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Mini review

Advances in the understanding of the structure and function of ER-α36,a novel variant of human estrogen receptor-alpha

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ABSTRACT

Estrogen receptors (ERs) belong to the nuclear receptor superfamily, whose members include ER-α66, ER-α36, ER-α46 and ER-β. Each receptor performs specific functions through binding with a specific ligand, such as estrogen. Recently, ER-α36, a novel variant of human estrogen receptor-alpha (ER-α), was identified and cloned. ER-α36 inhibits, in a dominant-negative manner, the transactivation of both the wild-type ER-α (ER-α66) and ER-β. As a predominantly membrane-based ER, ER-α36 mediates nongenomic estrogen signaling and is involved in the resistance of breast cancer to endocrine therapy, i.e., tamoxifen. This review summarizes recent studies on the structure and function of ER-α36 and the relationship of ER-α36 with cancer, with special emphasis on its function in the resistance of breast cancer to endocrine therapy.

1. Introduction

Estrogen, synthesized via the aromatization of androgens, is an essential component of the female hormone system [1]. The most dominant estrogen in the human body is 17β-estradiol (E2) [2]; however, the estrogens estrone and estriol are also present, although at lower levels. Estrogen can regulate growth, differentiation, and function in a large range of target tissues, such as the uterus, ovary, mammary gland and hypothalamic pituitary. However, the dysregulation of estrogen expression plays a role in many diseases, such as cancers of the breast, prostate and endometrium, resulting in abnormal cell proliferation [1,3]. The biological effects of estrogen are mediated through ligand-activated transcription factors known as estrogen receptor α (ERα) and β (ERβ), members of a large superfamily of nuclear receptors. ERα and ERβ are encoded by separate genes, ESR1 and ESR2, respectively. ERα is found on chromosome 6q, and its wild-type full-length mRNA encodes a 595 amino acid protein [4], ERβ is found on chromosome 14q and its wild-type full-length mRNA encodes a 530 amino acid protein [5,6]. Although the receptors have different ligand affinities, exist in different tissues and may act antagonistically [7], they share a common structure architecture that is composed of three independent but interacting function domains [8,9]. The functional domains include the variable N-terminal A/B domains, the C or DNA-binding domain and the D/E/F or ligand-binding domains. It is evident that the regulation of gene expression by ERs is a multifactorial process, involving both genomic and nongenomic actions [10–12]. ER genomic activity is termed nuclear-initiated steroid signaling (NISS), which is achieved through two mechanisms. The first mechanism involves the direct DNA binding of ER to its target gene. Estrogen binding to its receptor triggers a number of events starting with the migration of the receptor from the cytosol into the nucleus and dimerization of the receptor as well as the subsequent binding of the receptor dimer to specific sequences of DNA known as estrogen response elements (EREs). The DNA/receptor complex then recruits other proteins such as co-activators, which induce a conformational change and are responsible for the transcription of downstream DNA into mRNA and finally into protein to produce a change in cellular function [13]. The second mechanism involves the indirect interaction of the ER with responsive DNA sites, such as AP1 and Sp1, via a protein complex of transcription co-factors [14]. ER non-genomic activity [15] is termed rapid membrane-initiated steroid signaling (MISS) or membrane-initiated estrogen signaling (MIES), which involves a separate, membrane-associated receptor and the activation of various protein-kinase cascades. This rapid non-genomic signaling effect, induced by estrogen, may indirectly influence gene expression through the activation of signal transduction pathways that finally act on target transcription factors. Due to the alternative mRNA splicing of the ER gene, several ER isoforms are known to exist in cells. At least three ERα and five ERβ variants have been identified, such as ER-α36 [16], ER-α46

Abbreviations: E2, 17β-estradiol; ER, estrogen receptor; ERE, estrogen response element; AF, activation function; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; PI3K, phosphoinositide 3-kinase; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; MIES, membrane-initiated estrogen signaling.

