Review Article

Headway in resistance to endocrine therapy in breast cancer

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ABSTRACT Resistance to endocrine therapy is the major problem for ERα(+) breast cancer patients. Research in endocrine resistance, mainly based on breast cancer cell lines and transplantation animal models, has indicated that phosphorylation of estrogen receptors, high expression of SRC and high activation of ErbB/MAPK pathway are the 3 main mechanisms for occurrence of endocrine resistance. Restoration of ER expression and exploration of inhibitors to various biological targets are the 2 promising ways to solve this problem. Further research is needed to deeply explore relevant mechanisms and resolvents so as to guide clinical practice.

Key Words: breast cancer; endocrine resistance; AEs/AIs; SRC; ErbB /MAPK

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Introduction

Endocrine therapy of breast cancer dated back to the end of 19th century when premenopausal breast cancer patients began to receive curative bilateral ovariectomy. Tamoxifen was used in pre- and post-menopausal breast cancer patients since the 1980s, and it was better-tolerated than bilateral ovariectomy. Several drugs (including TAM and AIs) targeting ER are available now for ERα(+) breast cancer patients in clinical settings, interfering internal environment of breast cancer cells so as to inhibit tumor growth, reduce recurrence rate and increase survival rate (1,2). Most ERα(+) breast cancer patients could receive quite good effects from endocrine therapy initially, however a certain tumors would acquire resistance to endocrine therapy later and recurrence and/or metastasis might occur (3-5). Resistance to endocrine therapy is the major problem for ERα(+) breast cancer patients, hence it's of great importance to explore the mechanism and countermeasures for dealing with resistance to endocrine therapy. This review summarizes relevant research in resistance to endocrine therapy and presents an overview in this field.

No potential conflict of interest.

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Search method

Search strategy

Electronic searches were performed by databases of PubMed from its inception to June 2010. To achieve the maximum sensitivity of the search strategy and identify all studies about breast cancer and endocrine therapy resistance, we used appropriate free text and thesaurus terms including "breast cancer", "breast carcinoma", "breast tumour", "mammary cancer", "endocrine therapy", "drug resistance", "drug tolerance" and all other relative information about breast. The MeSH table was searched by "breast neoplasms" [MeSH Terms] AND ("endocrine system"[MeSH Terms] AND "therapy" [Subheading] OR "therapeutics" [MeSH Terms]) AND resistance [All Fields]. The reference lists of all retrieved articles were reviewed for further identification of potentially relevant studies.

Study selection

All studies assessing breast cancer, endocrine therapy and drug resistance published were included. No restrictions were placed on abstracts and conference proceedings. We excluded studies that were not directly relevant to drug resistance to breast cancer endocrine therapy, such as resistance to chemotherapy combined with endocrine therapy. Review about drug resistance to breast cancer endocrine therapy was excluded because we were about to explore the original mechanisms and countermeasures to drug resistance to breast cancer endocrine therapy, actually there is no reviews concentrating on drug resistance to breast cancer endocrine therapy.

Incidence of resistance to endocrine therapy in breast cancer patients

Approximately 70% breast cancer patients are ERα(+) and could get benefit from endocrine therapy through interfering mitosis of tumor cells and inhibiting tumor growth (6-7). TAM had been used in breast cancer endocrine therapy for 30 years. Many patients benefit from TAM while the problem of endogenic and exogenetic resistance to TAM indeed exists (8-9): among ERα(+) breast cancer patients, approximately 30%～40% have inherent resistance to TAM and can not benefit from the use of TAM at all (6-7); approximately 62% breast cancer patients, who take TAM orally after operation, would need further surgery because of recurrence and/or metastasis (10,11). The mechanism of fulvestrant (selective estrogen receptor down-modulator) is different from that of TAM, and breast cancer patients could benefit from fulvestrant after they fail the treatment of TAM although resistance to fulvestrant would eventually appear (12-14). Compared with TAM, the AIs could inhibit tumor growth more sustainably, but tumor cells would resist to AIs as well finally (4). Hence many of the breast cancer patients treated with endocrine therapies do not respond, and for those who do, many acquire resistance over time (19). In a word, drug resistance is the major problem of endocrine therapy, and it's of great importance to further explore it.

