breast (15), but their role in breast cancer development and progression is not yet clear, and it is believed that ER α , and not ER β , is the dominant receptor in breast cancer pathogenesis. Certainly, ER α is thought to be the primary predictive factor for endocrine response in breast tumors. Nevertheless, a number of groups have reported results regarding the

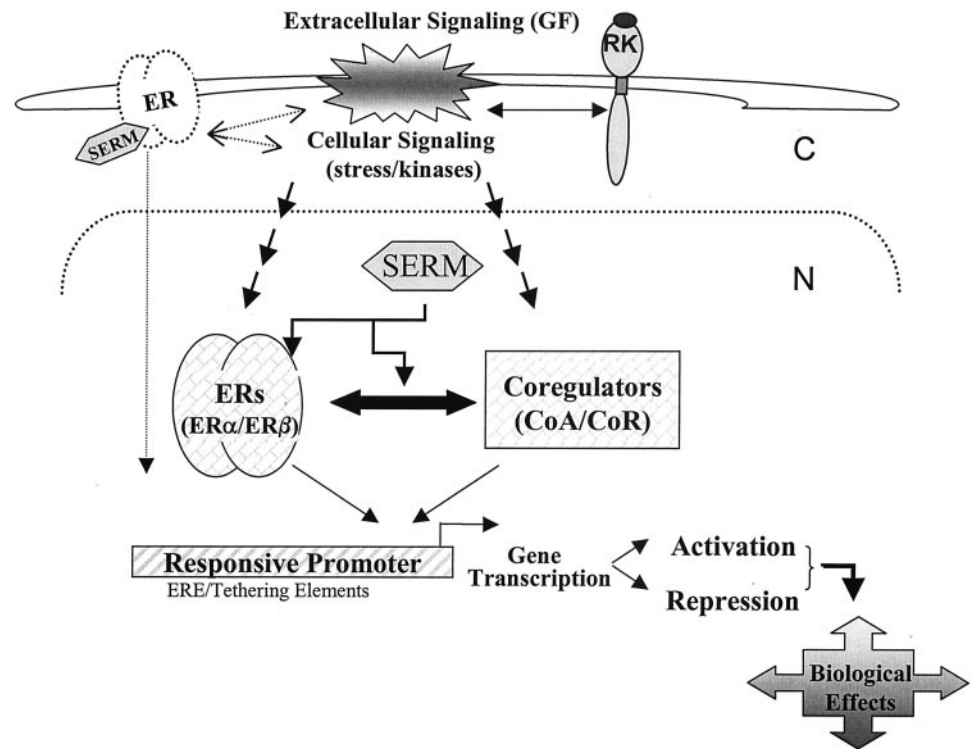


Fig. 2 The selective action of SERMs in a given cell or tissue is the combinatorial effect of a delicate balance of different factors acting in a concerted network to modulate the ER activity. These include the levels and ratios of the different ER subtypes (ER α :ER β), coregulatory proteins [coactivators (CoA)/corepressors (CoR)], extracellular signaling and their receptor kinases (RK), and the nature of promoters of key target genes at specific sites. ER's predominant effects are in the nucleus, but its activity in the membrane may also contribute to SERM activity. C, cell cytoplasm; N, cell nucleus.

possible function of ER β in breast cancer and its prognostic or predictive value, although the data are inconclusive and even somewhat contradictory (for recent reviews, see Refs. 15 and 29). Interestingly, however, several SERMs, including tamoxifen, have been shown to have agonistic activity preferentially on ER β , depending on the promoter (25). Thus, the two ERs signal in somewhat different ways depending on ligand and response element, a fact that may imply some additional predictive value for ER β . Noteworthy in this context is a study using reverse transcriptase-PCR that reported the detection of increased expression of ER β mRNA in tamoxifen-resistant breast cancer patients (30). This report, however, is based on a very small isolated study, so that the clinical implication of this observation is still uncertain.

The Role of Nuclear Receptor Coregulatory Proteins.

The ligand-bound ER, depending on the nature of the ligand, recruits and interacts with coregulatory proteins that can either enhance (coactivators) or repress (corepressors) its transcriptional activity (31). Most of these coregulators are not specific to the ER and operate also on other nuclear receptors and in some cases even on non-nuclear receptor transcription factors. However, some receptor-specific coregulators have been identified (32).