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[17], and ERβ2–ERβ5 [18], with ER-α66 and ERβ1 as the wild-type receptors of the ER. ER-α36 is a novel variant of ER-α that was cloned and identified by Wang et al. in 2005 and is predominantly localized to the plasma membrane and the cytoplasm, mediating a rapid membrane-initiated nongenomic signaling pathway [16,19,20]. Additional evidence indicates that ER-α36 has different subcellular localizations than its wild-type receptor and that it has its own specific functions. This review summarizes the recent studies on the structure and function of ER-α36 and its relationship with cancer, with special emphasis upon its function in the resistance of breast cancer to endocrine therapy.

2. The identification of ER-α36 and its expression pattern in breast cancer

ER-α36 has a molecular weight of 36-kDa. Prior to its successful cloning, previous reports had suggested the existence of this protein. In a study by Li et al. [21], ER-α46 was found to localize to the plasma membrane and modulate membrane-initiated estrogen actions, which stimulate rapid endothelial nitric oxide synthase (eNOS) activation in human endothelial cells. Unexpectedly, they found a predominant band of 35–39 kDa via Western blot analysis using antibodies raised against the ligand-binding domain of ER-α66. They predicted that the 36-kDa protein might be another ER-α66 variant. Wang et al. [16] confirmed this hypothesis by identifying a full-length clone from a normal human endometrium cDNA library (GenBank Accession No. BX640939) using molecular cloning techniques combined with an extensive GenBank homology search. This 5.4 kb cDNA encodes a 310 amino acid open-reading frame and a protein with a predicted molecular weight of 35.7 kDa. An expression vector containing this 5.4 kb cDNA was transiently transfected into human embryonic kidney (HEK) 293 cells, and the monoclonal antibody H222, raised against the ligand-binding domain of ER-α66, was used for Western blot analysis. The results showed that the open-reading frame encodes a 36-kDa protein that can be recognized by the anti-ER-α66 antibody. Because of the high homology with ER-α66, this protein was termed ER-α36 to distinguish it from ER-α66 and ER-α46. ER-α36 is expressed in all types of cancers, such as breast cancer [20,22], endometrial cancer [19], colorectal cancer [23], gastric cancer [24] and has also been demonstrated to be expressed in murine ovaries [25]. ER-α36 expression was not detected in the normal mammary cell line MCF10A. However, it was detected in established breast cancer cell lines, including the ER-α66-positive MCF-7, HB3396, and T47D cell lines and the ER-α66-negative MDA-MB-231 and MDA-MB-436 cell lines [20]. In breast cancer patients who are either ER-α66-positive or ER-α66-negative, ER-α36 expression was detected, and its expression appeared to be associated with decreasing nuclear and/or cytoplasmic ER-α66 expression [22,26]. Recent research data demonstrated that in 10 out of 12 triple-negative breast cancer (ER-a66-, PR- and Her2/neu-) cases, samples were found to exhibit ER-a36 expression, predominantly in a cytoplasmic and membranous pattern [27]. Adenoid cystic carcinoma and pure apocrine carcinoma, two rare types of breast cancer, are characteristically negative for ER-α66. Using immunohistochemical methods, ER-a36 was found to be expressed in 18 out of 19 pure apocrine carcinoma cases (94.7%, 95% CI 75.13–98.77) and in 8 out of 11 of adenoid cystic carcinoma cases (72.7%, 95% CI 42.81–90.08). Moderate to strong membranous and cytoplasmic expression was detected in both cancer types [28]. Together, studies on the ER-α36 expression pattern in breast cancer cell lines and tissues indicate that ER-α36 is expressed in both ER-positive and ER-negative breast cells, especially in ER-negative breast cancer cells that lack ER-α66 expression.