Research approaches of resistance to endocrine therapy in breast cancer

Research based on breast cancer cell lines

Several breast cancer cell lines have been applied in research of mechanism and countermeasures of drug resistance in breast cancer endocrine therapy, for example, MCF-7 cell line (6,15-17), MDA-MB-231cell line (6), LTLTCa cell line (4) and T47D cell line (15). Under a certain process, those cell lines could evolve various cell lines that have different characteristics but all of them have resistance to breast cancer endocrine therapy. For example, if cultured in medium rich in OH-TAM for 6 months, MCF-7 breast cancer cell line would evolve into CL6.8 cell strain, which has resistance to TAM (6); if cultured in medium rich in OH-TAM and Fulvestrant, cell strain resistant to OH-TAM and Fulvestrant would be evolved (6). And MCF-7/HER2-18 is ER positive cell lines with HER2 over-expression, which was built by stable transfecting MCF-7 with over-expressed HER2; while MCF-7 wild type (MCFwt) is positive ER cell lines without HER2 over-expression (19). Through assays of cell growth and cell migration&invasion, changes in different experimental group were evaluated (15); through western blotting, relevant proteins' level in cell lines with or without resistance to endocrine drugs could be compared, hence the mechanism of endocrine resistance could be further analysed (15). After inoculated with endocrine-responsive cell lines (for example MCF-7Ca cells), mice could be assigned into different groups when the tumors reached a measurable size. The mice could be killed and tumor tissues were collected with addition of endocrine drugs when: 1) tumor volume began to shrink; 2) tumor volume stopped shrinking and began to accrete; 3) tumor accreted to several times of origin volume. Immuno-blotting and other methods could be used to analyse relevant proteins' level of these tumor tissues and then the mechanism of endocrine resistance could be further analysed (4).

Building animal models with endocrine resistance

As to the problem of endocrine resistance, common research of animal models in exploring mechanisms and countermeasures is as follows: establishing animal models with endocrine resistance through subcutaneous inoculation of tumor cells with resistance to endocrine drugs, then in vivo observation of tumor volume which would reveal the difference of various drugs in inducing and reversing endocrine resistance to tumors was performed. Usually when tumors reached a sufficient size, for example, 150-200mm^3, the animals were randomly assigned to various treatment groups (19). Mice were frequently chosen to build animal models (18-19), for example, subcutaneous inoculation tumor cells in ovariectomized & immunosuppressed mice so as to simulate the postmenopausal breast cancer patients because the source of estrogen after menopause is from nonovarian tissue and is not under regulation by gonadotropins, which could be used to explore mechanisms of endocrine resistance and countermeasures to AIs (4). To explore mechanisms of endocrine resistance and countermeasures to TAM, premenopausal animal models could be built by subcutaneous heeling-in slow-release estradiol pellets in ovariectomized mice which could simulate the in vivo estrogen release (20). Ovariectomized athymic nude mice in the presence of estrogen could also be used in establishing xenografts (19). In vivo observation of resistance-relevant proteins expression levels would promote thorough analysis the mechanism of endocrine resistance.

