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REVIEW

ER- α 36: a novel biomarker and potential therapeutic target in breast cancer

Xingyun Su¹Xin Xu¹Guangliang Li^{1,4}Bingyi Lin²Jiang Cao³Lisong Teng¹

¹Department of Surgical Oncology, First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, People's Republic of China; ²Key Laboratory of Combined Multi-Organ Transplantation, Ministry of Public Health, Hangzhou, Zhejiang, People's Republic of China; ³Clinical Research Center, The 2nd Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, Zhejiang, People's Republic of China;

⁴Department of Medical Oncology, Zhejiang Cancer Hospital, Hangzhou, Zhejiang Province, People's Republic of China

Abstract: Estrogen receptor-alpha36 (ER- α 36) is a 36-kDa variant of estrogen receptor-alpha (ER- α) firstly identified and cloned by Wang et al in 2005. It lacks both transactivation domains (activation function 1 and activation function 2) and has different biological characteristics compared to traditional ER- α (ER- α 66). ER- α 36 primarily locates on plasma membrane and cytoplasm and functions as a mediator in the rapid membrane-initiated non-genomic signaling pathway. Additionally, it inhibits the traditional genomic signaling pathway mediated by ER- α 66 in a dominant-negative pattern. Accumulating evidence has demonstrated that ER- α 36 regulates the physiological function of various tissues. Thus, dysregulation of ER- α 36 is closely associated with plenty of diseases including cancers. ER- α 36 is recognized as a molecular abnormality which solidly correlates to carcinogenesis, aggressiveness, and therapeutic response of breast cancer. Additionally, special attention has been paid to the role of ER- α 36 in endocrine therapy resistance. Therefore, ER- α 36 provides a novel biomarker of great value for diagnosis, prognosis, and treatment of breast cancer. It may also be a potential therapeutic target for breast cancer patients, especially for those who are resistant to endocrine therapy. In this review, we will overview and update the biological characteristics, underlying mechanism, and function of ER- α 36, focusing on its biological function in breast cancer and endocrine therapy resistance. We will evaluate its application value in clinical practice.

Keywords: ER, ER- α 36, breast cancer, endocrine therapy resistance

Introduction

Estrogen is essential to the development and physiology of target organs, such as uterus, ovary, and breast. Dysregulation of estrogen results in pathological processes including osteoporosis, breast cancer, and endometrial cancer.¹ These pleiotropic effects of estrogen are related to estrogen receptors (ERs) which are generally regarded as ligand-activated transcription factors belonging to the nuclear receptor superfamily.^{1–3} Two different forms of ER have been discovered, ER- α and ER- β .^{3,4} They share similar architecture composed of three independent but interactional function domains.^{3,5} The N-terminal A/B domains possess a ligand-independent transactivation domain (activation function 1 [AF-1]), interacting with co-activators and activating transcription of target genes.⁶ The C or DNA-binding domain contains two zinc finger-like structures, allowing dimerization of receptors and then binding to target DNA sequences.⁶ The C-terminal D/E/F domains or ligand-binding domains mediate ligand binding, receptor dimerization, and nuclear localization and have a ligand-dependent activation domain (activation function 2 [AF-2]) (Figure 1B).

ER- α 36 is a 36-kDa variant of ER- α generating from full length ER- α by alternative splicing or different usage of promoters.^{6–9} Compared to ER- α 66, ER- α 36 retains

Correspondence: Lisong Teng
Department of Surgical Oncology,
First Affiliated Hospital, College of
Medicine, Zhejiang University, 79
Qingchun Road, Hangzhou 310003,
Zhejiang, People's Republic of China
Tel +86 571 8706 8873
Fax +86 571 8723 6628
Email lsteng@zju.edu.cn

Jiang Cao
Clinical Research Center, The
2nd Affiliated Hospital, College
of Medicine, Zhejiang University,
Hangzhou, Zhejiang Province,
People's Republic of China
Tel +86 571 8731 5201
Fax +86 571 8731 5201
Email caoj@zju.edu.cn



