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Resistance to endocrine therapy in breast cancer

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Abstract Endocrine therapy is the treatment of choice for patients with breast cancer expressing estrogen receptor (ER) and/or progesterone receptor. The efficacy of endocrine therapy is well established in the prevention, adjuvant and metastatic settings. However, either de novo or acquired resistance is frequently observed. Much effort has been made to elucidate the mechanisms of action underlying resistance to endocrine therapy in breast cancer, and several possible explanations have been suggested. Our previous studies have indicated that combined treatment with an antiestrogen, fulvestrant, and an inhibitor of the HER2 signaling pathway, trastuzumab, or an inhibitor of the HER1 signaling pathway, gefitinib, leads to an additive antitumor effect in breast cancer cells expressing ER and HER2 or HER1, respectively. It has also been suggested that the HER1 or HER2 signaling pathway is upregulated during the development of antiestrogen-resistant growth in breast cancer cells. These findings suggest that signal transduction inhibitors are effective for the treatment of antiestrogen-resistant breast cancer. A hypoxic microenvironment has been shown to promote malignant progression in cancer cells. Our previous study and others have suggested that hypoxia posttranscriptionally reduces ER expression and decreases sensitivity to hormonal agents in breast cancer cells. Our preliminary study has also shown that a hypoxic cytotoxin, tirapazamine, increases ER expression in breast cancer xenografts. Differential antitumor activity of tirapazamine on tumor cells under normoxic or hypoxic conditions may cause this phenomenon. These findings suggest that hypoxic cytotoxins may retard the development of endocrine resistance induced by hypoxia. Molecular mechanisms responsible for endocrine resistance in breast cancer are reviewed and possible therapeutic strategies against this resistance are discussed.

Keywords Resistance · Endocrine therapy · Signal transduction inhibitor · Hypoxia · Hypoxic cytotoxin · Breast cancer

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Introduction

Breast cancer, like prostate cancer and endometrial cancer, is classified as one of the hormone-dependent tumors. Estrogen plays important roles in the development and progression of breast cancer. Endocrine therapy, such as estrogen ablation [ovarian ablation or luteinizing hormone-releasing hormone (LH-RH) agonists for premenopausal patients; aromatase inhibitors for postmenopausal patients], antiestrogen therapy or progestin therapy, is the treatment of choice for patients with breast cancer expressing estrogen receptor (ER) and/or progesterone receptor (PgR). The clinical usefulness of endocrine therapy has been proven in the prevention, adjuvant and metastatic settings.

Approximately half of metastatic breast cancer expressing ER and/or PgR responds to endocrine therapy, and postoperative adjuvant endocrine therapy provides approximately a 50% reduction in the development of recurrent disease. These findings suggest that half of breast cancers expressing hormone receptors (HR) are de novo resistant to endocrine therapy. Furthermore, metastatic breast cancer, which initially responds to endocrine therapy, always acquires endocrine resistance (acquired resistance). Both de novo and acquired resistance to endocrine therapy are important problems in the management of breast cancer patients.

A tremendous effort has been made to elucidate the mechanisms of action underlying endocrine resistance in

breast cancer over the past two decades. Several possible mechanisms responsible for endocrine resistance have been reported. It should be noted that most of these explanations originated from studies on antiestrogen resistance. The reason for this is because antiestrogens have been most frequently prescribed and antiestrogen resistance has been most frequently observed in clinics. A proportion of antiestrogen-resistant breast cancer subsequently responds to aromatase inhibitors or progestin. However, such breast cancers ultimately acquire resistance to second- and third-line endocrine therapies. These findings suggest that there are multiple steps and complicated mechanisms responsible for endocrine resistance. Novel strategies modifying these steps may be able to retard the development of endocrine resistance and/or overcome endocrine resistance.

Molecular mechanisms responsible for endocrine resistance in breast cancer are reviewed and possible therapeutic strategies against this resistance are discussed.

Mechanisms of action underlying endocrine resistance

Several mechanisms of action have been suggested to be responsible for the development of resistance to endocrine therapy in breast cancer. According to mechanistic points of view, possible mechanisms of action are divided into three categories: (1) reduction in or loss of ER expression; (2) dysfunction of ER signaling; and (3) ligand-independent activation of ER.