Potential mechanisms of breast cancer endocrine resistance

Various mechanisms are relevant to inducing endocrine resistance (3). Different mechanisms have mutual correlation with each other and cause endocrine resistance together, while the molecular phenotype can change over time (19). Fig 1 conveys the basic signaling pathways and relevant targets together with their inhibitors associated with endocrine

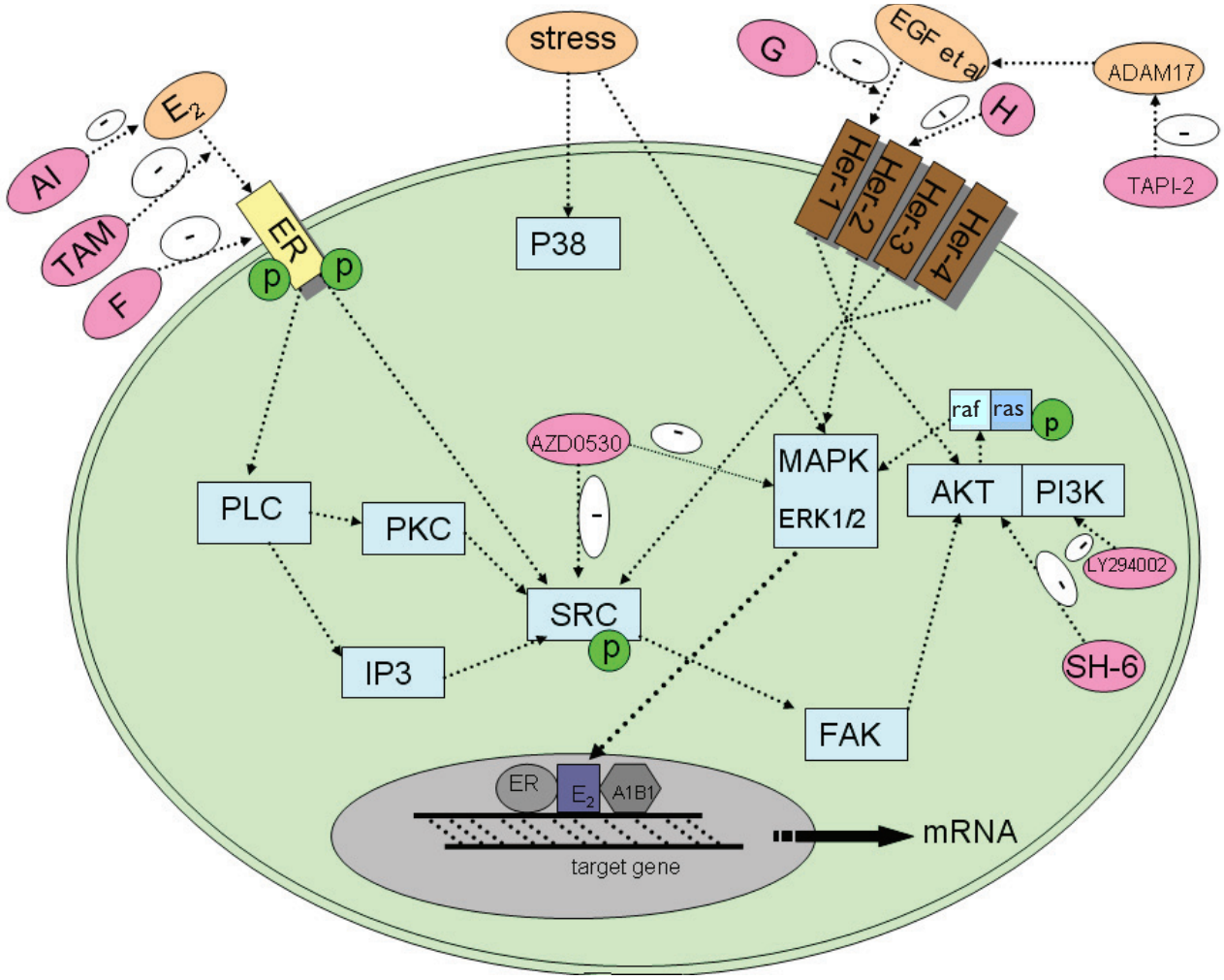

Fig 1. The basic signaling pathways and relevant targets together with their inhibitors associated with endocrine resistance
Estrogen receptor alpha (ER) and the growth factors (esp. Her-1 and Her-2) are the two main tumor markers used in the clinic to help predicting therapeutic response in breast cancer. The classical estrogen signaling pathway is responsible for growth, and TAM and AI and F are usually used to treat ER positive patients. TAM is selective estrogen receptor modulator inhibiting the combination of E2 and ER. AI could inhibit the peripheral convertion of other agents to E2. F could down-regulate the expression level of ER. Growth factor signaling via EGFR and Her-2 and stress-related pathways associated with p38 and ERK1,2 mitogen activated protein kinases have relationship with de novo and acquired resistance to endocrine therapy (19). Various treatments could be used in exploring the mechanisms of endocrine resistance, for example, E2 with the EGFR tyrosine kinase inhibitor gefitinib (E2 +G), estrogen deprivation (ED), estrogen deprivation plus the antiestrogen tamoxifen (ED+TAM),ED plus TAM and gefitinib (ED+TAM+G) or ED plus fulvestrant (ED+F), et al. SRC plays an important role in endocrine resistance, because it is the cross target for the two main signaling pathways associated with endocrine resistance. AZD0530 and TAM together showed improved growth inhibitory effects compared with either agent alone, and they could prevent the emergence of tamoxifen resistance; at the highest concentration of AZD0530(1μM), it will show corresponding inhibition of MAPK activity in the MCF-7 and T47D cell lines (15). In addition various targets and relevant inhibitors are shown in this figure.
Abbr: TAM, tamoxifen; ER, estrogen receptor; E2, estrogen; EGF, epithelial growth factor; Her-1(EGFR), epithelial growth factor receptor; G, gefitinib; F, fulvestrant (ER down-regulator); AI, aromatase inhibitor; MAPK, mitogen activated protein kinases; H, herceptin; ERK, extracellular signal-regulated kinase; MMP, matrix metalloproteinase; ADAM, a disintegrin and metalloproteinase; AREG, amphiregulin; PI3K, phosphatidylinositol-3-kinase.