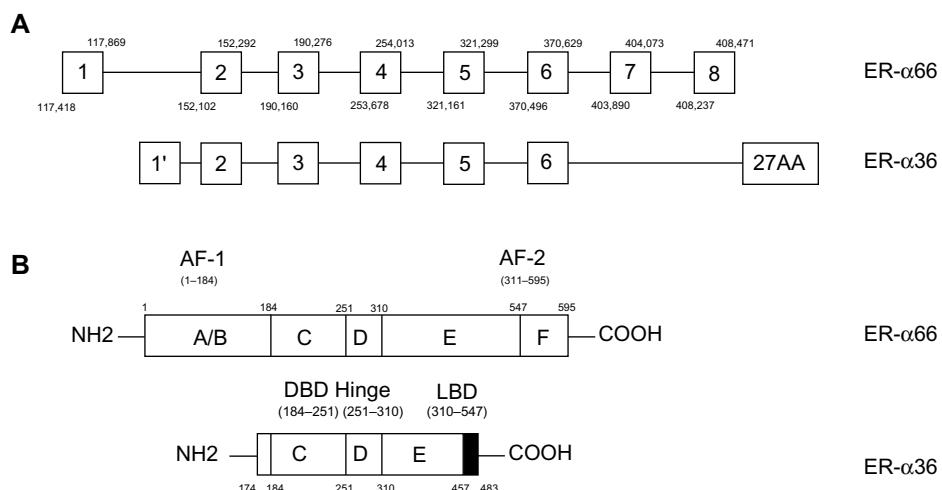


Figure 1 Structure of ER- α 66 and ER- α 36.

Notes: (A) Transcription of ER- α 36 initiates from a previously unidentified promoter in the first intron of ER- α 66. We labeled the first exon of ER- α 36 as “I” to distinguish it. Besides, ER- α 36 has an extra, unique 27-amino-acid sequence at C-terminus which may broaden ligand-binding spectrum of ER- α 36. (B) Compared to the protein structure of ER- α 66, ER- α 36 lacks both transactivation domains of AF-1 and AF-2 and retains DBD, Hinge, and LBD of ER- α 66. Therefore, ER- α 36 functions as a powerful competitor of ER- α 66.

Abbreviations: AF-1, activation function 1; AF-2, activation function 2; DBD, DNA binding domain; ER- α 36, estrogen receptor-alpha36; ER- α 66, estrogen receptor-alpha66; LBD, ligand binding domain.

DNA-binding, partial dimerization, and ligand-binding domains but lacks both transactivation domains (AF-1 and AF-2).^{3,14,15} ER- α 36 mainly locates on plasma membrane and cytoplasm mediating non-genomic estrogen signaling.^{6,10,11} Additionally, it inhibits traditional genomic estrogen signaling mediated by ER- α 66 in a dominant-negative pattern.¹⁰ Accumulating evidence has demonstrated that ER- α 36 participates in the development and physiological function of many tissues. Therefore, it is related to diseases such as postmenopausal osteoporosis,¹² airway hyperresponsiveness,¹³ and even malignancies including gastric,^{14–16} hepatic,¹⁷ endometrial,¹⁸ and breast cancers.¹⁹ ER- α 36 is crucial in the carcinogenesis and aggressiveness of breast cancer; in addition, it explains why antiestrogen therapy loses efficacy in ER-positive breast cancer patients.^{20–22} These achievements imply that ER- α 36 is a promising biomarker for diagnosis, prognosis, and treatment of various diseases, especially breast cancer.^{23–25} What's more, it provides a novel pharmaceutical approach to individualized treatment of breast cancer.

This review will overview and update the biological characteristics, molecular mechanism, and biological role of ER- α 36, focusing on its function in carcinogenesis, progression, and endocrine therapy resistance of breast cancer and its potential application value in clinical practice.

