Mechanisms of Endocrine Resistance in Breast Cancer

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Abstract

The estrogen receptor (ER) pathway plays a pivotal role in breast cancer development and progression. Endocrine therapy to block the ER pathway is highly effective, but its usefulness is limited by common intrinsic and acquired resistance. Multiple mechanisms responsible for endocrine resistance have been proposed and include deregulation of various components of the ER pathway itself, alterations in cell cycle and cell survival signaling molecules, and the activation of escape pathways that can provide tumors with alternative proliferative and survival stimuli. Among these, increased expression or signaling of growth factor receptor pathways, especially the EGFR/HER2 pathway, has been associated with both experimental and clinical endocrine therapy resistance. New treatment combinations targeting both ER and growth factor receptor signaling to block the crosstalk between these pathways and eliminate escape routes have been proven highly effective in preclinical models. Results of recent clinical studies, while partly supporting this approach, also highlight the need to better identify a priori the patients whose tumors are most likely to benefit from these specific cotargeting strategies.

INTRODUCTION

ER: estrogen receptor

HER2: human epidermal growth factor receptor 2

PR: progesterone receptor

The name endocrine therapy is given to those breast cancer treatments that target the estrogen receptor (ER) by blocking receptor binding with an antagonist or by depriving the tumor of estrogen. The ER, which has nuclear (genomic) and nonnuclear (nongenomic) functions, is the major driver in the majority of breast cancers. It is expressed in 75% of breast cancers overall, with its detection being slightly more frequent in tumors from postmenopausal women and less in younger women (1). ER expression is related to patient age and correlates with lower tumor grade, lower tumor proliferation, less aneuploidy, less frequent amplification of the c-erbB2 (HER2) oncogene and concomitant loss of the p53 tumor suppressor gene, positive expression of progesterone receptor (PR), metastases (preferentially to soft tissue and bone), and slower rates of disease recurrence (1–3). It is not related to initial nodal metastases, and thus it does not correlate with long-term disease recurrence and death after primary therapy (3).

These clinical factors, along with ER expression itself, are used to make treatment decisions in patients, especially those with metastatic disease. In some cases, multigene tests are performed on the primary breast tumor to assist in adjuvant therapy decision making and to distinguish which patients might benefit most from a combination of endocrine therapy plus chemotherapy, rather than endocrine therapy alone. The 21-gene and 70-gene profiles can classify ER-positive tumors according to their aggressiveness, risk of recurrence, and likelihood of benefiting from adjuvant endocrine or chemotherapy (4, 5). The stratification of ER-positive tumors on this basis indicates that some tumors are more resistant to endocrine therapy than others, despite expressing ER. In general, patients are more likely to benefit from endocrine therapy and less (if at all) likely to benefit from chemotherapy if their tumors have high levels of ER and PR, are negative for HER2 amplification, are slowly proliferating, are lower grade histologically, and have low-risk 21-gene or 70-gene profile scores. In contrast, patients with ER-positive tumors that are more aggressive, morphologically and genetically, are less likely to benefit from endocrine therapy, although there are exceptions. Additional recent molecular profiling studies have stratified ER-positive tumors into luminal A and luminal B subtypes. The more aggressive and endocrine-resistant tumors largely overlap with the luminal B subtype, whereas the more indolent and endocrine-responsive tumors generally correspond to the luminal A subtypes. Currently, however, no tests exist that can predict resistance to endocrine therapy with certainty, although tumors with absent ER and PR rarely respond. Most patients with ER-positive primary tumors are, therefore, treated with endocrine adjuvant therapy, whereas cases of ER-positive metastatic disease are treated with endocrine therapy initially and serially until the tumor demonstrates independence from estrogen.

Endocrine therapy is the most effective treatment for ER-positive metastatic breast cancer, but its effectiveness is limited by high rates of de novo resistance and resistance acquired during treatment. Only ~30% of patients with metastatic disease have objective regression of tumor with initial endocrine treatment, and another 20% have prolonged stable disease. Thus, ER is not the only survival pathway driving most of these tumors, and escape pathways when ER is targeted are already functioning or begin to function during treatment.