Coregulatory proteins are often present at rate-limiting levels in the nucleus, so that modifications of their level of expression and/or activity can lead to alterations in nuclear receptor signaling. A few key reports (33, 34) have shown that, at least in cultured cells, the differential expression or activity of coactivators and corepressors in a given cell can modulate the agonist *versus* antagonist activity of drugs, such as tamoxifen on

the ER. Ratios of coactivator:corepressor have also been shown to modify progesterone and glucocorticoid receptor-mediated transcription induced by their own selective receptor modulators (35) and to confer hyper or hyposensitivity to their ligands (36). This concept provides one explanation for the tissue-specific actions of SERMs and may also explain some clinical tamoxifen resistance, a concept that still remains to be proved in patients' samples. In addition, because the development of resistance to antiestrogen therapies is associated, at least in preclinical models, with an acquired hypersensitivity phenotype of the tumor cells (37), ratios of coactivators:corepressors might also be important in resistance to this kind of endocrine manipulation.

These observations, overall, further suggest that an assessment of coactivator and corepressor expression and activity in tumors may turn out to be essential in predicting prognosis and response to therapy in a given breast cancer patient (Figs. 2 and 3).

Corepressors and Coactivators in ER Responses to Ligands.

The number of NCoR proteins that may be important in ER pharmacology is steadily growing. Originally characterized by their ability to bind and repress the unliganded thyroid and retinoid acid receptors, the corepressors NCoR (38) and silencing mediator for retinoid and thyroid receptor (SMRT) (39, 40) are the most studied nuclear corepressors. These factors, at specific target promoter sequences, form multisubunit repressor complexes which include histone deacetylases and facilitate chromatin condensation and subsequent inhibition of gene transcription (31). These corepressors are also recruited to steroid receptor complexes but, as a rule, only when the receptors are bound with antagonist drugs. Thus, in the presence of SERMs with mixed agonist/antagonist activity, these corepres-

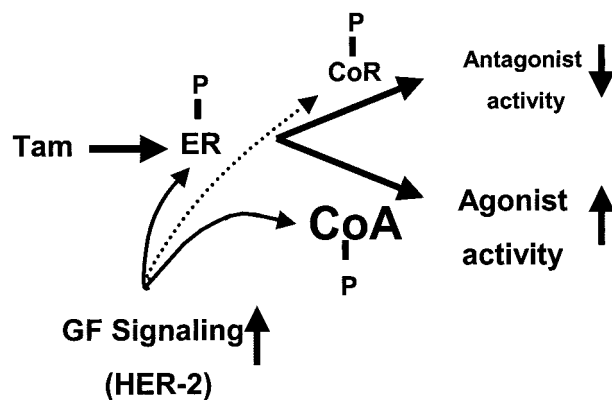


Fig. 3 Cross-talk of GF and ER pathways: the role of ER coactivators (CoA) and corepressors (CoR) in the activity of tamoxifen (Tam)-bound ER. Amplified signaling and high levels of CoA in tumor cells result in increased Tam agonistic activity and tumor growth.

ceptor complexes can be recruited to ER, resulting in partial repression of transcription (41). The possible significance of these corepressors in ER pharmacology has been suggested by us (42) and others (41) in studies showing that the development of tamoxifen resistance is associated with a decrease in the expression level of the corepressor NcoR.

The growing list of NcoAs already includes >30 identified factors. Coactivators bind to nuclear receptors when the receptors are occupied with agonistic ligands. These factors also form multiprotein complexes, which augment transcriptional activities by serving as adaptors to the basal transcriptional machinery, and by mediating chromatin remodeling through histone acetylase enzymatic activity either of the coactivators themselves or of other factors which they attract to the receptor complex (31). Kinase, methyltransferase, and ubiquitin ligase activities are other enzymatic activities described for various coactivators (43).

The SRC family of coactivators has been studied extensively and appears to have a wide spectrum of effectors that includes nuclear receptors along with other transcription factors, such as nuclear factor- κ B (44). Three members of this family, SRC-1/NCoA-1, SRC-2/GRIP1/TIF2/NCoA-2, and AIB1/SRC-3/RAC3/ACTR p/CIP, share a common domain structure and functions (45). However, although there is clearly some redundancy within the family, receptor, tissue, and biological function specificities do exist and are in part controlled by the specific sequences flanking the receptor-interacting motifs (45, 46) and maybe also by the phosphorylation status of the coactivators (47). A remarkable and clinically significant example of these SRCs' specificities is the critical role of SRC-1, but not of the other family members, in the agonist activity of tamoxifen in the uterus (48). Interestingly, this phenomenon occurs only on non-consensus responsive sites. Just how tamoxifen-bound ER can recruit and interact with coactivators is still unclear, but the process may involve, as suggested by the authors, "as-yet undetermined cell-specific factors that contribute to the spectrum of SERM actions" (48). One type of factor which one should consider is the particular set of extra and intracellular signaling

components within the microenvironment of specific cells or tissues that can modify both the ER and its coregulators' activity.