Basic characteristics of ER- α 36

Transcription of ER- α 36 initiates from a previously unidentified promoter in the first intron of ER- α 66, which continues

from exon 2 to exon 6 and skips the final exon 7 and exon 8 of ER- α 66 (Figure 1A).^{3,6,10,14,15} As a result, ER- α 36 lacks both transactivation domains (AF-1 and AF-2) and retains DNA-binding, partial dimerization, and ligand-binding domains of ER- α 66 (Figure 1B). The last 138 amino acids encoded by the final exon 7 and exon 8 are replaced by an extra, unique 27-amino-acid sequence at C-terminus. This extra sequence may broaden the ligand-binding spectrum of ER- α 36 so that it is able to interact with more factors other than estrogen.¹⁰ ER- α 36 primarily locates on plasma membrane (50%) and cytoplasm (40%) rather than nucleus.^{10,19,26–28} Because it has three potential myristylation sites near N-terminus instead of the nuclear-location signals of ER- α 66, ER- α 36 may be modified by palmitoylation posttranslationally and then locate on plasma membrane and cytoplasm just like ER- α 46, another variant of ER- α 66.^{6,10,11} Therefore, ER- α 36 is a potential regulator of membrane-initiated mitogenic signaling pathways responding to E2 α , E2 β , E3, E4, and even tamoxifen, an estrogen antagonist.^{6,10,24}

Though ER- α 36 exists in both ER- α 66-positive and ER- α 66-negative breast cancer cells,^{10,28} it is highly expressed in the majority of ER- α 66-negative breast cancer.^{19,29} Furthermore, overexpression of ER- α 36 is usually accompanied with a decrease of ER- α 66,^{10,24,28,30} indicating that ER- α 66 and ER- α 36 are mutually regulated and inversely associated.^{10,20,24,27–30} Wilms' tumor suppressor WT1 is one of the coordinators which activates promoters of ER- α 66 but suppresses ER- α 36 promoter activity.³¹ Another coordinator

is synuclein γ (SNCG), which binds to ER- α 66 or ER- α 36 depending on E2 concentration.^{32,33} In addition, ER- α 66 can negatively regulate the promoter activity of ER- α 36.³⁰ Deng et al found that proteasome inhibitors dramatically increased the steady level of ER- α 66, indicating that the proteasome system took part in the expression regulation of ER- α 36 and ER- α 66.³⁴ With the dynamic regulation of ER- α 66 and ER- α 36, genomic and non-genomic estrogen signaling pathways are also coordinated to maintain a balance.³¹ Although the explicit mechanism needs further investigation, it is obvious that an imbalance of ER- α 66 and ER- α 36 may result in abnormal proliferation and differentiation, finally leading to diseases including breast cancer.¹

Molecular mechanism underlying ER- α 36 action

Under normal conditions, ERs bind to estrogen and then transfer to the nucleus, subsequently binding to specific estrogen response elements and regulating transcription of downstream DNA (Figure 2).³⁵ Other than the traditional genomic pathway, ERs mediate the non-genomic pathway by membrane-associated receptors.^{36–39} The non-genomic pathway controls more genes than the genomic pathway, regulating cellular growth, survival, motility, invasion, and apoptosis. What's more, non-physiologic estrogen also activates the non-genomic pathway, which is important to

so-called “nontarget” tissue such as the cardiovascular system.^{40,41} The unique structure and intracellular location of ER- α 36 decide its molecular mechanism. Lacking both transcriptional activation domains, ER- α 36 binds to the same target DNA sequence as ER- α 66 does, but possesses no intrinsic transcriptional activity. Thus, ER- α 36 functions as a powerful competitor of the genomic signaling pathway mediated by ER- α 66.^{6,10} Additionally, ER- α 36 mediates rapid membrane-initiated estrogen signaling cascades, which regulate various biological functions and are associated with tumorigenesis, aggressive phenotype, and treatment sensitivity of carcinomas (Figure 2).^{16,23,24,42–44}

ER- α 36 and downstream kinases

The mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) pathway is the most popular mitogenic signaling in cancers, which can be rapidly activated by E2.^{45,46} Research has demonstrated that membrane-initiated signaling pathways mediated by ER- α 36 could stimulate the transcription of ELK1 in the nucleus.^{10,26,42,47,48} Tong et al found that ER- α 36 also activated ERK1/2 through the protein kinase C delta signaling pathway and then elevated expression of cyclin D1/cyclin-dependent kinase 4, a transcriptional coregulator that regulates cell cycle progression.⁴⁸ The MAPK/ERK signaling pathway mediated by ER- α 36 contributes to the potential invasion and metastasis of cancer