Understanding the pathways responsible for resistance in the metastatic setting may provide important clues to the mechanisms of resistance to adjuvant endocrine therapy given before or after primary surgery to eradicate distant micrometastases. Treatment in this setting is much more effective; the risk of recurrence is reduced by as much as 60% with estrogen deprivation therapies using aromatase inhibitors in postmenopausal women (6, 7). Unfortunately, biopsying patients with metastatic disease in the lung, bone, or liver is difficult and can be associated with high morbidity rates. However, such tissue is crucial for the molecular profiling of

resistant tumors in order to understand escape pathways. Despite these challenges, progress is being made in understanding potential mechanisms of resistance. The advances come largely from preclinical models of endocrine resistance as well as a greater understanding of the molecular mechanisms by which estrogen works to stimulate the growth of the tumor.

MECHANISM OF ESTROGEN ACTION

All biological networks have similar characteristics. In order to provide important functions in normal cells under a variety of conditions and stress factors, as well as to keep the cell alive, these networks must be complex with multiple levels of regulation, fine tuning capabilities, redundancy, and evolvability. Collectively these features allow the cell to adapt to cellular stress, toxins, and potentially hostile environments. Cancer cells exploit these normal functions, which are often altered genetically during oncogenesis, to provide them with a survival advantage and the ability to escape the effects of treatment. The ER signaling pathway is an example of a complex biological pathway that controls a variety of functions, such as cell proliferation, apoptosis, invasion, and angiogenesis, and is exploited by breast cancer cells to serve as a major survival pathway driven by the female hormone estrogen (**Figure 1**).

The classic function of ER is its nuclear function, also referred to as genomic activity, to alter the expression of genes important for normal cellular function and tumor growth and survival. ER modulates the expression of hundreds of genes, some by upregulation and others by downregulation (8). Upon binding to estrogen, ER dimerizes with another receptor monomer and attracts a complex of coactivators and corepressors to specific sites on DNA (3, 9). ER can also bind to other transcription factors such as AP-1 (activator protein-1) and SP-1 (specificity protein-1) at their specific sites on DNA, thereby functioning as a coregulator (3, 10). Coregulators serve as a fine-tuning mechanism by increasing or reducing the transcriptional activity of the receptor (11). Several coregulators have been implicated in cancer, most notably AIB1 (SRC-3), which is gene-amplified in a small percentage—but overexpressed in two thirds—of all breast cancers. Overexpression of this gene has been implicated in tamoxifen resistance (12).

The ER signaling pathway is also regulated by membrane receptor tyrosine kinases, including epidermal growth factor receptor (EGFR), HER2, and insulin-like growth factor receptor (IGF1-R) (13). These membrane kinases activate signaling pathways that eventually result in phosphorylation of ER as well as its coactivators and corepressors at multiple sites to influence their specific functions (13–16). This activation of ER by growth factor receptor signaling is sometimes referred to as ligand-independent receptor activation. Crosstalk between the growth factor receptor and ER pathways has been established through several other mechanisms as well. Estrogen can increase the expression of ligands such as transforming growth factor-α (TGFα) and IGF1 (10, 17–19), which can then activate the growth factor receptor pathway (13, 18, 20). On the other hand, estrogen signaling downregulates the expression of EGFR and HER2 while increasing the expression of IGF1-R (21–23). Activation of the PI3K/AKT and the p42/44 mitogen-activated protein kinase (MAPK) pathways by these receptors, in turn, downregulates the expression of ER and PR (24–29). Thus, while these receptor tyrosine kinases can activate the transcriptional function of ER, they can also reduce estrogen dependence by downregulating the expression of ER, perhaps contributing to the relative resistance to endocrine therapies in tumors amplified for HER2 (25, 30).