Reduction in or loss of ER expression

Endocrine therapy is not suitable for patients with breast cancer expressing no HR, because there is little chance of such breast cancers responding to endocrine therapy. If HR-positive breast cancer loses HR expression during endocrine therapy, such breast cancers become resistant to endocrine therapy. Three possible mechanisms responsible for reduction in ER expression have been reported.

Clonal selection

It is believed that breast cancer consists of estrogen-sensitive and -insensitive clones developing in a mosaic fashion. Endocrine therapy selectively kills HR-positive and estrogen-sensitive clones, while HR-negative and estrogen-insensitive clones grow predominantly. This leads to resistance to endocrine therapy. This mechanism of action appears to be reasonable and a large number of Japanese breast cancer experts believe in this mechanism [60].

Reduction in ER production

Hypermethylation of the promoter region of the ER gene causes transcriptional downregulation and decreases the production of ER protein [40]. Another transcriptional downregulation is caused by a decrease in the expression of transacting factors for ER, such as ER promoter B-associated factor 1 [69]. However, it is not yet known whether these changes directly cause the loss of ER or a loss of ER caused by other mechanisms subsequently induces these changes.

Degradation of ER under hypoxia

An inverse relation between the expression levels of ER and hypoxia-inducible molecules such as hypoxia-inducible factor (HIF)-1α and carbonic anhydrase IX has been reported in breast cancer tissues [9, 33, 35]. In addition, our previous study and others have suggested that hypoxic conditions lead to posttranscriptional downregulation of ER-α and its function [35]. Subsequently, it was revealed that the reduction in ER expression by a hypoxic environment is mediated by HIF-1α-driven proteasome-dependent degradation of ER [63]. These findings suggest that hypoxic microenvironments, which are frequently observed in breast tumors, cause a decrease in ER expression and function. It has been suggested that hypoxia promotes malignant progression of many types of human cancers [24]. Therefore, it might be possible that hypoxic microenvironments, which are caused by an increase in tumor size or induced by hormonal agents, provide selective pressure for the outgrowth of ER-poor and more aggressive clones, and finally cause a loss of ER expression in breast tumors.

Although loss of ER in ER-positive tumors is likely to be a main cause for the development of endocrine resistance, several studies have demonstrated that most antiestrogen-resistant breast cancers retain ER expression [30, 46]. These findings indicate that reduction in or loss of ER is not a main cause for the development of endocrine resistance.

Dysfunction of ER signaling

Recent studies have shown that ER-interacting proteins, called co-factors and include co-activators and co-repressors, have important roles in mediating transcriptional activation of target genes by the ER [55]. These findings suggest that quantitative or qualitative changes in ER-related co-factors could contribute to the development of endocrine-resistant breast cancer. However, no definitive relation has been elucidated between alterations of co-activators or co-repressors and endocrine resistance in breast tumors. There are,

however, some preclinical and clinical findings to support these mechanisms.

Quantitative changes in co-factors

Expression of steroid receptor co-activator (SRC)-1 was reported to increase the agonist activity of tamoxifen in experimental cell systems [59]. Additionally, two recent reports have suggested that SRC-1 expression was negatively associated with disease-free survival and positively correlated with human epidermal growth factor receptor (HER) 2 expression in a cohort of breast cancer patients [17, 45]. Similarly, a series of studies has revealed that tamoxifen behaves as an estrogen agonist in breast cancer cells that express high levels of SRC-3 [amplified in breast cancer (AIB)-1] and HER2, and patients whose tumors expressed high levels of both AIB1 and HER2 had worse outcomes with tamoxifen therapy [50, 57].

It was reported that expression of one of the co-repressors, nuclear co-repressor protein (NCOR) 1, was decreased in tamoxifen-resistant tumors in a mouse model system [41]. In addition, low NCOR1 expression was recently reported to be associated with significantly shorter relapse-free survival in breast cancer patients who only received tamoxifen [23].

Qualitative changes in co-factors

A novel co-activator, L7/SPA, has been reported and the complex of this co-activator and tamoxifen was shown to lead to an increase in transcription activation in target genes [65]. Overexpression of this type of co-activator may contribute to endocrine resistance. However, no clinical study has clarified this hypothetical mechanism.