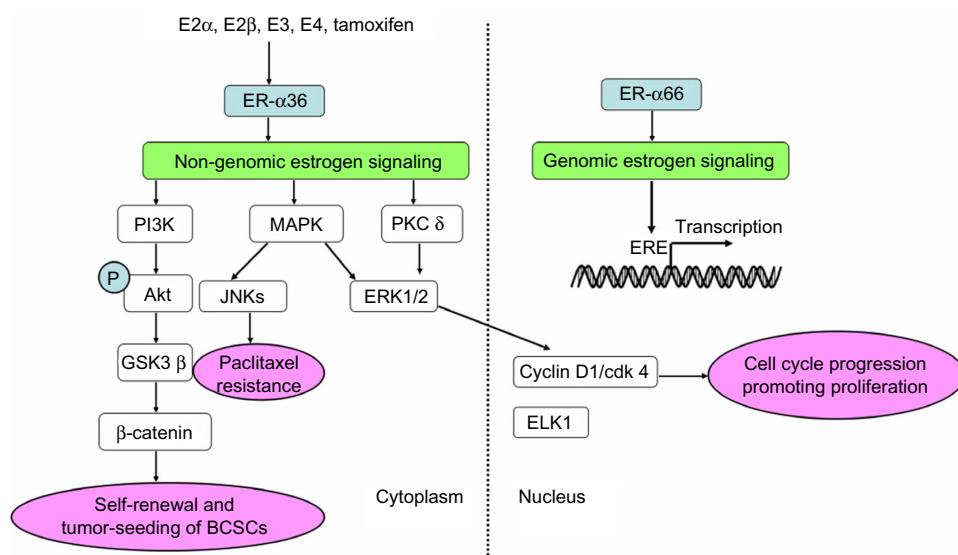


Figure 2 Genomic and non-genomic activities mediated by ERs.

Notes: ER- α 66 binds to estrogen and then transfers to the nucleus to interact with specific EREs and induce transcription of target genes. ER- α 36 has a broader ligand-binding spectrum than ER- α 66 so that it can respond to E2 α , E2 β , E3, E4, and even tamoxifen. ER- α 36 can mediate rapid MIES such as MAPK/ERK, PI3K/Akt, and PKC δ to regulate biological functions of cells.

Abbreviations: Akt, protein kinase B; BCSCs, breast cancer stem cells; cdk 4, cyclin-dependent kinase 4; ER- α 36, estrogen receptor-alpha36; ER- α 66, estrogen receptor-alpha66; EREs, estrogen response elements; ERK, extracellular signal-regulated kinase; ERs, estrogen receptors; GSK3 β , glycogen synthase kinase 3 beta; JNKs, c-Jun N-terminal kinases; MAPK, mitogen-activated protein kinases; MIES, membrane-initiated estrogen signaling; PKC δ , protein kinase C delta; PI3K, phosphatidylinositol 3-kinase.

cells.^{23,24,44} What's more, it induces paclitaxel resistance through c-Jun N-terminal kinases (JNKs), an important component of the MAPK family mediating paclitaxel-induced apoptosis and death (Figure 2).^{23,24,44}

Phosphatidylinositide 3-kinase/protein kinase B (PI3K/Akt) is another classical pathway associated with malignant cancers.^{49–51} Rapid phosphorylation of Akt, which is induced by E2, testosterone, and tamoxifen, can be blocked by a PI3K inhibitor or knockdown of ER- α 36, suggesting that the PI3K/Akt pathway is one of the non-genomic pathways mediated by ER- α 36 (Figure 2).^{26,47}

ER- α 36 associated proteins

Several proteins are involved in the stability and function of ER- α 36, thus promoting mitogenic estrogen signaling mediated by ER- α 36. Glucose-regulated protein 94, an endoplasmic reticulum chaperone, is a potential downstream effector of the ER- α 36-mediated pathway in gastric cancer.¹⁵ SNCG, previously regarded as breast cancer-specific gene *BCSG1*,⁵² binds to ER- α 36 without heat shock protein 90 to prevent degradation of ER- α 36 and significantly strengthen ER- α 36-mediated mitogenic estrogen signaling pathways.³³

