



# A Role for Estrogen Receptor alpha36 in Cancer Progression

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Estrogen receptor  $\alpha$  (ER $\alpha$ ) functions as a ligand dependent transcription factor that directly binds specific estrogen responsive elements, thus regulating the transcription of estrogen sensitive genes. ER $\alpha$  has also been shown to be associated with the plasma membrane (membrane associated ER $\alpha$ , mER $\alpha$ ), concentrated in lipid rafts, plasma membrane microdomains with a distinct lipid composition, where it transduces membrane-initiated estrogen-dependent activation of the mitogen-activated protein (MAP) kinase signaling pathway. Two isoforms of ER $\alpha$  have been described: the “traditional” ER $\alpha$ 66 (66 kDa) and a lower molecular weight variant: the ER $\alpha$ 46 (46 kDa). More recently, a novel ER $\alpha$  variant with a molecular mass of 36 kDa (ER $\alpha$ 36) has been discovered. Notably, ER $\alpha$ 36 has been found expressed in different human tumor cells, including both ER- positive and ER- negative breast cancer cells. Estrogen signaling at the cell membrane via ER $\alpha$ 36 appears as capable of activating multiple pathways of importance for cancer aggressiveness and metastatic potential. The presence of serum autoantibodies reacting with mER $\alpha$  (anti-ER $\alpha$  Abs) in a large percentage of patients with breast cancer has recently been reported by our group. These anti-ER $\alpha$  Abs seem to act as estrogen agonists rapidly triggering MAP kinase pathway activation thus inducing tumor cell proliferation and overcoming cell resistance to anti-estrogen drug tamoxifen. In this review, we describe the involvement of ER $\alpha$ 36 in different tumors. We also report the potential pathogenetic activity of anti-ER $\alpha$  Abs and their implication in drug resistance.

**Keywords:** estrogen, estrogen receptors, estrogen receptor 36, signaling, cancer, breast cancer, proliferation, autoantibodies

## INTRODUCTION

The biological effects of estrogen are mediated by specific receptors designated as estrogen receptors (ERs) (1). In humans, ERs play a key role in reproductive processes and are involved in the regulation of many physiological processes in several tissues, organs and systems such as central nervous system, cardiovascular and immune system. ERs belong to the steroid hormone superfamily of nuclear receptors, i.e., mainly detectable in the cell nucleus. However, ERs have also been found in the cytoplasm and even at mitochondrial level. Two different types of ERs have been identified: the estrogen receptor alpha (ER $\alpha$ ) (2, 3), and the estrogen receptors beta (ER $\beta$ ) (4, 5). Both ER $\alpha$  and ER $\beta$  are composed of several functional domains that serve specific roles (6, 7). Starting from NH<sub>2</sub>- to COO-terminus, the principal domains are (i) the N-terminal A/B domain (NTD); (ii) the DNA-binding domain (DBD); (iii) the ligand-binding domain (LBD). Two activation function (AF) domains, AF1 and AF2, located within the NTD and LBD, respectively, appear as responsible for regulating the transcriptional activity of ER. The regulatory mechanisms exerted by estrogens are mainly carried out *via* the control of gene transcription. This can

occur after activation and dimerization of ERs, by binding an estrogen response element (ERE, AGGTCA<sub>n</sub>TTGACCT). However, estrogen can also directly bind DNA through the involvement of cytoplasmic signaling proteins (8) and growth factor receptors (9, 10). Moreover, further estrogen signaling pathways have been observed. In fact, ERs can also be detected at the plasma membrane (membrane associated ER, mER), where they are embedded in lipid rafts, cholesterol enriched plasma membrane microdomains with a distinct lipid composition. These ERs can ignite non genomic pathways such as the activation of the mitogen-activated protein (MAP) kinase signaling pathway (11, 12). In the same vein, more recently, a seven-transmembrane receptor G protein-coupled receptor 30 (GPR30) structurally unrelated to the other ERs but able to mediate rapid non-genomic signals has also been identified (13).