resistance. The main biological targets of endocrine resistance are summarized in the following chart:

Phosphorylation of estrogen receptors (ERs)

Phosphorylation is one of the post-translational modification (PTMs) in cells including phosphorylation, glycosylation and acetylization, and ER is the main target of phosphorylation (21). Phosphorylation of ERs plays a pivotal role in acquiring endocrine resistance to TAM (22). In TAM-resistant MCF-7 Her-2/neu breast cancer cell lines and TAM-resistant breast cancer tumor tissues, it has been demonstrated by western blotting that specific ligand-dependent endogenous phosphorylation of ER occurs at S118 and S167 (22-25). Phosphorylated ER would lose ligand-dependance. While in vitro research revealed that different mechanisms of phosphorylation would lead to different biological characteristics of ER: ER phosphorylation induced by steroid receptor coactivator (SRC) and protein kinase A (PKA) would increase ER's affinity to estrogen (E2); ER phosphorylation induced by mitogen-activated protein kinase (MAPK) would reduce ER's affinity to trans-hydroxytamoxifen (TOT) (22). Phosphorylated-ER's affinity to estrogen response elements (ERE) would be significantly reduced with stimulation of exogenous TOT no matter by which mechanisms ERs were phosphorylated (22). With presence of E2, ER phosphorylation induced by AKT, MAPK and PKA would all increase the mutual interaction between DNA combining receptors and SRC3 receptors (22). Compared with TAM, although it takes a much longer time for AIs to induce breast cancer endocrine resistance, phosphorylation of ERs still plays an important role in acquiring endocrine resistance to AIs (3,21). It has been demonstrated that expression level of phosphorylated-ERα in tumor tissues resistant to letrozole is much higher than that in control group, which is sensitive to letrozole; while expression level of ERα(not phosphorylated) is much lower than that of control group (4). Meanwhile, expression level of phosphorylated-ERα will be even higher in tumor tissues resistant to letrozole if tumors continue growing under a certain concentration of letrozole, and expression level of ERα (not phosphorylated) will be even lower, while expression level of MAPK will be higher and expression level of PR will be constant (4). There are many phosphorylation sites of ERα, and different sites were phosphorylated by different mechanisms (21).