Many investigators discovered a significant co-expression of ER- α 36 and epidermal growth factor receptor (EGFR) in primary breast cancers, indicating that ER- α 36 took part in EGFR-related carcinogenesis.¹⁹ Further studies elucidated

that epidermal growth factor (EGF) induced phosphorylation of ERK1/2 via ER- α 36 in a time- and dose-dependent pattern.¹⁸ A positive feedback loop was confirmed that EGFR signaling activated transcription of ER- α 36 through an activator-protein-1-binding site in the promoter of ER- α 36. In turn, ER- α 36 interacted with the EGFR/Src/Shc complex to strengthen the EGFR signaling pathway and stabilize EGFR protein (Figure 3).²⁰ A similar feedback loop between ER- α 36 and human epidermal growth factor receptor 2 (HER-2), a member of the EGFR family, was reported.⁵³ Interestingly, our previous study found that HER-2 expression didn't increase in tamoxifen-resistant cells, which overexpressed ER- α 36 and EGFR.²³ In the Src/EGFR/signal transducer and activator of transcription 5 (STAT5) pathway mediated by ER- α 36, Src functions as a switch to adjust phosphorylation of EGFR and then recruits STAT5 as a downstream effector, which regulates activation of the MAPK/ERK signaling pathway and expression of cyclin D1 (Figure 3).^{16,20,54} Therefore, the positive feedback loops between ER- α 36 and growth factor receptors are important for cellular function, although the definite mechanism has not been clarified.

Biological roles of ER- α 36

ER- α 36 participates in the development and function of target tissues. For example, expression and subcellular localization of ER- α 36 are necessary for folliculogenesis,

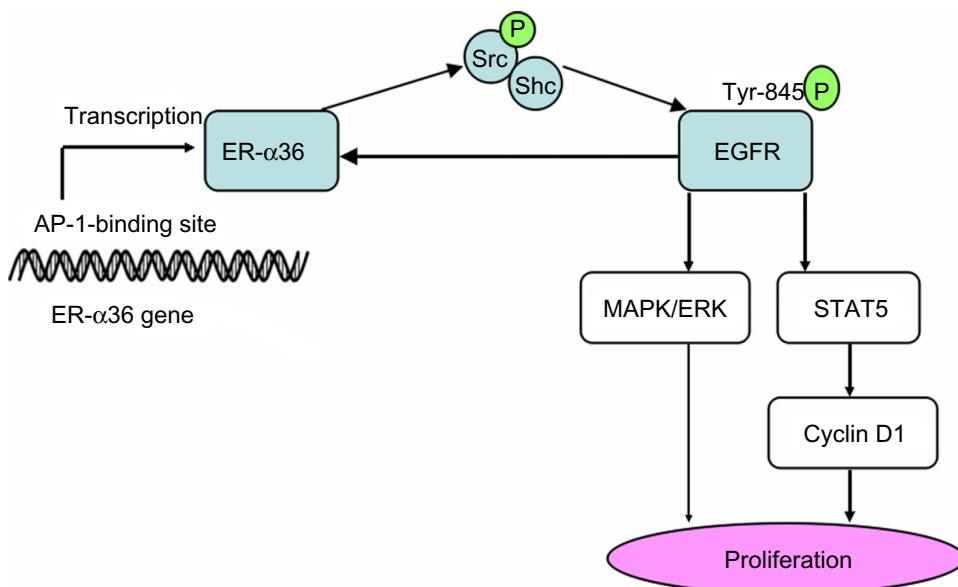


Figure 3 Positive feedback loop between EGFR and ER- α 36.

Notes: A positive feedback loop has been confirmed that EGFR signaling activates transcription of ER- α 36 through an AP-1-binding site in the promoter region of ER- α 36. In turn, ER- α 36 is able to stabilize EGFR protein and mediate MAPK/ERK and Src/EGFR/STAT5 pathways. In the Src/EGFR/STAT5 pathway, Src functions as a switch by phosphorylation to enhance proliferation and malignant properties of cancer.

Abbreviations: AP-1, activator protein 1; EGFR, epidermal growth factor receptor; ER- α 36, estrogen receptor-alpha36; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinases; STAT5, signal transducer and activator of transcription 5.

oocyte meiotic maturation, ovulation, and luteinization of the postnatal ovary, thus facilitating maturation of the female reproductive system.⁵⁵ Additionally, ER- α 36 maintains the bone density of postmenopausal women.¹² However, dysregulation of ER- α 36 leads to various dysfunctions and diseases, such as osteoporosis,¹² airway hyperresponsiveness,¹³ and even cancers.^{14,17–19} Since the mammary gland is estrogen-dependent, ER- α 36 plays a particularly vital role in breast cancer. Therefore, we will focus on its role in breast cancer and endocrine therapy resistance and its potential application value in clinical practice.