In humans, the ER $\beta$  is 530 amino acids in length, with a molecular weight of 59 kDa and it is encoded by the gene estrogen receptor 2 (ESR2), located on chromosome 14, locus 14q23.2 (14). To date, three other truncated shorter isoforms at 54, 49, and 44 kDa and one elongated isoform at 61 kDa are known. The ER $\alpha$  is encoded by the ESR1 gene located on chromosome 6, locus 6q25.1 (15). The full-length size of ER $\alpha$  is 595 amino acids with a molecular weight of 66 kDa (ER $\alpha$ 66). In the last few years, two further shorter isoforms (at 46 and 36 kDa) have been characterized. ER $\alpha$ 46, the 46 kDa isoform of ER $\alpha$ , lacking of the N-terminal A/B or of the transcriptional activation domain 1 (AF-1), is expressed in various cell types, as macrophages (16), vascular endothelial cells (17), osteoblasts (18) and also in cancer cells. The other isoform, ER $\alpha$ 36, the 36 kDa isoform of ER $\alpha$ , differs from the classic ER $\alpha$ 66 in the lack of the AF-1 and AF-2 transcriptional activation domains but it retains the DNA-binding domain as well as the partial dimerization and ligand-binding domains (19). ER $\alpha$ 36 shares a common overall structure with ER $\alpha$ 46 but it is characterized by a unique 27 amino acids domain that replaces the last 138 amino acids encoded by both ER $\alpha$ 46 and ER $\alpha$ 66 gene. This unique amino acid sequence in ER36 may alter the ligand binding domain, which explains why ER36 has a different binding affinity. This receptor is mainly located in cytoplasm and at the plasma membrane of several different cancer cell types (19–21) and even in healthy tissues, among which ovarian, breast, kidney, lung, heart and bone (22).

## ESTROGEN RECEPTOR ALPHA 36 MOLECULAR MECHANISMS

ER $\alpha$ 36 is mainly involved in the initiation of non-genomic signaling pathways to activate the phosphatidylinositol-3-kinase/AKT (PI3K/AKT) and the mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) (19, 23). The interaction of ER $\alpha$ 36 with 17 $\beta$ -estradiol (E2) causes Src activation inducing downstream cascades: MEK activation, phosphorylation of ERK and paxillin (PXN), which induces a third messenger expression, cyclin D1 (24). ER $\alpha$ 36 also activates ERK1/2 through the protein kinase C (PKC) delta signaling pathway, and phospholipase D (PLD), leading to an increase in

the expression of cyclin D1/cyclin-dependent kinase 4, which regulates cell cycle progression, leading to an increase in the proliferative rate and to an enhancement of metastatic potential (25). Through ER $\alpha$ 36, E2 and tamoxifen induce the activation of MAPK/ERK and PI3K/AKT pathways that, in turn, regulate c-Myc protein expression, contributing to the cancer metastatic potential (26, 27). More in general, several studies suggest that ER $\alpha$ 36 could act as a negative dominant regulator of estrogen genomic signaling promoted by ER $\alpha$ 66 and ER $\beta$  (28). However, there is a positive feedback mechanism between ER $\alpha$ 66 and ER $\alpha$ 36. In fact, ER $\alpha$ 66 appears as able to suppress the ER $\alpha$ 36 activity. The loss of ER $\alpha$ 66 expression related to an increase in ER $\alpha$ 36 expression represents one of the mechanisms leading to the acquisition of resistance to antiestrogen therapy, e.g., by tamoxifen (28). This review highlights the effects of the ER $\alpha$ 36 on several different cancer cells types.

## ER $\alpha$ 36 IN DIFFERENT TYPES OF TUMORS

It is now known that estrogens, through their receptors, play an important role in the pathogenesis of many types of tumors. In particular, ER $\alpha$ 36, has been demonstrated to be involved in tumor progression and growth, metastatic potential, resistance to treatment and poor prognosis (29). A high expression of this isoform has been described in some types of tumors, such as renal cell carcinoma, papillary thyroid carcinoma, laryngeal carcinoma, endometrial carcinoma, hepatocarcinoma, gastric cancer, neuronal tumors (neuroblastoma and glioblastoma), and breast cancer. However, whether ER $\alpha$ 36 could play a role in other human cancers is still scarcely investigated and cannot be ruled out.