Interaction between ER-α and ER-β or ER-βcx

A recently discovered ER subtype, ER-β, and its isoforms, such as ER-βcx, may play an important role in the development of endocrine resistance. An early series of studies suggested that the mRNA expression levels of wild-type ER-β predict a poorer prognosis and tamoxifen resistance in breast cancer [61, 62]. In contrast, two recent independent studies have proposed that low protein expression levels of ER-β predict resistance to tamoxifen therapy in breast cancer patients [15, 25]. An experimental study suggested that ER-βcx reduces ER-α-mediated transactivation in a dominant-negative fashion [42]. In contrast, a recent study has theorized that protein expression levels of ER-βcx positively correlated with a favorable response to endocrine therapy [51]. The clinical significance of ER-β and its isoforms in the management of breast cancer patients remains controversial and is yet to be determined.

Ligand-independent activation of ER

Classically, transactivation of target genes by ER requires ligand binding to ER. Experimental studies have revealed that several growth factors and their intracellular signaling pathways can stimulate ER activity in the absence of ligand. In addition, it has been suggested that this ligand-independent ER activation is mainly caused by the phosphorylation of different sites in the ER protein [49]. These phenomena may relate to the development of endocrine-resistant breast cancer.

Overexpression of HER1 and/or HER2

It has been reported that HER1 and/or HER2 are overexpressed in 20–30% of primary breast cancers. Clinical studies have suggested that overexpression of HER1 and/or HER2 is related to tamoxifen resistance [26, 47]. Experimental studies have also demonstrated that the intracellular signaling pathway activated by HER1 or HER2, RAS/RAF/MEK/ERK1/2, phosphorylates the serine 118 residue located in the ER protein and leads to ligand-independent transactivation of target genes [6, 31]. Interestingly, it was reported that increased ERK1/2 activity correlates with endocrine resistance and shorter survival in patients with breast cancer [21]. Moreover, experimental studies have suggested that the blockade of these signaling pathways by signal transduction inhibitors may restore the antitumor effect of antiestrogens [54].

Upregulation of AKT activity

It has been proposed that the phosphatidylinositol-3-OH-kinase (PI3K)/AKT (protein kinase B) pathway plays important roles in cell survival and apoptosis [19]. It was reported that phosphorylation of the serine 167 residue located in the ER protein by AKT results in ligand-independent activation of ER [7]. In addition, it has been demonstrated by two different groups that increased AKT1 activation contributes to the aggressive phenotype of tamoxifen-resistant ER-positive breast cancers, and that the overexpression of AKT3 in breast cancer cells results in E2-independent tumor growth; further, the growth of these tumors is enhanced by tamoxifen [16, 64]. It has also been reported that blockage of the AKT signaling pathway by mammalian target of rapamycin (mTOR) inhibition effectively restores the susceptibility of breast cancer cells to tamoxifen [14]. Interestingly, it has been suggested that a negative regulator of AKT, a tumor suppressor PTEN, is frequently inactivated in breast cancer and that there is a strong association between downregulation of PTEN expression in ER-positive tumors and failure of tamoxifen treatment [56].

Upregulation of protein kinase A activity

Protein kinase A (PKA) induced by G-protein-coupled receptors and the 90-kDa ribosomal S6 kinase are also known to be involved in ER phosphorylation [8, 29]. It has recently been reported that phosphorylation of the serine 305 residue in the hinge region of ER-α by PKA induced resistance to tamoxifen, and PKA activity thus induced a switch from antagonistic to agonistic effects of tamoxifen on ER-α [43]. This report also indicated that downregulation of a negative regulator of PKA, PKA-RIα, was associated with tamoxifen resistance in clinical samples.

Mutations of the ER gene

A missense mutation in the tyrosine 537 residue in the ligand-binding region of ER has been detected in metastatic breast cancer tumors [66]. This mutant ER was reported to be equally active in the absence of ligand or in the presence of estradiol or tamoxifen. Substitution of this residue by different amino acids results in the ligand-independent activation of ER together with co-activator recruitment [67]. However, other studies have suggested that only 1% of primary breast cancers carry gene mutations of ER in the coding region, most of which did not result in alterations at the protein level [53]. Therefore, the clinical significance of ER gene mutations is questionable.