Studies also suggest that ER may work by nontranscriptional mechanisms. Low levels of ER have been found outside the nucleus in the membrane, cytoplasm, or even mitochondria, although the exact location for this receptor remains controversial (31). Some of the nongenomic action of estrogen appears to be too rapid for a transcriptional effect to activate growth factor receptor signaling, including

AIB1: amplified in breast cancer–1

SRC: steroid receptor coactivator

EGFR: epidermal growth factor receptor

IGF1-R: insulin-like growth factor receptor

PI3K: phosphoinositide 3-kinase

MAPK: mitogen-activated protein kinase

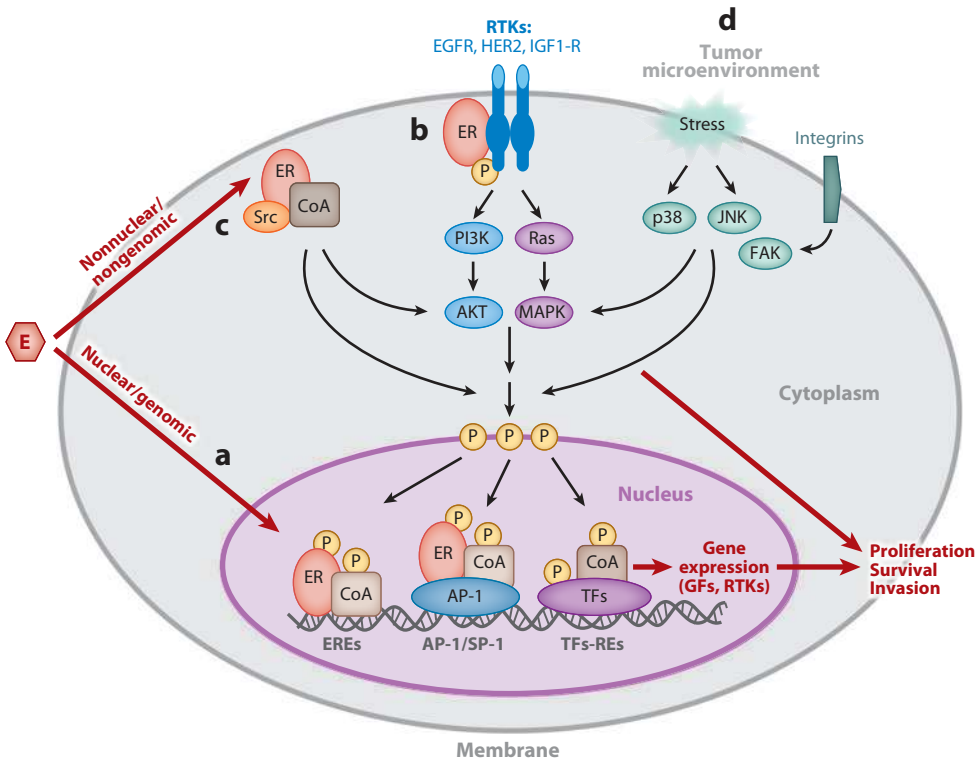

Figure 1

Mechanisms of estrogen receptor (ER) action in breast cancer. Estrogen (E)-bound ER, acting as a transcription factor in the nucleus (nuclear/genomic activity), binds to DNA sequences in promoter regions of target genes either directly [at estrogen response elements (EREs)] or indirectly via protein-protein interaction with other transcription factors at their cognate DNA-responsive sites (e.g., members of the AP-1 or the SP-1 transcription complexes at AP-1 or SP-1 sites). Upon estrogen binding, ER generally recruits coactivator complexes (CoA) to induce or modulate gene transcription, including genes encoding growth factors (GFs) and receptor tyrosine kinases (RTKs) (*a*). A small subset of the cellular pool of ER localized outside the nucleus and/or at the cell membrane associates in response to estrogen with GF RTKs (e.g., EGFR, HER2, and IGF1-R) (*b*) and with additional signaling and coactivator molecules (e.g., the Src kinase) (*c*). This interaction, similar to GF activation of these pathways, activates multiple downstream kinase pathways (e.g., SRC, PI3K/AKT, and Ras/p42/44 MAPK), which in turn phosphorylate various transcription factors (TFs) and coregulators, including components of the ER pathway that enhance gene expression on EREs and other response elements (REs). The nonnuclear/nongenomic activity, which can also be activated by tamoxifen, is enhanced in the presence of overexpression and hyperactivation of RTKs and can contribute to endocrine therapy resistance. Overall, the nuclear/genomic and nonnuclear/nongenomic ER activities work in concert to provide breast tumor cells with proliferation, survival, and invasion stimuli. Signaling from the microenvironment activates stress-related pathways and members of the integrin family. These pathways then trigger downstream kinase pathways [e.g., FAK (focal adhesion kinase), JNK (c-Jun N-terminal kinase), and p38 MAPK] that can further modulate components of the transcriptional machinery, including ER (*d*). Alterations in each of these transcriptional and signaling elements can mediate resistance to endocrine therapy either by modulating ER activity or by acting as escape pathways to provide alternative proliferation and survival stimuli.