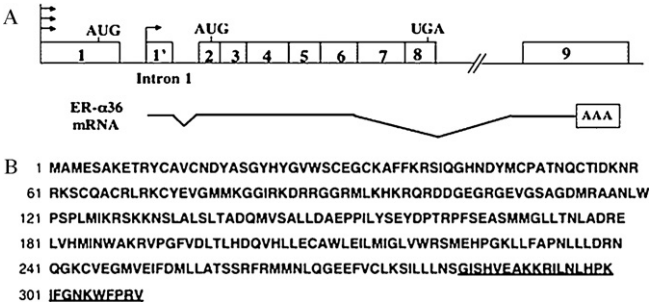

Fig. 1. Structure of the human ER-α36 gene. (A) Genomic organization of the human ER-α36 (hER-α36) gene. The common exons between the hER-α36 and hER-α66 genes are shown as open numbered boxes. The extra exon of the hER-α36 gene that is beyond the 8 exons found in hER-α66 gene is numbered as 9 in the open box. Intron 1 of the hER-α66 gene is shown with exon 1′ of the hER-α36 gene in the open box. The lower panel shows the mRNA structure of the hER-α36 isoform. (B) The predicted amino acid sequence of the ER-α36 open-reading frame. The last 27 amino acids that are unique to hER-α36 are underlined.
Adapted from [16].

3. The structure and function of ER-α36

ER-α66 was the first successfully cloned estrogen receptor. The ER-α66 gene contains 8 exons and 7 introns, and the mRNA encodes for 595 amino acids, resulting in a protein with molecular weight of 66 kDa [29]. As a novel variant of ER-α66, the transcription of ER-α36 is initiated from a previously unidentified promoter located in the first intron of the ER-α66 gene. To distinguish it from the ER-α66 original exon 1, this exon is named exon 1. "Exon 1" of ER-α36 is directly spliced into exon 2–6 of the ER-α66 gene, and then exon 6 is spliced to exon 9 located 64,141 bps downstream of the ER-α66 gene (Fig. 1A). As ER-α36 skips exons 7 and 8, it possesses a unique 27 amino acid C-terminus (Fig. 1B).

Both the ER-α36 and the ER-α46 proteins are initiated from a favorable Kozak sequence in exon 2 [17]. Compared with classic ER-α66, ER-α46 lacks the first coding exon of the ER-α66 gene, which encodes the first 173 amino acids (AF-1) of ER-α66 [17]. Different from ER-α46, ER-α36 lacks both transcription activation domains (AF-1 and AF-2) but retains the DNA-binding domain and the partial dimerization and ligand-binding domain, followed by the unique 27 amino acid domain at the C-terminus as described above (Fig. 2).

Because of the specific structure of ER-α36, it possesses different transcriptional activity than ER-α66. ER-α66 negatively regulates the promoter activity of ER-α36 [30]; however, ER-α36 can inhibit the estrogen-independent and estrogen-dependent transactivation activities of ER-α66 [20]. Zou et al. [30] isolated and cloned a DNA fragment of approximately 751 bps containing the promoter

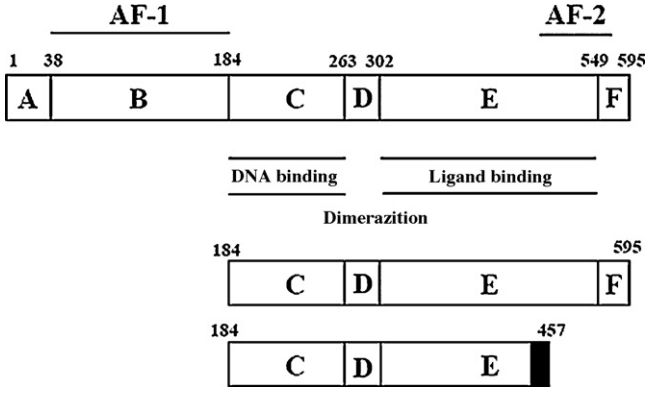

Fig. 2. Comparison of the domain structure of human ER-α isoforms.
Adapted from [16].

