Molecular Pathways

Adaptive Changes Result in Activation of Alternate Signaling Pathways and Acquisition of Resistance to Aromatase Inhibitors

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Abstract

Hormone therapy is an effective approach for the treatment of breast cancer. Although the antiestrogen tamoxifen has had a major impact on the treatment of the disease, aromatase inhibitors (AIs), which reduce estrogen synthesis, have recently proved to be more effective. These agents are now used as first-line therapy for postmenopausal breast cancer. Nevertheless, despite the efficacy of these agents, resistance to treatment eventually may occur in some patients. In an effort to overcome this resistance and extend the benefits of AIs, investigators have studied the mechanisms involved in resistance to AIs. Adaptive changes that result in activation of alternate signaling pathways in AI-resistant tumors have been identified in xenograft and cell line models. Expression of estrogen receptor α and aromatase was shown to be decreased in tumors after long-term treatment with AIs. In contrast, increased expression was observed in tyrosine kinase receptors such as Her-2 and insulin–like growth factor receptor, as well as in downstream signaling proteins such as mitogen–activated protein kinase. Functional activation of the mitogen–activated protein kinase pathway and dependency on growth factor receptor signaling have been observed in AI-resistant cells and tumors.

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Background

Estrogen is the major stimulus for breast cancer progression in both pre- and postmenopausal patients. The actions of estrogen on tumors are mediated by estrogen receptor alpha (ERα). Normally, in the absence of ligand, ERα resides in a large molecular complex with multiple heat shock proteins acting as chaperone proteins. The most potent ligand for ERα is 17β estradiol (E2), and the weaker estrone (E1) and estriol (E3) activate ERα by similar mechanisms. Estrogens, owing to their fat-soluble nature, rapidly diffuse through the plasma membranes and bind to ERα at the hormone-binding domain, as depicted in Fig. 1. This binding of estrogen to ERα results in the dissociation of heat shock proteins, which in turn leads to conformational change and dimerization of the receptor. This allows phosphorylation of ERα at several serine residues within its N-terminal portion. The receptor dimer then translocates to the nucleus and binds to the estrogen response element located upstream of the proximal TATA box. The DNA-bound dimer recruits coactivators such as steroid receptor coactivator-1 (SRC-1), amplified in breast cancer–1 (AIB-1), and cAMP response element binding (CREB) protein (CBP), which allow the activation of estrogen-responsive genes such as progesterone receptor (PGR), p82, cyclin D1, and c-myc (Fig. 1). These events lead to cell cycle entry and progression following expression of cell cycle regulating genes. In addition, several genes associated with cell survival such as Bcl-2 and tumor growth factor alpha (TGFRα) are also up-regulated in estradiol-treated cells in vitro and ERα-positive tissues in vivo, with increased estrogen-induced cell survival contributing significantly to breast cancer growth in response to estrogens.

In young women, the main source of estrogen is the ovary. After menopause, ovarian production declines and extragonadal sites (e.g., adipose tissue), which are not under the control of the pituitary, become the main source of circulating estrogen. However, tissue concentrations within the breast are comparable to those of premenopausal women as a result of local estrogen synthesis and uptake. In breast cancer patients, tumor ERα concentrations are higher after menopause, resulting in cancers that are sensitive to even low levels of estrogens. About two thirds of the breast cancer patients are postmenopausal women with ER-positive (ER+) tumors. Because estrogen signaling is of primary importance in the proliferation and progression of breast cancer, investigators have developed two types of breast cancer treatment to target this signaling pathway. The antiestrogens (AEs), such as tamoxifen, target ERα, whereas the more recently developed aromatase inhibitors (AIs) target the biosynthesis of estrogen by directly
interacting with the enzyme aromatase. Synthesis of estrogen is the last step in the steroid biosynthesis pathway, and therefore inhibition of aromatase does not affect the production of any other steroids.