the PI3K/AKT and the Ras/p42,44 MAPK pathways (13). Thus, ER—through this nongenomic activity—can alter the expression of genes normally regulated by growth factors (13, 31, 32). Finally, the stress kinase pathway via p38 and JNK (c-Jun N-terminal kinase) can also modulate ER function by phosphorylation of ER and its coregulators (33, 34). The microenvironment and its associated integrin signaling may exert a similar activity (35). Thus, ER activity and signaling are modulated by a variety of pathways that could also contribute to resistance to ER-targeted therapies, especially when the pathways display aberrant activity in a cancer cell.

MECHANISM OF ACTION OF ENDOCRINE THERAPIES

Various endocrine therapies work by different mechanisms to antagonize the growth-promoting activity of estrogen. Selective estrogen receptor modulators (SERMs) such as tamoxifen bind ER and antagonize the effects of estrogen on specific target genes (8, 36). Tamoxifen also has some estrogen-agonist effects on certain genes and tissues, and augmentation of this property may play a role in resistance (8, 37). Estrogen deprivation is another mechanism utilized to antagonize ER (38). In premenopausal women, tamoxifen or pharmacological or surgical ovarian ablation is standard, and in postmenopausal women, aromatase inhibitors are prescribed to block the conversion of weak androgens of adrenal origin to estrogen in peripheral tissues as well as breast cancer tissue itself. Fulvestrant is an ER downregulator and a more potent antiestrogen that reduces ER levels in cells (39). Older endocrine therapies such as high-dose or physiological-dose estrogens and androgens work by less well-known mechanisms, although it has been proposed that high-dose estrogen can induce apoptosis by activation of the Fas ligand (40). Tamoxifen, but not estrogen deprivation or fulvestrant, activates the nongenomic ER, another property that could contribute to endocrine resistance in some cases.

CLINICAL CLUES TO ENDOCRINE THERAPY RESISTANCE

Several clinical observations provide clues to potential mechanisms for resistance to endocrine therapy (**Table 1**). ER loss over time in the tumor occurs in ~20% of patients treated with endocrine therapy (41–43). Such tumors would no longer be driven by estrogen, but the escape pathways that take over with loss of estrogen dependence have not been well defined. Upregulation of HER2 by either acquisition of gene amplification or overexpression has been shown to occur in some tumors (42, 44, 45). HER2 may subsequently assume the driving role in tumor progression by serving as an alternative survival pathway or by reducing the level of ER, thus rendering the tumor less responsive to estrogen (25, 46). Preclinical and clinical data suggest the possibility that tumors can alternate between ER and HER2 as the dominant pathway, with targeted therapy against one pathway causing reactivation of the other (23, 25, 42, 44, 45, 47–51). PR, on the other hand, is lost more frequently than ER with intervening endocrine therapy, and with this loss the tumor becomes more aggressive and patients have a worse survival outcome than patients who maintain PR expression after resistance to one endocrine therapy (52, 53). In this case, PR loss might be associated with increased growth factor signaling and upregulation of the PI3K pathway, which downregulates PR and ER expression (27, 29, 54).

Table 1 Clinical clues to mechanisms of resistance to endocrine therapy

Decrease or loss of ER
Upregulation of HER2 in some patients after endocrine therapy
Loss of PR after progression on endocrine therapy
Response to sequential endocrine therapies
Shorter response duration and less frequent responses with sequential endocrine therapies
Withdrawal response after high-dose estrogen therapy
Eventual loss of dependence on estrogen with resistance to all endocrine therapies
Lower clinical benefit to endocrine therapy in high-grade, highly proliferating, high 21-gene profile score ER-positive tumors

Abbreviations: ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; PR, progesterone receptor.