### Renal Cell Carcinoma

Dysregulated estrogen signaling contributes to the initiation and progression of renal cell carcinomas (30), but the mechanism has not been well established. Wang et al., in a retrospective study, hypothesized that ER $\alpha$ 36 may be involved in tumor progression (31). In this study the authors described a different expression level and cell localization of ER $\alpha$ 36 in malignant and benign renal tumor cells. In particular, a greater expression of ER $\alpha$ 36 in malignant tumor cells compared to benign ones with a predominantly cytoplasmic localization in the latter has been shown. Furthermore, the high expression levels of ER $\alpha$ 36 have been found related (i) to renal cell carcinoma necrosis, and (ii) to increased metastatic aggressiveness. Further studies appear as mandatory in order to evaluate the potential role of ER $\alpha$ 36 expression levels as prognostic markers in renal cell carcinoma and to differentiate benign from malignant tumors (31).

### Papillary Thyroid Carcinoma

Papillary thyroid cancer (PTC) represents about 80% of malignant thyroid tumors and is three times more common in women than in men suggesting a critical role of estrogen in its occurrence and development (32–34). Dai et al., analyzed the expression levels of ER $\alpha$ 36, in association with the epidermal growth factor receptor (EGFR) and HER2, in PTCs, nodular

hyperplasias and normal thyroid tissues (35). The results obtained highlight the existence of a significant correlation between the high levels of expression of ER $\alpha$ 36 in PTC tissues and the progression and increase of tumor metastases. In particular, by correlating the expression levels of ER $\alpha$ 36, EGFR and HER2 with the clinicopathological characteristics of PTC, high levels of these receptors significantly correlate with extrathyroidal extension and lymph node metastasis have been found. Conversely, there is no correlation between ER $\alpha$ 36 expression and the histologic subtype, age, gender and tumor size of PTC patients. Hence, ER $\alpha$ 36, in association with the expression of EGFR and HER2, could represent, if further validated, a possible marker of the tumor stage of PTC (35).

## Laryngeal Carcinoma

Significant differences between sexes have been described as concerns this carcinoma, with a male to female ratio of 11: 1 (36–38). This, suggests that sex hormones may be involved in the tumorigenesis of this form of cancer. Even though it is not uniformly accepted as a hormone-dependent tumor, laryngeal cancer expresses ER $\alpha$ 36. By *in vitro* studies using laryngeal carcinoma epithelial cells (Hep2), the binding of ER $\alpha$ 36 with E2 has been shown to induce a rapid activation of PKC and PLD, with an increase in the proliferative rate and in resistance to chemotherapy-induced apoptosis. In Hep2 cancer cell line, ER $\alpha$ 36 is located in caveolae (sphingolipid and cholesterol rich plasma membrane microdomains) in association with caveolin-1 and, after E2 treatment, induces an upregulation of angiogenic and metastatic factors (39). By immunohistochemical analysis of human laryngeal tumors, an association between the amount of ER $\alpha$ 36 and vascular endothelial-derived growth factor (VEGF) has been observed, supporting a role of ER $\alpha$ 36 in vascularization and metastasis (39).

## Endometrial Carcinoma

Endometrial carcinoma is one of the most frequent gynecological malignancies in women (40, 41). Among the most common risk factors are polycystic ovary syndrome, obesity (42) and prolonged exposure to endogenous estrogens (43). Estrogens, in addition to increasing the risk of tumor onset, play a key role in the development and progression of endometrial carcinoma (40). Comparing endometrial cancer cells to normal cells, an increase in aromatase activity, the enzyme that converts androgens into estrogens, has been observed (44).

Interestingly, endometrial carcinoma cells (Hec1A) express ER $\alpha$ 36 at plasma membrane and cytoplasm level (45). A positive correlation between ER $\alpha$ 36 and EGFR expression levels has been observed in Hec1A cultured cells suggesting the involvement of ER $\alpha$ 36 in the activation of the extracellular signaling linked to EGFR in endometrial carcinoma (45). Moreover, as observed in other tumors, ER $\alpha$ 36 promotes the agonist activity of tamoxifen in endometrial cancer cells (23). In fact, both estrogen and tamoxifen are able to promote the activation of the MAPK/ERK and PI3K/AKT pathways through ER $\alpha$ 36 (23). Furthermore, treatments with estrogens or tamoxifen induce the expression of the proto-oncogene c-Myc in Hec1A cells (23, 25). Therefore,

ER $\alpha$ 36 could be considered as a potential prognostic biomarker of endometrial carcinoma (46).