region of the ER-α36 gene located in the first intron of ER-α66 and constructed a luciferase reporter vector from the ER-α36 promoter. Upon the transient transfection of this construct into HEK293 cells, the promoter activity of this fragment possessed a high promoter activity. Furthermore, upon co-transfection of the construct and an ER-α66 expression vector into HEK293 cells, the promoter activity of ER-α36 was suppressed even in the presence of E2. These studies suggested that ER-α66 could suppress ER-α36 promoter activity in an E2-independent manner. Although ER-α36 lacks both AF-1 and AF-2 domains, it is deduced from its possession of the DNA-binding domain to influence the trans-activation of ER-α66 when it co-exists with ER-α66 in specific breast cancer cells. Wang et al. [30] constructed a reporter plasmid in which two EREs were located upstream of the thymidine kinase promoter. Upon co-transfection of this vector with the ER-α36 expression vector into HEK293 cells, no luciferase activity was detected in the cells in the presence or absence of estrogen, suggesting that ER-α36 lacks transcription function. Furthermore, when the reporter plasmid and the ER-α36 and ER-α66 expression vectors were transiently co-transfected into HEK293 cells, low levels of luciferase activity was noted. All of the data demonstrate that ER-α36 could inhibit the E2-independent and E2-dependent transactivation functions of ER-α66. ER-α36 may compete for binding to the EREs of the estrogen-response gene with ER-α66 and lead to the inhibition of genomic estrogen signaling mediated by ER-α66. Therefore, ER-α36 may be a potential inhibitory factor in the nuclear-initiated steroid signaling of ER-α [30].