The third member of the SRC family is AIB1. This coactivator is overexpressed in >50% of breast tumors, and it is gene amplified in ~5–10% of breast tumors (49–51), giving rise to its name and strongly suggesting that it is important in breast cancer pathogenesis. The mouse AIB1 homologue SRC-3 is expressed in a tissue-specific fashion and required for the normal development of mammary glands (52). Knockout studies have further suggested a physiological role for SRC-3/AIB1 distinct from that of SRC-1 and have again substantiated the diversity among coactivator family members (52). AIB1 is also highly expressed in cultured MCF-7 human breast cancer cells, and its activity is crucial for growth of these cells *in vitro* and *in vivo* (53). When expressed in cultured cells, AIB1 enhances tamoxifen agonistic activity (54), again suggesting its potential role in breast cancer endocrine resistance.

ER Pathway Cross-Talk with GF and Cellular Kinase Pathways. Compelling evidence suggests that cross-talk between ER and GF-receptor pathways such as the EGFR/HER2 family and IGF-I receptor contributes to endocrine resistance.

Numerous studies suggest that in breast tumors, estrogens and ERs promote the expression and/or activation of several of the proteins in these pathways. Examples include receptors (IGF family), autocrine growth factors (tumor growth factor- α and IGF-II), and signaling intermediates (insulin receptor substrate-1 and 2; Refs. 55 and 56 and references therein). Through its activity in the membrane, ER, in a ligand-dependent mode, can also directly activate different GF receptors and their downstream kinases and signaling molecules (27). Thus, it is possible that abnormally increased signaling within these GF signaling pathways may account for loss of some estrogen dependence, resulting in antiestrogen-resistant tumors. Indeed, acquired resistance of MCF-7 cells *in vitro*, after long-term treatment with tamoxifen, is associated with increased levels of EGFR and MAPK activity (57).

But cross-talk of GF signaling with ER and endocrine response operates in both directions. Thus, molecular communication from several GF or intracellular kinase signaling pathways to the ER pathway can alter its function and thereby contribute to tumor growth. Diverse signaling pathways and various agents that raise intracellular kinase activities induce ER activation in the presence of tamoxifen or in the absence of any activating ligand (42). ER itself is now known to be subject to and activated by phosphorylation at multiple sites and by multiple kinases (58–63). However, phosphorylation of ER coregulators is probably at least equally important in mediating these GF effects on the ER pathway. Experimental support for this issue comes from studies showing that: (a) phosphorylation site mutants of ER can still be activated by various signaling (64); and (b) phosphorylation site mutations of coactivators substantially reduce their ability to enhance transcription of signaling kinase-potentiated ER (65). It therefore appears that coregulators are essential to allow GFs to fully exert their influence on the ER pathway. It may well be that this regulatory mode reflects a more global biological means to control and target diverse extracellular signaling to specific transcription paths (66, 67). Indeed, in the case of ER, key signaling kinases, including p42/44MAPK, AKT, the stress-related c-Jun NH₂-

terminal kinase and p38 MAPKs, and protein kinase A (61–63, 68), have all been reported to activate the ER pathway by direct phosphorylation of the ER, its coactivators, or both. This cross-talk could therefore markedly increase tamoxifen's agonist properties in a cell that expresses high levels of both a coactivator and kinase signaling (Fig. 3). Notably, our preclinical studies (69, 70), as well as those of others (37, 57), have already shown that *de novo* and/or acquired tamoxifen resistance is associated with increased levels of these different kinases.

Phosphorylation of ER and other nuclear receptors has been the subject of numerous studies. Phosphorylation of ER on serine residues clustered in its NH₂-terminal region increases both ER nuclear translocation and ligand-independent transcriptional activity of the AF-1 function, including in the presence of tamoxifen (58–63). The precise mechanisms by which phosphorylation activates ER coactivators are not yet clear. Recent studies suggest, however, that phosphorylation can enhance nuclear sublocalization of the coactivators (44) and their interaction with the ER (71) and may directly increase their own acetyltransferase activity or stimulate their ability to recruit other transcriptional coactivators or integrators to the receptor complex (64).