Biological function of ER- α 36 in breast cancer

In order to investigate the biological function of ER- α 36 in breast cancer, Zhang et al established ER- α 36-knockdown MDA-MB-231 cell lines in which expression of ER- α 36 decreased obviously. They found knockdown of ER- α 36 suppressed proliferation, migration, and invasion and increased sensitivity to paclitaxel in MDA-MB-231 cells.²⁴ Chaudhri et al got similar results that ER- α 36 was able to enhance cellular proliferation and metastasis and protect against apoptosis.⁴³ Recently, Yu et al studied the relationship between ER- α 36 and chemotherapy response in 120 breast cancer patients and found that ER- α 36-negative patients had better response to anthracycline and/or paclitaxel than ER- α 36-positive patients.²⁵

ER- α 36 and breast cancer stem cells (BCSCs)

BCSCs, a subpopulation of stem/progenitor cells that possess the ability to self-renew, play a key role in occurrence, recurrence, metastasis, and chemotherapy resistance of breast cancer.^{56,57} CD44⁺/CD24^{-low} and aldehyde dehydrogenase 1 (ALDH1) were identified as markers of BCSCs.^{56,58} Kang et al found that ER- α 36 was highly expressed in ALDH1-positive SK-BR-3 cells. Additionally, the depletion of ER- α 36 reduced the growth rate and proportion of ALDH1^{high} cells, indicating that ER- α 36 contributed to the proliferation and maintenance of stem-like cells.⁵³ Deng et al observed that estrogen significantly increased the population of CD44⁺/CD24^{-low} cells in MCF-7 and T47D breast cancer cells, but failed to do so after knocking down ER- α 36, suggesting that estrogen positively regulated BCSCs through the ER- α 36-mediated signaling pathway.³⁴ Furthermore, they found that overexpression of ER- α 36 tended to promote self-renewal and tumor-seeding efficiency of BCSCs through the PI3K/

AKT/glycogen synthase kinase 3 beta/ β -catenin signaling pathway (Figure 2).³⁴

ER- α 36 and endocrine therapy resistance

Endocrine therapy is a dominant anticancer method for ER-positive breast cancer patients. Although aromatase inhibitors are increasingly applied in postmenopausal women,⁵⁹ antiestrogens are still used as first-line therapy, especially when aromatase inhibitors are ineffective. However, less or no response after long-term treatment is the major obstacle in antiestrogen treatment.^{60,61}

Tamoxifen

Tamoxifen is a competitive antagonist of ER- α , effectively blocking traditional genomic signaling pathway mediated by ER- α 66.^{61,62} However, it can activate different kinase cascades and produce second messengers, thus acting as an agonist that is equal to estrogen.⁶³ For example, tamoxifen strongly activates the MAPK/ERK signaling pathway to stimulate cell growth; additionally, it activates the p38/MAPK and SAPK/JNK pathways to induce apoptosis.^{10,64} In recent years, a large number of explorations have concentrated on the association between ER- α 36 and tamoxifen resistance. Due to the unique 27-amino-acid domain, ER- α 36 has a broader ligand-binding spectrum than ER- α 66 so that it can respond to tamoxifen.^{10,24} Usually, ER- α 36 is highly expressed in ER- α 66-negative breast cancer, which is insensitive to endocrine therapy.^{10,28,29} Clinical research demonstrated that high expression of ER- α 36 was associated with decreased tamoxifen sensitivity and poorer survival in ER- α 66-positive breast cancer patients who received tamoxifen as adjuvant therapy.²⁹ Additionally, laboratory studies revealed that the tamoxifen-resistant MCF-7 cell line (MCF-7/TAM) highly expressed ER- α 36.^{22,23,65} However, knocking down ER- α 36 in MCF-7/TAM cells could not restore their sensitivity to tamoxifen completely.²³ This may be because the expression level of ER- α 36 in MCF-7/TAM cells is too high to be completely blocked; besides, biological reprogramming during development of tamoxifen resistance is much more complicated than upregulation of ER- α 36. Furthermore, proteins maintaining the stability and function of ER- α 36 such as SNCG can strengthen tamoxifen resistance mediated by ER- α 36.³³ Therefore, ER- α 36 is an underlying cause of tamoxifen resistance in breast cancer patients.