Response to one form of endocrine therapy after progression on another is a historically recognized observation that is the key to management of patients with metastatic disease. Tumors in such patients are still estrogen dependent but have become resistant to the ER-targeted therapy being given. Responses to an aromatase inhibitor or fulvestrant after progression on tamoxifen are good examples of this phenomenon (55, 56). Subsequent responses to serial endocrine therapy tend to be shorter, coincident with a decline in ER level, suggesting a gradual shift from dependence on ER to an alternative escape pathway. High-dose estrogen therapy was the first additive endocrine therapy for breast cancer, later replaced by tamoxifen and aromatase inhibitors owing to their more favorable toxicity profile. It is important to note that patients responding and then progressing on high-dose estrogen therapy frequently respond simply to estrogen withdrawal. Occasionally the disease can be controlled for many years by initiating and then sequencing high-dose estrogen with estrogen withdrawal over time. In one such patient treated by Dr. Osborne, metastatic bone disease was controlled for more than eight years by alternating high-dose estrogen with estrogen withdrawal three separate times. Eventually all tumors completely lose estrogen dependence, even when ER is still expressed, by mechanisms that are poorly understood and are likely to be multiple as the tumor progresses to a more aggressive phenotype.

These observations suggest several types of endocrine therapy resistance (**Table 2**). Resistance can occur de novo (existing before any treatment is given) or be acquired (developing during a given therapy after an initial period of response). Some tumors lose estrogen dependence with loss of ER expression, although preclinical data suggest that ER can sometimes be reexpressed during subsequent treatment (25, 49, 51). Other tumors lose estrogen dependence while still expressing ER, indicating that an escape pathway has developed to replace ER. Still other tumors continue to express ER but have not lost estrogen dependence and will respond to an alternative form of endocrine therapy. These tumors have developed resistance to the specific ER-targeted therapy. The fact that subsequent remissions tend to be shorter and the fact that ER levels decline over time suggest that other survival pathways are beginning to exert their effects or that an endocrine-resistant clone is slowly emerging over time. Whether tumor cells with stem cell–like qualities play a role in the development of endocrine resistance remains to be clarified.

Table 2 Types of endocrine therapy resistance

De novo
Acquired during treatment
Loss of estrogen dependence due to loss of estrogen receptor
Loss of estrogen dependence despite presence of estrogen receptor
Resistance to a specific therapy; tumor still estrogen dependent

SIGNALING MOLECULES AND PATHWAYS IMPLICATED IN RESISTANCE TO ENDOCRINE THERAPY

Multiple pathways and molecules have been implicated in the diverse mechanisms responsible for endocrine resistance. These pathways and their gene networks, recently reviewed elsewhere (57), have mostly been investigated in the preclinical setting with a focus on tamoxifen. However, several alternative pathways have been shown or suggested to play a more general role in resistance to various other forms of endocrine therapy. Deregulation of these pathways most often arises from genetic or epigenetic changes in the tumor cells themselves. These changes influence uptake and metabolism of the endocrine agents and cellular responses to their inhibitory effects.

Tumor Microenvironment and Host-Associated Mechanisms of Resistance

The importance of the tumor microenvironment as a modulator of these processes and contributor to endocrine sensitivity has been

recognized in recent years. Evidence to support this notion has emerged from studies involving gene expression profiling and biomarkers associated with endocrine therapy responses (58, 59), and from more sophisticated in vitro and in vivo experimental model systems (35). Components of the microenvironment implicated in endocrine resistance include stromal cells (e.g., fibroblasts, endothelial, and immune system cells), structural elements of the extracellular matrix (ECM), and soluble factors (e.g., growth factors and cytokines), as well as additional microenvironmental conditions such as hypoxia and acidity (60). The role of tumor cell pathways engaged in mediating these microenvironmental and extracellular matrix stimuli, especially the integrin family and other adhesion molecules (e.g., CAS^{p130}), has also recently been documented (61, 62), suggesting novel signaling axes (e.g., integrin/FAK/SRC kinase) that may be targeted to circumvent endocrine resistance. In addition, as a result of recent pharmacogenomic and high-throughput studies, the list of additional host genome–associated factors governing endocrine sensitivity is growing.