In addition to its functions as a dominant-negative effector of ER-α66 transactivation functions through the genomic activity of the nuclear ER, ER-α36 mediates membrane-initiated signaling that is associated with its predominant expression on the plasma membrane and in the cytoplasm [19,20,22,31]. Wang et al. [20] isolated nuclear, plasma membrane, and cytosol subcellular fractions of HEK293 cells stably expressing exogenous ER-α36 and examined the localization of ER-α36 in these cell fractions by Western blot analysis, using the monoclonal antibody raised against ER-α36. The results demonstrated a high percentage of ER-α36 (50%) in the plasma membrane fraction and lower, but significant, amounts in the cytosol (40%) and the nuclei (10%) fractions. These data demonstrate that ER-α36 is highly expressed on the plasma membrane and is a membrane-based ER. Likewise, Lee et al. [22] performed immunohistochemistry assays using a specific ER-α36 antibody on tissue samples from 31 breast cancer patients. ER-α36 was predominantly expressed in the cytoplasm and plasma membrane, with little or no expression in nuclei. To further verify this conclusion, Lin et al. [19] carried out an immunofluorescence assay in MCF-7 and Hec1A cells with similar results. Together, these studies suggested that ER-α36 is primarily expressed on the plasma membrane and in the cytoplasm. Increasing evidence demonstrates that ER-α is located in the plasma membrane and cytoplasm and is involved in membrane-initiated nongenomic signaling pathways. It has been well documented that the ER located in the membrane and/or cytoplasm is able to activate growth factor tyrosine kinase receptors (TKRs), such as the epidermal growth factor receptor (EGFR) [32], human epidermal growth factor receptor 2 (HER2) [33], and the insulin-like growth factor I receptor (IGF-IR) [34], and cellular kinases, such as c-Src [35]. The interaction between the membrane/cytoplasmic ER and TKRs turns on TKR pathways and downstream kinases, e.g., mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and phosphoinositide 3-kinase (PI3K)/Akt and intracellular Ca2+ mobilization [36]. TKR-induced kinases may phosphorylate the nuclear ER and its co-activators as well as other transcription factors, resulting in enhanced gene expression and finally generating the specific physiological effect [37,38]. The previous study demonstrated that membrane ERα46 in human endothelial cells efficiently modulated MIES, such as eNOS activation [21]. ER-α36 is also membrane-based; thus, its involvement in similar membrane-initiated nongenomic activity was deduced and gradually verified. Using Western blot analysis, Wang et al. [20] detected E2-induced ERK1/2 phosphorylation in HEK293 cells that were transfected with an ER-α36 expression plasmid. The result demonstrated that ERK1/2 phosphorylation was increased approximately 10 fold in a time-dependent manner after E2 treatment for 5 min in cells transfected with an ER-α36 expression plasmid, with lower levels of phosphorylation in control cells transfected with an empty vector. To verify that ERK1/2 activation is initiated by a membrane-initiated estrogen-signaling pathway, strong activation of ERK1/2 phosphorylation was also observed when cells were treated with E2-BSA, a membrane-impermeable form of E2. Mek1 locates upstream of ERK1/2 in the MAPK/ERK signaling pathway, and upon phosphorylation, it can activate ERK1/2 [39]. In the same assay, Mek1 phosphorylation was detected in an apparent time-dependent manner [20]. Similarly, Kang et al. [31] detected ERK1/2 phosphorylation in SK-BR-3 cells treated with E2 but not in cells co-treated with E2 and the anti-ER-α36 antibody. When the SK-BR-3 cells were transfected with small hairpin RNA (shRNA) specific for ER-α36, the E2-induced phosphorylation of ERK1/2 was abrogated. Elk, a downstream effector of ERK1/2 in the MAPK/ERK signaling pathway, is involved in transcription activation [40]. Upon co-transfection of HEK293 with the ER-α36 expression vector, the Gal4-LUC vector (a luciferase reporter plasmid containing Gal4 DNA-binding sites) and the Gal-ELK vector (an expression vector containing an ELK transcriptional activation domain fused with the Gal4 DNA-binding domain), a consistent increase in the Elk_Gal4 fusion protein-mediated transactivation of the reporter gene was induced by E2 treatment [20]. These data strongly support the conclusion that ER-α36 is a membrane-based ER, which mediates E2-dependent activation of the MAPK/ERK signaling pathway. The PI3K/Akt signaling pathway is crucial in many cellular processes, existing in many cell types, and plays an important role in cell proliferation and survival [41]. Lin et al. [19] detected the activation of the PI3K/Akt signaling pathway in MCF-7 cells transfected with or without an ER-α36 expression vector; however, while E2-induced Akt phosphorylation could be detected in ER-α36-transfected cells, Akt phosphorylation was abrogated in the same cells pretreated with the PI3K inhibitor LY294002. Meanwhile, upon siRNA knockdown of ER-α36 expression, E2 treatment could not induce Akt phosphorylation in Hec1A cells. Together, these studies suggest that ER-α36 also mediates E2-dependent activation of the PI3K/Akt signaling pathway. It was reported that rapid calcium mobilization could be induced by estrogen and the GPR30-selective agonist G1 in GPR30-transfected cells [42], which was considered to be strong evidence for GPR30 acting as a novel ER, mediating nongenomic estrogen signaling [43–45]. However, the study of Kang et al. [31] demonstrated that the previously reported activities of GPR30 in response to estrogen were actually through its ability to induce expression of ER-α36. ER-α36, but not GPR30, acts as an extranuclear ER to mediate nongenomic estrogen signaling. SNCG (synuclein γ), previously identified as a breast cancer-specific gene, is highly expressed in breast cancer cells and predicts poor diagnosis. Studies demonstrated that SNCG participates in the functional molecular chaperone protein heat shock protein 90 (Hsp90)-based multichaperone complex for steroid receptors and stimulates ER-α66 transcriptional activity but does not affect ER-β signaling [46]. However, Shi et al. [47] demonstrated that SNCG is a dependent molecular chaperone protein for ER-α36 in the presence and absence of Hsp90, which can bind to ER-α36 both in in vitro cell-free systems and in breast cancer cells, and also is able to interact with ER-α36 in the presence of 17-AAG, which disrupts Hsp90 from its client protein ER-α66. SNCG prevents the degradation of ER-α36 and restores the down-regulation of MIES induced by Hsp90

disruption as well as significantly stimulates the mitogenic estrogen membrane-initiated signaling mediated by ER-α36. This evidence suggests that SNCG can maintain the stability and function of ER-α36, protect and stimulate ER-α36-mediated MIES and render cells resistant to tamoxifen.