Previous explorations have demonstrated that ER- α 36 is a potent mediator of membrane-initiated signaling pathways associated with agonist effects of tamoxifen, which leads to

tamoxifen resistance.^{10,24} For example, the MAPK/ERK and PI3K/AKT signaling pathways that are mediated by ER- α 36 induce the expression of protooncogene c-Myc, which has profound mitogenic effects (Figure 4).^{10,44} ER- α 36 also mediates the Src/EGFR/STAT5 pathway regulating activity of the MAPK/ERK signaling pathway and expression of the growth-promoting cyclin D1 (Figure 4).⁶⁶ However, it is still unknown whether other non-genomic pathways, such as protein kinase C, protein kinase A, and calcium channels are involved in tamoxifen resistance.

Further research has revealed that overexpression of EGFR with downregulation of ER- α 66 is responsible for tamoxifen resistance.^{67–69} Li et al established a tamoxifen-resistant MCF-7 cell line (MCF-7/TAM) that had an accelerated proliferation rate as well as enhanced migratory and invasive ability. In MCF-7/TAM cells, ER- α 36 upregulated EGFR expression and downregulated ER- α 66 expression, switching growth status from estrogen-dependent to growth-factor-dependent, which was a critical step in the development of tamoxifen resistance (Figure 4).²³

ICI 182,780 (Fulvestrant, Faslodex[®])

ICI 182,780 (Fulvestrant, Faslodex), a ‘pure’ antiestrogen, not only accelerates degradation but also impairs dimerization and nuclear localization of ER- α 66.^{70,71} However, several studies reported that ICI 182,780 failed to induce

degradation of ER- α 36,^{6,72} presumably because ER- α 36 has a truncated ligand-binding domain lacking the last four helices (helix 9–12) of ER- α 66, which are essential for protein degradation induced by ICI 182,780.^{6,73} Therefore, failure of ER- α 36 degradation is a highly possible reason for ICI 182,780 resistance.

Application value of ER- α 36

Metastasis and recurrence are the most intractable issues in the management of cancer; thus, using molecular alteration to assess tumor degree and prognosis is of great significance. A large number of studies have confirmed that ER- α 36-positive breast cancers tend to have more lymph node metastasis, advanced degree, less sensitivity to chemotherapy or endocrine therapy, and poorer outcome than ER- α 36-negative breast cancer. Therefore, ER- α 36 is a promising biomarker for malignant behavior, therapeutic response, and outcome of breast cancer, guiding clinical decisions in early diagnosis, prognosis estimation, and personalized treatment. For example, we can combine the expression of ER- α 66 and ER- α 36 to predict whether endocrine therapy is suitable and effective for breast cancer patients.

Given its important role in carcinogenesis, progression, and therapeutic response of breast cancer, ER- α 36 is emerging as a potential therapeutic target for breast cancer. The surface localization of ER- α 36 makes it possible to generate