Tumor-Associated Mechanisms of Resistance

As suggested above, however, most pathways potentially involved in endocrine resistance stem from the tumor cells themselves. These pathways fall broadly into three conceptual categories with overlapping components and mechanisms.

ER and ER coregulators. The first category consists of the ER itself, its coregulators, and additional factors that deregulate ER activity and modulate the receptor functions in response to endocrine therapy. As mentioned, loss of ER expression (i.e., the ERα isoform) in refractory endocrine tumors, though uncommon, results in an endocrine-insensitive phenotype (41, 42). Importantly, however, therapies inhibiting growth factor receptor pathways known to downregulate ER can restore ER expression and endocrine sensitivity in both preclinical and clinical settings (25, 49, 51). The expression of ER splicing variants, specifically the newly identified short variant ERα36 (63) and estrogen-related receptors, has also been implicated in reducing endocrine response. In addition, evidence indicates that negative (corepressors) and positive (coactivators) ER coregulators, which directly influence the balance of agonistic versus antagonistic activities of SERMs such as tamoxifen and the ligand-independent activity of the ER, are critical in determining endocrine sensitivity and resistance (16 and references therein). Overexpression of the ER coactivator AIB1 (also known as SRC3 or NCoA3) is associated with clinical and experimental tamoxifen resistance (12, 14), and downregulation of the corepressor NCoR was documented in tamoxifen-refractory experimental tumors (64). ER and its coregulators are also intimately regulated by posttranslational modifications. Growth factor receptors [e.g., EGFR/HER2, IGF1-R, and FGFR (fibroblast growth factor receptor)] and additional cellular and stress-related kinases [e.g., AKT, p42/44, JNK, and p38 MAPKs, PKA (protein kinase A), PAK1 (p21-activated kinase), IKK (IκB kinase), SRC, and CDK7 (cyclin-dependent kinase)] regulate multiple posttranslational modifications (16, 57, 65 and references therein). Phosphorylation, methylation, ubiquitination, and additional posttranslational modifications of ER and its coregulators have all been shown to influence ER activity and sensitivity to various endocrine therapies (57). ER can also reside outside the nucleus, engaging with cytoplasmic and membrane signaling complexes, and can activate and regulate various growth factor receptors and other cellular signaling pathways as a result (13, 14, 31, 32). Intriguingly, hyperactivation of these signaling pathways increases nonnuclear ER localization and its nongenomic activity, thus creating a positive feedback loop of crossactivation between the ER and growth factor receptor pathways. Importantly, this nonnuclear ER activity can be activated by both estrogen and tamoxifen, thus contributing to resistance (13, 14, 32). Other

endocrine therapies, however, such as the more potent ER degrader fulvestrant or strategies of estrogen deprivation, fail to trigger this nongenomic ER activity. Last, increased levels of transcription factors, such as NFkB and AP-1, that tether ER to specific gene promoters, have also been associated with endocrine resistance (66–68).

Cell cycle signaling molecules. The second category of endocrine resistance–related pathways includes molecules involved in the cellular and biological responses to endocrine therapy such as inhibition of cell proliferation and induction of apoptosis. Most of the evidence for the role of these pathways stems from preclinical studies. Both upregulation of positive regulators of the cell cycle, especially those controlling G1 phase progression, and downregulation of negative regulators of the cell cycle have been documented to interrupt and block the antiproliferative effects of endocrine therapy, leading to resistance (57, 69). For example, overexpression of the positive regulators MYC and cyclins E1 and D1 results in endocrine resistance, either by activating cyclin-dependent kinases critical for G1 phase or by relieving the inhibitory effects of the negative cell cycle regulators p21 and p27 (69, 70). Likewise, reduced expression, stability, or activity of p21 and p27 (71, 72), as well as inactivation of the RB (retinoblastoma) tumor suppressor, are also associated with poor response to endocrine therapy, especially tamoxifen. Of note, multiple growth factor receptors and their downstream signaling pathways, by modulating specific transcription factors or microRNAs, or by protein phosphorylation, downregulate expression or activity of these negative cell cycle regulators. Overexpression of HER2 and hyperactivation of AKT and SRC kinase are prominent examples of such pathways. Consistent with the cytotoxic effect of endocrine therapy, upregulation of cell survival signaling and antiapoptotic molecules, such as BCL-X_L, and decreases in expression of proapoptotic molecules, such as BIK (BCL2-interacting killer) and caspase 9, can also lead to endocrine resistance (73). As before, activation of growth factor receptor signaling via the PI3K/AKT pathway is an important modulator of these apoptotic/survival molecules, but additional molecules such as NFκB (nuclear factor κB) have also been implicated (66). Finally, whether autophagy, recently shown to mediate cell survival, plays a more general role in endocrine resistance is yet to be determined (74).