Both AEs and AIs are effective treatments that are well tolerated in comparison with cytotoxic chemotherapy. AIs that reduce estrogen production have now been shown to be an effective treatment for breast cancer. Triazole compounds such as letrozole and anastrozole, and steroidal exemestane have been found to be superior to the AE tamoxifen and have few side effects (1–5). Furthermore, the benefits of hormone therapy are long-lasting. Patients generally receive hormone therapy for 5 years; however, some patients may eventually relapse during treatment. Various investigators have studied the mechanisms involved in the development of resistance to AIs to gain a clearer understanding of how tumors adapt and survive the pressure of suppressive treatment, with the ultimate goal of devising treatment strategies to overcome this resistance. Results obtained to date indicate that over time, tumors adapt to a low-estrogen environment by switching to alternate signaling pathways that allow them to escape the antiproliferative effects of AIs.

**Role of ER in acquired resistance to AIs**

Although there is no change in the ability of AIs to inhibit aromatase, ample evidence suggests that ER-mediated pathways play a role in the growth of breast cancer cells and tumors despite showing resistance to endocrine agents such as tamoxifen or AIs. Some tumors respond to the use of another agent (e.g., steroidal AI after nonsteroidal AI), and tamoxifen-resistant tumors may respond to AIs.

Model systems such as LTED, UMB-1Ca, and LTLC exhibit up-regulation of ER upon acquisition of resistance (6–8), whereas the long-term letrozole-treated (LTLT-Ca) model shows that ERα levels are down-regulated and the Her-2/mitogen–activated protein kinase (MAPK) pathway is up-regulated (9–11). When LTLT-Ca cells were treated with MAPK inhibitor (PD98059), ER expression was increased to levels in MCF-7Ca cells. This suggests that hormone sensitivity may be restored by inhibition of the MAPK pathway (12). Similar results were also obtained with the anti-Her-2 agent trastuzumab (11). Other studies showed that hyperactivation of MAPK results in loss of ERα, and abrogation of MAPK activity reverses ERα down-regulation (13–16). In addition, it has been shown that upon cessation of letrozole treatment, ER protein levels increase and response to letrozole is restored (17, 18).

More-recent clinical studies confirmed changes in Her-2 and ERα status in secondary tumors and metastatic lesions compared with primary tumors (19, 20).

Other mechanisms of resistance centering on ER signaling pathways include ERα mutation (21) and truncated ERE variant (ERez36) (22). Moreover, up-regulation of ER-related transcription factors such as activator protein 1 (AP1) (23) and NF-xB (24–26), as well as coactivators of ER such as AIB1 (27), has been reported to confer resistance to endocrine therapy (25, 26). Nonetheless,
these results were described as resistant mechanisms against tamoxifen, and the role of these mechanisms in AI resistance remains unclear.

**Role of growth factor receptor–mediated pathways in resistance**

The Her-2 receptor is the protein product of the Her-2 proto-oncogene and a member of the epidermal growth factor receptor (EGFR, Her-1) family of transmembrane receptor tyrosine kinases, which also includes Her-3 and Her-4. Her-2 is known as an orphan receptor because a ligand for Her-2 has not been identified. Amplification of Her-2 has been implicated as an important event in the genesis of human cancer. Extensive clinical studies (28) have demonstrated that expression of high levels of Her-2 in patients with node-positive breast cancer correlates with a poor prognosis. Upon binding of ligand to the EGFR, Her-3 (erbB3), Her-4 (erbB4), or Her-2 is recruited as the preferred partner of these ligand-bound receptors into an active, phosphorylated heterodimeric complex that activates several signaling pathways involved in the proliferation and enhanced survival of tumor cells. This results from the induction of receptor autophosphorylation in several COOH-terminal tyrosines and the recruitment of signal transducers and adaptor molecules that promote cellular proliferation, differentiation, motility, adhesion, protection from apoptosis, and cellular transformation. In addition, overexpression of Her-2 on the cell surface results in homodimerization and activation. Amplification and overexpression of Her-2 is observed in 20–30% of human breast cancer patients and is inversely correlated with survival. Therapeutic monoclonal antibodies that target extracellular domains of Her-2 [e.g., trastuzumab (Herceptin)] has been approved for adjuvant treatment with chemotherapy for breast cancer patients with Her-2–positive disease.