To summarize, ER-α36 is generated from a promoter located in the first intron of the ER-α66 gene. As a novel variant of ER-α66, ER-α36 lacks both transcription activation domains of the wild-type receptor and functions as a dominant-negative effector of the transactivation activities of ER-α66 through complete inhibition of the genomic activity of ER-α66. ER-α36 primarily localizes to the plasma membrane and cytoplasm and mediates membrane-initiated nongenomic signaling pathways. ER-α36 may be involved in cell growth, proliferation and differentiation in carcinomas through these genomic and nongenomic mechanisms and may play an important role in the carcinogenesis and progression of tumors.

4. ER-α36 and breast cancer

4.1. ER-α36 may be involved in the carcinogenesis and progression of breast cancer

As described previously, ER-α36 is predominantly located on the plasma membrane and in the cytoplasm, and it can be detected in ER-positive and ER-negative human breast cancer [22]. Until now, the role of ER-α36 expression in the carcinogenesis and progression of breast cancer was unclear. Zheng et al. [48] analyzed ER-α36 messenger RNA levels in 74 paired samples of breast cancer and matched normal tissue using a PCR assay and then correlated the PCR results with the clinicopathological characteristics of these patients. Compared with the matched normal tissue, ER-α36 mRNA levels in breast cancer tissues were lower regardless of their ER-alpha66 expression status. Furthermore, the down-regulation of ER-alpha36 mRNA was correlated with local progression, lymph node metastasis, and advanced cancer stage, indicating that down-regulation of ER-α36 may be involved in the carcinogenesis and progression of breast cancer. Zhang et al. [27] investigated the role of mitogenic estrogen signaling mediated by ER-a36 in the malignant growth of triple-negative breast cancer (ER-a66-, PR- and Her2/neu-) MDA-MB-231 and MDA-MB-436 cells that express high levels of ER-a36. They found that ER-a36 mediates nongenomic and mitogenic estrogen signaling in these ER-negative breast cancer cells both in vitro and in vivo [27]. Through this function of ER-a36, E2 stimulated the malignant growth of the breast cancer cells, and knockdown of ER-a36 expression in these cells using the small hairpin RNA method diminished their responsiveness to estrogen. In addition, ER-a36 was found to interact physically with the EGFR/Src/Shc complex and mediated the estrogen-induced phosphorylation of EGFR and Src. EGFR signaling activated ER-a36 transcription through an AP1 site in the ER-a36 promoter, and ER-a36 expression was able to stabilize the EGFR protein. These results revealed a novel crosstalk mechanism between ER-α36 and the EGFR protein that allows these two receptors to regulate positively each other's expression. Furthermore, it was demonstrated that ER-a36 mediates nongenomic estrogen signaling through the EGFR/Src/ERK signaling pathway in ER-negative breast cancer cells, which may play an important role in the malignant growth of ER-negative breast cancer.