Growth factor receptor pathways. The third category of pathways involved in endocrine resistance comprises those that can provide alternative proliferation and survival stimuli to the tumors in the presence of effective inhibition of the ER pathway. Importantly, these pathways—such as growth factor and other cellular kinase pathways—can also circumvent the inhibitory effects of endocrine therapy via bidirectional crosstalk and modulation of the ER. However, many of these pathways can, either initially or eventually during the course of treatment, emerge to act as ER-independent drivers of tumor growth and survival, thus conferring resistance to all types of endocrine therapy. Pathways such as the HER tyrosine kinase receptor family and receptors for insulin/IGF1, fibroblast growth factor (FGF), and vascular endothelial growth factor (VEGF), as well as cellular Src, AKT, and stress-related kinases, have been implicated (75–78). These pathways can be activated by amplification and/or overexpression of the receptors or their cognate ligands. Pathway activation can also be achieved by deregulation of downstream signaling moieties, such as activating mutations in the PI3K catalytic subunit or loss of expression of the PTEN tumor suppressor of this pathway (79). The androgen receptor and potentially additional nuclear receptors have also been implicated as alternative growth stimulators that can bypass ER inhibition and lead to resistance (80). Importantly, although these pathways and mechanisms are varied and span a wide range of signaling cascades and gene networks, EGFR and HER2 have been recognized as important contributors to endocrine resistance. As a result, many clinical strategies have focused on cotargeting

this pathway together with ER to circumvent endocrine resistance and improve patient outcome.

CLINICAL TRIALS DESIGNED TO OVERCOME ENDOCRINE RESISTANCE

Many clinical trials have begun to test the idea that growth factor receptor signaling contributes to de novo or acquired endocrine resistance (75, 81, 82). Some of these trials were short-term neoadjuvant trials, some phase II, some randomized phase II, and some phase III in patients with metastatic disease. Some focused on HER2-positive patients whereas others included all ER-positive patients, regardless of HER2 status.

The TAnDEM trial randomized patients with HER2-positive tumors to anastrozole alone or anastrozole plus trastuzumab (83). The results clearly showed an advantage for the combination, although both arms did poorly, exemplifying the difficulty of controlling ER-positive HER2-positive disease. Despite targeting both major pathways, remissions are few and brief owing to the rapid development of more dominant survival pathways in metastatic disease.

Because preclinical studies suggest a major role for the EGFR (HER1) in acquired endocrine resistance, many trials incorporated gefitinib into the endocrine therapy regimen with tamoxifen or an aromatase inhibitor (23). Some of these studies have not yet been published but have been presented at meetings and published as abstracts. Two randomized phase II trials of somewhat similar design randomized patients with ER-positive metastatic breast cancer to either tamoxifen +/− gefitinib or anastrozole +/− gefitinib (84, 85). One of these trials was terminated early because of slow patient accrual, but it showed a numerical advantage in clinical benefit rate and progression-free survival with the addition of gefitinib to anastrozole (85). The other larger trial with 290 patients also showed a numerical advantage in favor of gefitinib added to tamoxifen (84). Both trials reported that the advantage was confined to previously untreated patients and suggested that an advantage was seen even in patients whose tumors were initially negative for HER2 overexpression (84, 85). These studies concluded that the strategy of combining an EGFR inhibitor with an endocrine agent was of sufficient interest to warrant further study and that studies designed to select the appropriate patients for combined treatment were paramount. A much smaller trial in more heavily treated patients failed to confirm these data and showed a high rate of patient withdrawal from the study (30%) owing to side effects of gefitinib (86). Finally, another trial comparing anastrozole plus gefitinib with fulvestrant plus gefitinib showed that the regimens were tolerable and suggested a slight advantage for the anastrozole combination (87).