Spliced variants of ER mRNA

An alternatively spliced mRNA lacking specific exons, which encode truncated and potentially constitutively active forms of ER, has been discovered [13]. However, analysis of breast tumors failed to confirm a significant role of the variants in the development of endocrine resistance [4]. The pathological significance of the ER mRNA variants remains to be determined.

Strategies against endocrine-resistant breast cancer

There are many possible mechanisms of action underlying endocrine resistance in breast cancer. Some appear to be irreversible and impossible to overcome. However, others appear to be reversible and might be overcome by adequate strategies. Clinically testable strategies are discussed.

Inhibition of growth factor signaling pathway

It has been suggested that upregulation of growth factor-mediated signaling pathways causes ER phosphorylation, induces ligand-independent ER activation and leads to endocrine-independent and endocrine-resistant growth of breast cancer cells [49]. Recent clinical studies have also suggested that ER-positive breast cancer overexpressing HER1 and/or HER2 is likely to be resistant to tamoxifen [26, 47]. These findings suggest that inhibitors of growth factor signaling pathways may decrease ER phosphorylation and ligand-independent ER activation, and may re-induce estrogen-dependent growth in breast cancer.

Blockade of HER2 signaling pathway

The humanized anti-HER2 monoclonal antibody, trastuzumab, is the only agent that inhibits the growth factor-mediated signaling pathway and has been introduced into clinical use [58]. Our previous experimental study and others suggested that combined treatment with an antiestrogen and trastuzumab effectively inhibits the growth of breast cancer cells expressing both ER and HER2 [2, 34]. It has been reported that herstatin, a secreted HER2 gene product, which binds to the HER2 ectodomain and blocks HER2 activation, enhanced sensitivity to tamoxifen in breast cancer cells overexpressing HER2 [28]. Inhibition of the mitogen-activated protein kinase (MAPK) pathway driven by HER2 signaling may also be inhibited by MEK inhibitors and this may re-induce tamoxifen sensitivity in breast cancer [39]. Several clinical studies have been launched worldwide to test the clinical usefulness of combined treatment with trastuzumab and endocrine therapeutic agents [68]. However, no synergistic antitumor effect of these combinations has been reported in breast cancer patients.

Blockade of HER1 signaling pathway

A HER1-specific tyrosine kinase inhibitor, gefitinib, was recently introduced as a treatment for nonsmall cell lung cancer [10]. This agent effectively inhibits the signaling pathway mediated by HER1. It has been reported that approximately 20% of breast cancers overexpress HER1, and breast cancer overexpressing HER1 is more aggressive and resistant to endocrine therapy. It has been also suggested that ER-positive breast cancer overexpressing HER1 more rapidly acquires endocrine resistance [3, 38]. Moreover, experimental studies have suggested that gefitinib is not only effective in breast cancer cells overexpressing HER1 but also effective in breast cancer overexpressing HER2 [1]. These findings suggest that gefitinib is a promising agent for combined use with endocrine therapeutic agents. Our previous study and others have suggested that the combined use of gefitinib and an antiestrogen additively inhibited the growth of breast cancer cells expressing both ER and HER1, and that tamoxifen-resistant breast cancer cells associated with HER1 overexpression preferentially responded to this combination therapy [22, 48]. Although limited information is available on the efficacy of gefitinib in the treatment of breast cancer, one preliminary

study suggested that gefitinib was active in patients with tamoxifen-resistant breast cancer [52]. Further clinical studies are needed to clarify the antitumor activity of gefitinib in tamoxifen-resistant breast cancer.

Blockade of other intracellular signaling pathways

Activation of the PI3K/AKT pathway, which is not only driven by HER1/HER2 signaling but also by AKT overexpression or PTEN inactivation, may cause tamoxifen resistance in breast cancer. PKA activity is also suggested to induce a switch from antagonistic to agonistic effects of tamoxifen on ER. These findings suggest that selective blockade of the PI3K/AKT or PKA pathways may overcome tamoxifen resistance in breast cancer [14, 43].