Steroid receptor coactivator (SRC)

It has been stated above that SRC plays an important role in the process of ER phosphorylation. SRC acts as a non-receptor tyrosine kinase, and its overexpression has a close correlation with various malignant tumor genesis including breast cancer (26-27). In vitro research has revealed that SRC has correlation with development of breast cancer endocrine resistance (17). Overexpression of SRC will weaken tumor cells' sensitivity to TAM in MCF-7 breast cancer cells which are with positive ERα and sensitive to TAM; while using inhibitor of SRC could reduce its expression and restore tumor cells' sensitivity to TAM in breast cancer endocrine resistant cells developed from TAM-sensitive MCF-7 breast cancer cells. The expression level of SRC in cytoplasma of breast cancer tumor cells is much higher than that in cytoplasma of breast cells besides tumor in human ($p<0.01$); while in nucleolus the expression level of SRC is just the other way. This implies that the correlation between the reactivity of breast cancer cells to endocrine drugs and SRC expression levels in cytoplasma or nucleolus might be poles apart. AZD0530 could inhibit SRC kinase activity dose-dependently, as shown by a decrease in phosphorylaiton of SRC at Y419 (15). In addition, AZD0530 together with TAM significantly suppress expression of the Ki-67 antigen, and has correlation with expression of both cyclin-D1 (necessary for the progression of cells from G1 to S phase) and C-Myc (a positive regulator of cellular proliferation) (15).

ErbB family

The ErbB family has 4 members, including ErbB1, ErbB2, ErbB3 and ErbB4, and all of them are tyrosine kinase receptors. Research conducted by Ghayad et al (6) has demonstrated that ErbB1, ErbB2 and ErbB3 are activated and ErbB4 is highly expressed in endocrine resistant breast cancer cells, while ErbB heterodimers and various ligands relevant to ErbB are also highly expressed. AKT and MAPK are the main biological targets in the downstream of ErbB family associated signal transduction pathway, and activated AKT and MAPK have a close correlation with breast cancer endocrine resistance (16). The activated AKT has correlation not only with endocrine resistance but also with poor prognosis (28). In breast cancer cell lines with resistance to TAM and Fulvestrant, the MAPK pathway is highly activated (29-30), which will make ERαfurther phosphorylated and make cells even more resistant to endocrine drugs through various ways (31,32). It has been demonstrated by western blotting that the biological target MAPK and PI3K/AKT are activated in breast cancer cells resistant to endocrine drugs, while highly expressed MAPK has close correlation with phosphorylation of serine at site 118 in region AF1 of ERα, and highly expressed PI3K/AKT has close correlation with phosphorylation of serine at site 167 in region AF1 of ERα (6). In clinical settings, patients usually benefit less from TAM if the tumors are with positive ERαand MAPK highly phosphorylated (33).

Outlook of resolution of endocrine resistance

Restoration of ER expression

Table 1. Biological targets and relevant inhibitors associated with resistance of breast cancer endocrine therapy

Biological target	Inhibitor	Biological target	Inhibitor
MEK (6)/MAPK (4)	PD98059/U0126	SRC (15)	AZD0530
PI3K (6)	LY294002	EGFR/HER-2 (18)	gefitinib
HER-2	herceptin	ADAM10 (36)	INCB3619
HSP90 (6)	17–DMAG	AKT (6)	SH-6
ADAM17 (36)	TAPI-2	ADAM17 (36)	INCB3619

Breast cancer patients with negative ERα will not benefit from endocrine therapy at all, which is primary resistant to endocrine resistance. Breast cancer patients with positive ERα will benefit from endocrine drugs at first, but will acquire resistance to it later, which is secondary resistant to endocrine therapy. Primary resistance to endocrine therapy is mainly due to methylation of promoter in ERα encoding gene and remodeling of chromatin, which is quite different from the mechanisms of secondary resistance, however there are some relationship between primary and secondary resistance to endocrine therapy.