4.2. ER-α36 is an important determinant of breast cancer resistance to endocrine therapy

Breast cancer is a heterogeneous disease in which multiple genetic alterations, such as in ER and HER2, affect the clinical behavior of the disease and the response of patients to therapeutic interventions [49,50]. The status of ER and HER2 are considered two of the most important prognostic markers and predictors in the response of patients to endocrine therapy [51]. Three kinds of therapeutic approaches have been developed based on these particular molecular alterations in the tumors of breast cancer patients. The monoclonal antibody termed trastuzumab targets the receptor protein HER2 and is used for patients whose tumors express HER2 [52,53]. Endocrine therapy is used for patients whose tumors express the ER [54,55]. Cytotoxic chemotherapy is the best therapeutic approach for patients who develop resistance to trastuzumab and endocrine therapy and the other patients [56]. Currently, endocrine therapy of breast cancer includes a selective ER modulator (SERM) (such as tamoxifen) [57,58], the pure ER antagonist ICI 182 780 (fulvestrant) [59], and aromatase inhibitors (such as letrozole) [60,61]. Until now, the selective ER modulator tamoxifen was the most common endocrine therapy used for breast cancer. Unfortunately, at the time of diagnosis, approximately 40% of ER-positive tumors and all ER-negative tumors fail to respond to tamoxifen therapy. Therefore, breast cancer resistance to endocrine therapy is classified as either intrinsic resistance or acquired resistance [62]. Intrinsic resistance is de novo upon the initial exposure to endocrine therapy, while acquired resistance arises over time after an initial response to endocrine therapy. Regardless of any resistance, ER nongenomic signaling pathways may be one of the most important mechanisms for the development of endocrine therapy resistance in breast cancer [63]. Membrane and cytoplasmic ER-initiated estrogen signaling pathways can lead to the activation of downstream kinases in the EGFR/HER2 pathway such as PI3K/Akt and MAPK. This kinase activation can then lead to phosphorylation of the nuclear ER and its coactivators, thus up-regulating genomic ER activity and enhancing gene expression, including target genes in the EGFR/HER2 pathways. These gene products further augment EGFR/HER2 signaling, thus completing the cooperative cycle between the two activities of the ER and their crosstalk with the EGFR/HER2 signaling pathway. In HER2-overexpressing cells, the resulting activation of downstream kinases make the function of tamoxifen similar to that of estrogen in stimulating cell growth and finally lead to tamoxifen resistance [64–66]. Furthermore, resistance to aromatase inhibitors frequently occurs in clinical settings, possibly because estrogen withdrawal results in an adaptation to the environment that causes the ER in the tumor cells to be overly sensitive to estrogen or independent of estrogen [66,67]. The mechanisms of breast cancer resistance to endocrine therapy remain to be elucidated, and understanding the function of the key genetic alterations involved in the resistance will facilitate the development of new pharmaceutical compounds targeted at specific molecular components of the endocrine resistance pathways.

Because ER-α36 predominantly mediates nongenomic signaling pathways, it is suggested that ER-α36 plays an important role in breast cancer resistance to endocrine therapy. When ER-α36-transfected MCF-7 cells were treated with tamoxifen, a rapid phosphorylation of ERK1/2 and Akt was detected. However, in ER-α66-positive but ER-α36-negative MCF-7 cells, tamoxifen-induced phosphorylation of ERK1/2 and Akt was abrogated [20,22]. These studies demonstrated that tamoxifen can activate the ER-α36-mediated MAPK/ERK signaling pathway and the PI3K/Akt signaling pathway responsible for cell proliferation and survival and suggested that ER-α36-mediated non-genomic activity is involved in tamoxifen resistance. Thus, tamoxifen in ER-α36-positive breast cancer cells functions not as a drug for treatment but an agonist of ER-α36. The contrary effect of tamoxifen in breast cancer patients who highly express ER-α36 may explain why these patients require chemotherapy but not endocrine therapy [20,22]. Shi et al. [26] investigated whether ER-α36 expression is associated with

outcome in breast cancer patients treated with tamoxifen. In the first cohort of patients with ER-66-positive tumors who received tamoxifen treatment, the overexpression of ER-α36 was associated with a poorer disease-free survival (DFS) and disease-specific survival (DSS). However, in patients with ER-α66-positive tumors who were not treated with tamoxifen or with ER-α66-negative tumors regardless of tamoxifen treatment, expression of ER-α36 was not associated with survival. In another cohort of patients who only received tamoxifen as adjuvant therapy and among those with ER-α66-positive tumors, the overexpression of ER-α36 was significantly associated with a poorer DFS and DSS, and ER-α36 was shown to be an independent unfavorable factor for both DFS and DSS by a multivariate analysis. These studies concluded that patients with ER-α66-positive tumors that show high levels of expression of ER-α36 are unlikely to benefit from treatment with tamoxifen. These studies demonstrated that expression of ER-α36 could be involved in resistance to tamoxifen in breast cancer patients. Similarly, in breast cancers with ER-α36 overexpression, ER-α36 may also mediate aromatase inhibitor therapy resistance through a rapid membrane-initiated steroid signaling [31]. In ER-negative breast cancer cells that express endogenous ER-α36, high levels of ER-α36 expression may be involved in estrogen hypersensitivity. These cells, which are hypersensitive to estrogen, may provide an explanation for the failure of ER-negative breast cancer, which retains nongenomic estrogen signaling, to respond to aromatase inhibitors.