The largest trial by far (1,286 patients) compared letrozole with and without lapatinib in patients with ER-positive metastatic breast cancer (88). The addition of lapatinib conferred a significant advantage in progression-free survival and response rate in the HER2-positive subset. In the HER2-negative subset, there was no overall significant benefit from lapatinib, but a preplanned Cox regression analysis showed a 23% reduction in the risk of progression with the addition of lapatinib. The benefit was seen in those who had discontinued tamoxifen therapy within six months of entering the study. A more recent analysis showed that patients with tumors exhibiting lower ER levels received the most benefit (89). This result, which will require confirmation from other trials, is consistent with data suggesting that growth factor signaling downregulates ER expression (3, 25, 29). Perhaps those tumors with lower ER expression are also those that rely on the HER pathway when ER is blocked by endocrine therapy. These tumors would be expected to respond well to a HER inhibitor.

Two randomized neoadjuvant studies have compared anastrozole and gefitinib (90, 91). One small study in patients selected for higher expression of EGFR evaluated gefitinib alone versus gefitinib plus anastrozole (91). Both

treatment regimens reduced phosphorylation of EGFR, Ki67 index, and tumor size, suggesting that gefitinib is effective in tumors selected by EGFR expression. The other study compared anastrozole alone to anastrozole plus gefitinib in patients selected only by ER status (90). If EGFR expression is important for the response to gefitinib, and if expression rises over time in patients treated with endocrine therapy, then the neoadjuvant setting, where EGFR levels would be low, may not be optimal to investigate this new strategy. Like the preclinical studies that led to testing this strategy in patients (23), gefitinib would be expected to delay the onset of acquired resistance but exert a minimal impact on initial response. In fact, this neoadjuvant trial showed no benefit from gefitinib, and even showed a trend for a reduced antitumor effect on Ki67 and tumor response with the combination (90).

Other trials in metastatic disease evaluated inhibitors of signaling molecules downstream from HER receptors. Two trials of mTOR inhibitors have been reported in combination with endocrine therapy (92, 93). A randomized phase II study compared letrozole alone to letrozole plus temsirolimus in hormone receptor–positive metastatic breast cancer and suggested a benefit. The trial was expanded to a phase III trial that was terminated early owing to lack of efficacy of the combination (92). The phase III portion of the study used a lower dose than the phase II portion because of toxicity with the higher dose. A randomized phase II trial of letrozole with or without everolimus, using an optimal dose of the inhibitor in the neoadjuvant setting, showed a statistically significant increase in response with the combination (93).

CONCLUSIONS

ER-targeted therapy has improved the quality of life and survival of millions of women with breast cancer around the world in the past three decades, but resistance to therapy continues to be a major problem. The ER signaling pathway is a complex network with many levels of control including extensive crosstalk with growth factor signaling pathways, thus offering several possible mechanisms of resistance. Clinical trials in patients suggest that HER2-overexpressing, ER-positive breast cancers should be treated with a combination of ER-targeted and HER-targeted therapies. Early results from clinical trials also suggest that subsets of patients with ER-positive, HER2-negative breast cancers may benefit from a combination of a growth factor pathway inhibitor with ER-targeted therapy such as tamoxifen or an aromatase inhibitor. Further studies are needed to confirm and expand these observations and to identify a priori those patients most likely to benefit from this approach. Finally, ongoing and planned additional studies combining ER-targeted and growth factor pathway–targeted therapy will determine whether this strategy is of value.

It is likely that there are many causes of resistance to endocrine therapy once a tumor becomes independent of estrogen. One factor limiting our understanding of these varied mechanisms is the lack of tumor tissue for detailed studies before treatment and after resistance has developed. This type of study will be crucial if we are to learn which escape pathways become activated in endocrine resistance and which targeted therapies can prevent or overcome this type of tumor progression.

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