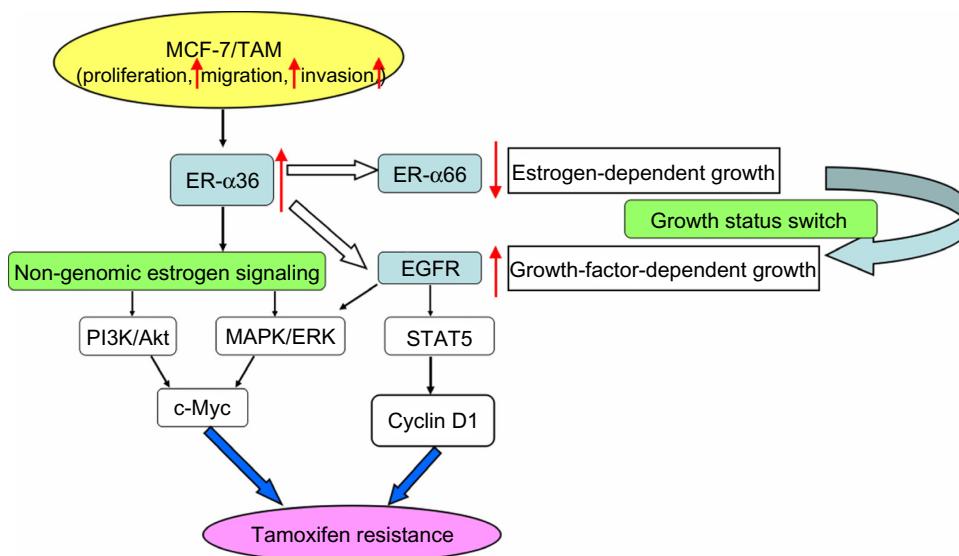


Figure 4 ER- α 36 and tamoxifen resistance.

Notes: ER- α 36 is an important cause of tamoxifen resistance. On the one hand, it activates membrane-initiated signaling pathways, such as MAPK/ERK, PI3K/AKT, and Src/EGFR/STAT5 pathways, causing the agonist effect of tamoxifen. On the other hand, ER- α 36 upregulates EGFR and downregulates ER- α 66 switching growth status from estrogen-dependent to growth-factor-dependent. Therefore, ER- α 36 leads to tamoxifen resistance.

Abbreviations: Akt, protein kinase B; EGFR, epidermal growth factor receptor; ER- α 36, estrogen receptor-alpha36; ER- α 66, estrogen receptor-alpha66; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinases; MCF-7/TAM, tamoxifen-resistant MCF-7 cell line; PI3K, phosphatidylinositol 3-kinase; STAT5, signal transducer and activator of transcription 5.

specific surface epitopes for treatment intervention with small molecules or biologics which directly down-modulate the ER- α 36 protein or indirectly block its downstream signaling pathways, such as biological antagonists, monoclonal antibodies, and small kinase inhibitors. Inhibition of ER- α 36 can suppress tumor growth and progression, benefitting breast cancer patients, especially those who are resistant to endocrine therapy. Though few studies have focused on the therapeutic application of ER- α 36 and no clinical trials currently, a targeted agent suitable for clinical testing will be available in the near future. Broussoflavonol B, a flavonoid purified from the bark of *Broussonetia papyrifera* (Moraceae), exhibits a more potent growth inhibition effect than tamoxifen in ER-negative breast cancer.^{74,75} It decreases the steady level of ER- α 36 and EGFR and restricts growth of stem-like cells in MDA-MB-231 cells.⁷⁵

Conclusion

ER- α 36 is a variant of ER- α with different characteristics and functions than ER- α 66. It locates on plasma membrane and cytoplasm. Therefore, ER- α 36 mediates the non-genomic signaling pathway and suppresses the traditional genomic signaling pathway. ER- α 36 plays a key role in breast cancer, leading to an accelerated proliferation rate together with enhanced migratory and invasive ability. Additionally, it mediates the agonist action of tamoxifen and switches the growth status of breast cancer cells from estrogen-dependent to growth-factor-dependent, both of which can be the molecular mechanisms of tamoxifen resistance. Furthermore, failure of ER- α 36 degradation is a possible reason for Fulvestrant resistance. Therefore, ER- α 36 is a promising biomarker for tumor behavior and therapeutic response, being a new indicator to select optimal candidates for therapeutic strategies. Additionally, it is a potential therapeutic target to improve survival of breast cancer patients.

These achievements make the heterogeneity of the estrogen effect more clear and put a new insight into the properties of ER- α 36. Additionally, studies of ER- α 36 deepen our knowledge of the carcinogenesis and progression of breast cancer and provide an alternative explanation for endocrine therapy resistance. However, there are still many challenges in the future. More research is needed to clarify the biological function and deep mechanism of ER- α 36. Additionally, developing an effective and specific inhibitor of ER- α 36 is a difficult work. Before the inhibitor is widely used, the inhibitory consequences should be validated regarding proliferation, invasion, migration, and survival of tumor cells and clinical trials with large sample sizes and long-term observation are also needed.

Acknowledgments

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Disclosure

The authors declare no conflicts of interest in this work.

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