Retardation of the development of acquired endocrine resistance

Even if it is difficult to overcome endocrine resistance, it may be possible to prolong the antitumor effect of endocrine therapy or retard the development of endocrine resistance. Two promising strategies are discussed.

Intermittent, alternating and combined endocrine therapies

Acquired endocrine resistance has been recognized as an adaptive response of breast cancer cells to endocrine therapy. Consequently, intermittent treatment with a single endocrine therapeutic agent, alternating use of two different agents, or combined treatment with two different agents may interfere with the adaptation process and retard the development of endocrine resistance.

Prostate-specific antigen is a sensitive and specific tumor marker. A new strategy in which intermittent treatment with a LH-RH agonist is given to patients with advanced prostate cancer has been tested in a clinical trial [70]. Unfortunately, there is no such sensitive and specific tumor marker in the management of breast cancer. However, approximately 70% of metastatic breast cancer patients have at least one elevated tumor marker, such as CA 15-3, CEA or NCC-ST-439 [37]. If a certain endocrine therapy is effective and an elevated tumor marker decreases during the treatment of metastatic breast cancer patients, endocrine therapy can be stopped until the tumor marker increases again. This intermittent treatment may prolong the efficacy of endocrine therapy.

Recent clinical studies have demonstrated that alternating treatment with tamoxifen and an aromatase inhibitor is more effective than tamoxifen alone in the adjuvant setting for postmenopausal patients with early breast cancer [5, 11]. It is possible that the sequential administration of an aromatase inhibitor effectively destroys breast cancer cells acquiring tamoxifen resistance. However, the mechanisms of action underlying the efficacy of alternating treatment of two different endocrine therapies remain to be elucidated.

Concurrent use of tamoxifen and a LH-RH agonist has been reported to be more effective than respective agents alone in premenopausal patients with metastatic breast cancer [32]. Recent clinical studies have also indicated that this concurrent use is as effective as chemotherapy, such as the cyclophosphamide, methotrexate, fluorouracil (CMF) regimen, in the adjuvant setting for premenopausal breast cancer patients [27]. It has recently been reported that concurrent use of a third-generation aromatase inhibitor and a LH-RH agonist provides a high rate of clinical benefit to premenopausal metastatic breast cancer patients [18]. Concurrent use of an aromatase inhibitor and a LH-RH agonist is also under investigation in the adjuvant setting for premenopausal breast cancer patients [44].

Targeting hypoxic tumor cells

Our previous study and others have suggested that an epigenetic change, such as a hypoxic microenvironment, may play an important role in the development of endocrine resistance in breast cancer [12, 35, 63]. If so, hypoxic cytotoxins, such as tirapazamine which selectively kills cancer cells under hypoxic conditions [20], may be useful for retarding the development of endocrine resistance. Our preliminary study suggested that the administration of hypoxic cytotoxins restored ER expression in human breast cancer xenografts transplanted into athymic nude mice [36]. It can be speculated that the hypoxic cytotoxin destroyed hypoxic breast cancer cells with a low level of ER, and that normoxic breast cancer cells with a high level of ER preferentially survived in the xenografts. If this is the case, normoxic tumor cells should be responsive to endocrine therapy. Further experimental studies are clearly needed to clarify this interesting phenomenon.

Conclusions

Although there are many possible mechanisms of action responsible for the development of endocrine resistance, a common cascade has not yet been discovered. In other words, there appears to be no common cascade. Multistep or complicated mechanisms may be involved in the development of endocrine resistance. Generally, endocrine therapy is less toxic and provides better quality of life to patients with breast cancer in comparison with cytotoxic chemotherapy. Therefore, clinical researchers have to make a continuous effort to develop new strategies for enhancing or prolonging the efficacy of endocrine therapy.

In consideration with clinical implications, strategies for retarding endocrine resistance, such as intermittent, alternating or combined endocrine therapy, are testable in clinical trials. In addition, concomitant or sequential administration of signal transduction inhibitors, such as trastuzumab or gefitinib, may not only prolong the efficacy of endocrine therapy but also overcome a part of endocrine resistance in the near future. Well-designed clinical trials supported by scientific rationale are essential to clarify the usefulness of novel strategies as described above.

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