**Letrozole resistance model.** Studies done by Long et al. (29, 30) involved the use of the xenograft mouse model, which was previously developed to study the antitumor effects of sequencing and combining AIs and AEs in post-menopausal breast cancer. In this model, human ER-positive breast cancer cells (MCF-7Ca) were inoculated into ovariectomized mice and grown as tumors under the influence of estradiol produced by aromatization of androstenedione (10). The mice were then treated with AI letrozole for an extended period of time (56 weeks) until tumor growth was no longer inhibited by the treatment and tumors were actively growing (31). Cells were then isolated from these resistant tumors and designated LTTLT-Ca cells (32–35). The LTTLT-Ca cells formed tumors in immunosuppressed, ovariectomized mice without estrogen stimulation and were unresponsive to AI treatment. Western blot analysis of tumors collected during the course of letrozole treatment revealed that ERα expression was increased in tumors that responded to letrozole, but was progressively reduced as the tumors became resistant. After 56 weeks of letrozole treatment, ERα expression decreased and Her-2 expression increased. In addition, levels of downstream proteins of Her-2 signaling, including the MAPK pathway, were all significantly increased (36–39).

Similar decreases in ERα and increases in Her-2 were also seen in cells isolated from the resistant tumors. In addition, most of the proteins in the downstream signaling pathway were increased in the resistant tumors. When LTTLT-Ca cells were incubated with a series of doses of MEK-1/2 inhibitor (UO126) and MAPK inhibitor (PD98059), a dose-dependent inhibition of proliferation of LTTLT-Ca cells was observed, whereas there was no effect on the proliferation of parental MCF-7Ca cells. These results indicate that the LTTLT-Ca cells were dependent on the MAPK pathway and no longer dependent on estrogen stimulation for proliferation. When LTTLT-Ca cells were treated with MAPK inhibitor (PD98059), ER expression increased to levels found in the MCF-7Ca cells. This suggests that inhibition of the MAPK pathway may restore hormone sensitivity. In addition, this finding also emphasizes the involvement of ERα in the growth of endocrine-resistant cells and tumors.

Treatment with trastuzumab caused a marked dose-response inhibition of proliferation of LTTLT-Ca cells accompanied by inhibition of pH2-2 and p-MAPK expression, whereas ERα expression was increased. Further evidence for the restoration of ER was obtained when LTTLT-Ca cells were pretreated with trastuzumab for 72 hours followed by 1 hour of E2 treatment. ERα transcriptional activation was induced in the LTTLT-Ca cells to the same extent as in parental MCF-7Ca stimulated with E2 only. This was also evident from the increase in recruitment of ERα to the pS2 promoter, and confirmed that Her-2 regulation of ERα mediated transcription of downstream genes, such as pS2. These finding indicates that Her-2 is a negative regulator of ERα. In addition, treatment of LTTLT-Ca cells with trastuzumab and E2 resulted in recruitment of ERα to the aromatase I.3/II promoter and led to up-regulation of aromatase, which is essential for tumor response to AIs. Similar results were also obtained in SKBr3 cells, suggesting that these results are not unique to MCF-7Ca cells (16, 40–42). Thus, interaction between ERα and Her-2, and Her-2 regulation of ER are not limited to MCF-7 cells and may be relevant for treating AI-resistant breast cancer patients.

**Cross-talk between the pathways**

Various studies have demonstrated that other signaling pathways, such as MAPK and PI3K/Akt pathways, can engage in cross-talk and activate ERα signaling pathways in a ligand-independent manner. MAPK has been shown to phosphorylate ERα directly or indirectly via Elk-1 and p90RSK and result in the transcription of genes involved in growth regulation and tumor progression. As depicted in Fig. 1, both MAPK and Akt can directly phosphorylate ERα within the hormone-independent activation function (AF-1) domain at Ser-118 and Ser-167, respectively (43, 44). In addition to phosphorylating ERα itself, these two pathways can also stimulate ER signaling pathway by phosphorylating an ER coactivator such as AIB1.

Several reports (45) have suggested that translocation of ERα to the membrane may be responsible for the cross-talk
with EGFR family members in endocrine-resistant phenotypes (46–49), whereas others have indicated that EGFR family transmembrane receptors, such as Her-2, can translocate to the nucleus and act as transcription factors (50–52).