5. ER-α36 and other tumors

Previous studies have reported that gastric tumor tissues have negative or low ER expression levels [68]. However, recent studies show that ER-α36 is highly expressed in gastric tissues [24]. An indirect immunofluorescence assay with a specific anti-ER-α36 antibody revealed that ER-α36 is primarily expressed on the plasma membrane and in the cytoplasm of gastric cancers cell and that the high expression of ER-α36 is associated with lymph node metastasis. Thus, ER-α36 is considered to be a marker of gastric cancer metastasis.

Endometrial cancer is one of the most common gynecologic cancers, and studies revealed that ER-α36 is mainly expressed on the plasma membrane of ER-negative endometrial cancer Hec1A cells and mediates the testosterone-stimulated MAPK/ERK and PI3K/AKt signaling pathway [19,69], suggesting that ER-α36 may be involved in the carcinogenesis and progression of endometrial cancer. Tu et al. [70] further verified ER-α36 expression in the Hec1A endometrial cancer cell line. Furthermore, ER-α36 expression was increased in high-stage ($P = 0.03$) and high-grade ($P = 0.224$) tumor samples from endometrial cancer patients. This expression significantly correlates positively with EGFR expression, while the positive rate of phospho-ERK in the ER-α36 positive group and the EGFR positive group was higher than that in the ER-α36 negative group and the EGFR negative group. All of these results showed that ER-α36 mediated the EGF-stimulated ERK activation in Hec1A cells. Tong et al. [71] investigated the function and the underlying mechanisms of ER-a36 in the growth regulation of endometrial Ishikawa cancer cells. Their studies demonstrated that E2 activated the PKCδ/ERK pathway and enhanced cyclin D1/cdk4 expression via ER-α36-mediated MIES, suggesting that ER-a36 is a novel and important player in endometrial carcinogenesis.

6. Conclusions

ER-α36, a novel variant of ER-α66, lacks both AF-1 and AF-2 domains but retains the DNA-binding domain and partial dimerization and ligand-binding domains. In breast cancer and in many other tumor tissues or cells, ER-α36 is predominantly located in the plasma membrane and the cytoplasm and mediates the MIES in these tissues or cells. Additional data indicate that ER-α36 is involved in the resistance of breast cancer to endocrine therapy through its MIES activity.

A recent study demonstrated that ER-α36 could be expressed in mouse airway epithelial and smooth muscle cells in a predominantly membranous pattern and that its expression level could be up-regulated by allergen exposure, which was associated with allergen-induced airway hyperresponsiveness [72]. ER-α36 was also found to be strongly expressed in osteoblasts and osteoclasts from normal postmenopausal women, mediating a bone-sparing effect of E2 in postmenopausal women [73]. These results suggest that ER-α36 may be expressed in many other cells or tissues in addition to tumors and may be involved in mediating additional biologic functions.

The current research on ER-α36 has deepened our knowledge on its structure and function. However, the expression pattern of ER-α36 during carcinogenesis and progression of various cancers, including breast cancers, remain unclear. Moreover, little is known about the effect of ER-α36 on the clinical behavior and the responses to therapeutic interventions or the relationship of ER-α36 with the wild-type or other variants of ERs. As resistance often occurs in endocrine therapy of breast cancer, further studies undertaken to understand the mechanism of ER-α36 in the clinical endocrine therapy resistance of breast cancer will provide important clues to develop strategies for new therapy.

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