As with coactivators, various signaling pathways also modulate and attenuate corepressor activities on ER (42, 72). Phosphorylation and consequent nuclear export is one potential mechanism responsible for GF-induced, kinase-mediated inhibition of corepressor function (73).

Thus, the cumulative data suggest that ER coregulators are important contributors to estrogen-mediated tumor growth and, potentially, to sensitivity or resistance to endocrine therapy. Because both the level and activity of coregulators, which are highly controlled by diverse signaling pathways, can specify endocrine sensitivity (Fig. 3), both coactivators and corepressors and the interactions among them may need to be measured simultaneously in tumors to accurately predict endocrine response of breast cancer patients.

Clinical Significance: The Interaction of AIB1 and HER2 in Predicting Tamoxifen Resistance of Breast Cancer

HER2, a member of the EGFR family, is gene amplified and/or overexpressed in 20–30% of breast cancers and appears to be associated with a more aggressive phenotype (74). Experimental data suggest that overexpression of HER2 or its stimulation by heregulin (75) leads to tamoxifen resistance. Ten years ago, we first demonstrated in our xenograft model that human breast cancers overexpressing HER2 are resistant to tamoxifen (76). High HER2 expression has also been shown to correlate with tamoxifen resistance in patients in some studies, but this association is not strong, and other studies have failed to confirm the association (74, 77, 78). Therefore, the predictive value of HER2 is still in question, and best current practice in adjuvant breast cancer therapy does not deny tamoxifen to patients with HER2-positive tumors.

The ER coactivator AIB1, like ER itself, is phosphorylated and activated by different signaling kinases, including the p42/44 MAPK, which can be activated by HER2 (64). Therefore, high levels of activated AIB1 could profoundly reduce the

antagonist effects of tamoxifen in tumors that also overexpress GF receptors, such as HER2. We thus hypothesized that tumors with a relative abundance of coactivators, such as AIB1, especially those with enhanced HER2 signaling, which can activate AIB1, should be less responsive to tamoxifen therapy because of increased estrogen agonistic activity of tamoxifen-bound ER (Fig. 3). Hence, AIB1 levels may be the “missing component” to complement and augment the predictive value of HER2.

To test this hypothesis, we conducted a study which correlated AIB1 and HER2 protein expression, separately or in combination, with DFS in tumors from patients treated with adjuvant tamoxifen compared with those receiving no adjuvant therapy (79). The data from this study are among the first to clearly demonstrate that an ER coregulator is important in the pathophysiology of disease in humans. Using Western blot assays, AIB1 levels were quantified in 316 samples from patients with long-term clinical follow-up (79). Interestingly, high AIB1 expression was associated with better outcome in the untreated subset of patients. This result suggests a “protective” role for AIB1 in these untreated tumors, perhaps because of its supportive role to the ER pathway in mediating more differentiated and indolent tumors. In contrast, in tamoxifen-treated patients, high AIB1 predicted significantly worse DFS, confirming that tamoxifen offers little or no benefit and may be detrimental in this subset. But most important, in a combined analysis, only those tumors expressing high AIB1 together with high HER2 had poor outcome with tamoxifen. Tumors with high HER2 only or high AIB1 only had excellent DFS. These clinical data strongly support our hypothesis that increased signaling from the EGFR/HER2 family activates the p42/44 MAPK, which in turn activates ER and AIB1. Phosphorylated AIB1 then probably translocates to the nucleus (44), where it can interact with ER. In the presence of high levels of activated AIB1 to complex with ER, the agonist activity of tamoxifen is increased, eliminating any benefit to the patient. Whether AIB1 and/or HER2 are also important in resistance to therapies designed to reduce estrogen levels is still an open question, because resistance in this setting is mediated by the acquisition of estradiol hypersensitivity (37).

Conclusions and Prospects

Breast cancer endocrine resistance, especially to SERMs such as tamoxifen, is a major clinical problem and a leading cause of treatment failure and, thus, of mortality from this disease. The degree and direction of SERM signaling in a given cell, tissue, or tumor are ruled by a delicate balance of different factors acting in a concerted network to modulate the ER activity. These include the levels and ratios of the different ER subtypes, coregulatory proteins, extracellular signaling, and nature of promoters of key target genes at specific sites. Causative mechanisms for endocrine resistance are probably multifactorial. Clinical and basic molecular studies suggest that signaling from GF, stress, and other cellular pathways directly to ER and/or some key coregulators is especially important in mediating resistance. Thus, in addition to ER itself, ER coregulator and specific intermediate signaling molecules in other pathways are all potentially important predictive biomarkers and new clinical intervention targets. Monoclonal antibodies to the de-

fine specific phosphorylated sites on these key molecules should be especially useful tools.