A strong inverse correlation has been observed between the ERα and Her-2 proteins and pathways activated by these proteins (53). Stable transfection of hormone-dependent cells with members of a growth factor signaling pathway, such as Her-2, EGFR, or Raf, was reported to lead to loss of ERα and the estrogen refractory phenotype (54). Of interest, resistance to trastuzumab is associated with up-regulation of genes such as VEGF and TGFα, which are also known as ERα target genes (55). Fig. 1 shows how these pathways may interact.

Clinical-Translational Advances

Emerging clinical data support the notions that up-regulation of the Her-2/MAPK pathway corresponds to AI resistance, and that dual targeting of the ER signaling pathway with AIs and other GFR signaling cascades may confer a better clinical outcome. Lipton et al. (53) demonstrated that approximately 26% of patients treated with letrozole converted from serum Her-2 negative to positive at the time of disease progression. Serum Her-2, the extracellular domain of Her-2 protein (which is shed into serum), has been shown to correlate with the overexpression of Her-2 protein in tumor cells and can be used to predict response to trastuzumab. The dynamic interaction between ER and the Her-2/MAPK signaling pathway was illustrated in a small clinical study of 10 patients with ER-negative/HER-2–positive advanced breast cancer treated with trastuzumab and chemotherapy. Intriguingly, the tumors of 3 patients in this trial converted from ER-negative to ER-positive after treatment with trastuzumab, and subsequently responded to letrozole (56–58). For dual inhibition with AIs and Her-2 inhibitors, three clinical trials have demonstrated that a better clinical outcome can be obtained by combining Her-2–targeted therapy with AIs. A phase II trial of letrozole and trastuzumab in ER-positive/Her-2–negative metastatic breast cancer patients demonstrated that the combination was well tolerated, with a clinical benefit rate of 50% (56).

In a randomized phase III trial (the TAnDEM trial), addition of trastuzumab to anastrozole showed a significant improvement in PFS from 3.0 months to 8.2 months (HR = 0.71; P = 0.019) upon addition of lapatinib to letrozole in patients with Her-2–positive breast cancer. No significant improvement in PFS was noted in patients with Her-2–negative tumors. Of interest, the preplanned Cox regression analysis of patients with Her-2–negative tumors who relapsed \(<6\) mo after tamoxifen discontinuation demonstrated a nonsignificant trend toward improvement in PFS for the combination (HR = 0.78; P = 0.117) (60). This result highlights the significance of dual inhibition of the Her-2 and ER signaling pathways in tumors that have been resistant to endocrine therapy, even though the initial tumors may lack Her-2 overexpression. Furthermore, given that no benefit was obtained by combining letrozole and lapatinib up front in ER-positive and Her-2–negative tumors, this implies that coblockage of Her-2 and estrogen deprivation from the beginning may not delay the emergence of resistance. In addition, cotargeting of the downstream signaling of the PI3K/Akt pathway with mTOR inhibitors has been examined in multiple clinical trials. A recent randomized phase II study of letrozole ± everolimus for 4 months in the preoperative setting demonstrated a significant increase in tumor shrinkage as determined by ultrasound (58% vs. 47%; P = 0.03) and a greater reduction in the cell proliferation index (Ki67, 57% vs. 30%) (61).

These results suggest that inhibition of both the estrogen pathway and growth factor signaling may be an effective strategy to overcome resistance of breast cancer to AI therapy. Several trials are currently being conducted on the basis of these preclinical findings. In addition to Her-2 with trastuzumab and lapatinib, multiple novel agents that can be used to target the MAPK and PI3K/Akt signaling pathways are under clinical development. These agents include MEK inhibitors, Raf inhibitors, PI3K inhibitors, mTOR inhibitors, and Akt inhibitors.

Conclusion

The above data suggest that an inverse and compensatory relationship exists between GFRs and ERα, and that inhibition of one pathway leads to activation of the other. Strategies to block both estrogen signaling and GFR signaling may enhance breast cancer treatment by reversing resistance, restoring sensitivity to AI therapy, and delaying the need for more-toxic chemotherapy. However, most of the results obtained to date suggest that careful preselection of patients based on specific biomarkers is necessary for the success of these newer strategies.

Delaying the use of chemotherapy by the sequential use of endocrine therapies, which are relatively well tolerated, offers significant quality-of-life advantages for breast cancer patients, especially elderly patients and those with advanced disease.

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