The down-regulation of ERα expression induced by highly activated MAPK can be reversed: inhibition of MAPK activity would make ERα expression up-regulated; and ERα expression would be down-regulated again if the activity of MAPK is restored (7). Research conducted by Brinkman et al demonstrated that the mechanisms of ERα expression deficiency has correlation not only with methylation of promoter in ERα encoding gene but also with high activation of MAPK induced by EGFR and ErbB2 overexpression. Hence MAPK could be selected as the potential target for re-expression of ERα and restoration of sensitivity to endocrine therapy (34). A clinical trial including 10 negative ERα and positive ErbB2 breast cancer patients demonstrated that 3 patients became to be positive ERα and then acquired continuous sensitivity to letrozone after intravenous injection of Herceptin for a period of time (35). In transplantation animals based on positive ERα and positive ErbB2 MCF-7 breast cancer cell lines, high expression of MAPK is associated with deficiency of ERα expression, while ERα would be re-expressed and sensitivity to endocrine therapy would be restored if inhibitor of MAPK was applied (18).

Combination of various drugs

Based on mechanisms of endocrine resistance, inhibitors of different biological targets could be used in treatment of breast cancer alone or in combination, which might suppress the occurrence of endocrine resistance and restore sensitivity to endocrine drugs. Table 1 below shows various biological targets and relevant inhibitors associated with resistance of breast cancer endocrine therapy. Various inhibitors to biological targets are mainly used in basic research while scarcely used in clinical settings, and much more research is needed to develop drugs which could be used in clinical practice.

EGFR-MAPK is the main target associated with breast cancer endocrine therapy, and activation of EGFR is regulated by various cytokines, including EGF, TGF–α, ADAM17/AREG, HB–EGF, BTC, epiregulin, epigen and so on (20). After EGFR is activated by the above mentioned cytokines, downstream of the pathway will be activated by forming EGFR homodimers or forming heterodimers with ErbB2, ErbB3 and ErbB4. Combination of endocrine drugs and EGFR/ ErbB2 inhibitors would be a novel regimen which might solve the problem of endocrine resistance and elevate therapeutic effects (18).

For LTLTCa cells, which are separated from tumor tissues with resistance to letrozole, the expression level of ERα could be restored to the original status and sensitivity to AIs and AEs could also be restored if it is cultured in medium rich in Herceptin, which is inhibitor of ErbB2 signal pathway (37). This indicates that there are intimate crosstalk between the signal pathways of ER and ErbB2. However, compared with the patients who take letrozole alone as adjuvant therapy, those who take letrozole and Herceptin in combination show no better prognosis, which indicates that combination of Herceptin and endocrine drugs would be a better choice than each one alone in patients with recurrence or metastasis, therefore in adjuvant settings taking Herceptin and endocrine drugs in combination is not recommended (4). At present, phase Ⅲ clinical trial is carried out to explore the difference between neratinib group and neratinib plus AIs group in patients with recurrence or metastasis. Besides, AZD0530, which is inhibitor of SRC, combined with TAM could effectively prevent the occurrence of endocrine resistance based on research in breast cancer cell lines, indicating that SRC probably is the potential target of preventing occurrence of endocrine resistance (15).

In summary, endocrine resistance is one of the problems for ERa positive breast cancer patients. Research on mechanisms of endocrine resistance mainly focused on one signal pathway. As mechanisms of endocrine resistance involve multiple signal

pathways and multiple targets, and there are complicated crosstalks among those pathways, therefore further research work is needed to further explore relevant mechanisms so as to guide clinical practice.

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