These new tools could be an important supplement to the currently available predictive markers in breast cancer. Therapies that will include specific inhibitors or down-regulators targeting coactivators, such as AIB1, as well as important kinases and other signaling intermediates involved in endocrine resistance are potential new treatment avenues for breast cancer patients. Combining endocrine treatment with these new targeted therapies is a promising approach to improve present treatment strategies and overcome endocrine resistance and should be investigated in future preclinical and clinical studies.

Open Discussion

Dr. Mitch Dowsett: Can you do anything to abrogate the function of the AIB1?

Dr. Kent Osborne: Yes. We've now got about three or four clones of the ribozyme under an inducible system, and we're just ready to start those experiments. So presumably, if acquired tamoxifen-stimulated growth is similar to estrogen-stimulated growth at the outset, then we should be able to abrogate that tamoxifen-stimulated growth either by blocking the GF pathway with Iressa or other drugs or by blocking AIB1. That's the experiment we're trying to do now.

Dr. Stephen R. D. Johnston: You say overexpression of AIB1 in the normal hormone-sensitive cells is a good prognostic factor, and in your MCF-7 cells, if you put in HER2, they become tamoxifen resistant. Do you think HER2 phosphorylates different sites in AIB1? Have you done any work to see if actually the activation of AIB1 is altered when you've got HER2 in the system or whether it has nothing to do with AIB1 and it's coactivation of ER in the context of a high level of AIB1?

Dr. Osborne: Right, phosphorylation of AIB1 is very important, but there are multiple phosphorylation sites, and they probably don't all do the same thing. Others have shown that you functionally activate AIB1 when you phosphorylate it. You get more augmentation of ER signaling.

Dr. Dowsett: Phosphorylation of the ER by these GFs seems to sensitize the cell to estrogen. But does that then make the cell more sensitive to estrogen deprivation? If you give your HER2 cells fulvestrant, is that then going to abrogate any AIB1 effects?

Dr. Osborne: If you treat with fulvestrant in a HER2-overexpressing cell, you would expect it to work because it degrades the receptor, and in fact, it does. Fulvestrant works great, just like estrogen withdrawal does, in the HER2-overexpressing tumors. In the wild-type MCF-7, it takes a very long time to develop resistance to fulvestrant. It's twice as long as it is for estrogen withdrawal. In the HER2-overexpressing model, however, it's shorter.

Dr. Steven Come: You also get rid of all of the ERs in your model, don't you, with fulvestrant?

Dr. Osborne: Estrogen-treated tumors have ~100 fmol/mg protein. Fulvestrant lowers it down into the teens, and it stays there even at the time of resistance. The receptors don't start rejuvenating again, but fulvestrant doesn't get rid of them completely. We're going to do a dose response of fulvestrant to

see if getting rid of the ER entirely would prevent the resistance. One could argue that it might, and it gets to the issue about the dose in clinical patients. Very infrequently do you totally down-regulate ER in clinical specimens at the dose that we're giving people.

Dr. Johnston: The issue of cross-talk, which we're going to come back to tomorrow, suggests that ultimately it may all come down to the ER still. If you take ER out of that model with an ER down-regulator, such as fulvestrant, you get a net effect. So it comes back to the issue of whether any of these signaling agents are going to be of use in this situation if ultimately the signal is all still coming down through ER, and what we've got to do is effectively block ER's action with AIB1.

Dr. Osborne: Even if you just had a little bit of receptor, if that receptor was superactive, it could be bad if, for instance, there was a lot of AIB1 around. I'm beginning to wonder whether the resistance that we see to fulvestrant (there are, as you know, 15 fmol/mg protein of ER still there) might occur through fulvestrant acting as a weak agonist when AIB1 is activated by GFs.

Dr. Dowsett: It's amazing to see what low concentrations can be stimulatory in the cell line setting after the cells have been deprived of estrogen; it's at 10^{-14} molar when the kDa of the receptor is something like 10^{-9} or 10^{-10} . So the amplification we're getting through these coactivators must be massive.

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