## Hepatocarcinoma

Hepatocellular carcinoma (HCC) is one of the most common malignancies and is the third cause of cancer-related death worldwide. The risk is increased in presence of chronic hepatitis and cirrhosis. The incidence of this carcinoma is 3 times higher in men than in women suggesting that sex hormones, estrogen in particular, could play a critical role in its development (47). In fact, some studies suggest that estrogens could exert a protective role in the development of HCC (48). Accordingly, the incidence of this carcinoma can be significantly lowered by estrogen treatment in post-menopausal women (49).

As concerns ERs, Miceli et al. (50) suggest that a switch from the expression of ER $\alpha$ 66 to the expression of ER $\alpha$ 36 could be associated with development and progression of human HCC. ER $\alpha$ 36, poorly expressed in normal hepatocytes, is instead well expressed by hepatocarcinoma cells and is localized at the plasma membrane as well as in the cytoplasm, supporting the idea that it could be involved in HCC development and/or progression (50). Other studies highlight more complex role of ER $\alpha$ 36. Its expression seems in fact to be higher in primary HCC in comparison with secondary HCC and it appears as inversely correlated to ER $\alpha$ 66 expression (21). Hence, these authors hypothesize that the expression level of ER $\alpha$ 36 might be considered a useful tool to differentiate the primary and secondary HCC. Further insights also derive from studies carried out with epigallocatechin-3-gallate (EGCG), a natural product that exerts its anti-cancer in HCC by inhibiting ER $\alpha$ 36 (51). Hence, ER $\alpha$ 36 appears to play a role in the pathogenesis of HCC, but further studies are needed to better understand the exact role of the different ER isoforms in this cancer.

## Gastric Cancer

To date, the molecular and cellular mechanisms involved in the development of gastric cancer are still to be elucidated. Some studies suggest a protective role for estrogen in the development of gastric cancer (52–54). In fact, the incidence of this cancer is higher in men than in women before menopause and tends to increase in women after menopause (53). Other data highlight an involvement of estrogen in the tumorigenesis of gastric cancer (55). In this regard, ER $\alpha$ 36 appears to be highly expressed in gastric cancer cell lines with a mainly cytoplasmic and/or surface localization. In addition, ER $\alpha$ 36 expression is significantly associated with lymph node metastasis but not with the other clinicopathological features of gastric adenocarcinoma. Therefore, the increase of ER $\alpha$ 36 expression in gastric adenocarcinoma and its association with metastasis could suggest that the evaluation of ER $\alpha$ 36 level could represent a prognostic biomarker for gastric cancer progression (56). However, opposite results have been reported by another study conducted by Wang et al. These authors reported a lower expression of ER $\alpha$ 36 in tumor tissues than in normal tissues and an expression level of ER $\alpha$ 36 negatively correlated with the tumor size and the number of metastases (57). On these bases, the need of further studies aimed at clarifying the effective

role of ER $\alpha$ 36 in gastric cancer onset and progression appears well evident.

## Neuroblastoma

Neuroblastoma is a very aggressive solid tumor that occurs most frequently in children after leukemia and brain cancer. It is an embryonic tumor that originates from the sympathetic nervous system (58). In recent years, several lines of evidence have demonstrated that ER $\alpha$  can contribute to neuroblastoma tumorigenesis. In particular, the expression of ER $\alpha$  appears to be related to neuronal differentiation and to the survival rate of patients with neuroblastoma (59, 60). *In vitro* studies in human neuroblastoma SH-SY5Y cells, the knocking down of the ER $\alpha$ 36 gene with the specific siRNA lead to a reduction of cell proliferation and an increase in apoptotic susceptibility. In particular, the silencing of ER $\alpha$ 36 seems to be associated to a reduction in protein phosphatase 2A (PP2A) activity (of importance in cellular homeostasis and tumor suppression) and an increase in phosphorylation of the tau protein (of importance in cytoskeletal integrity and function). In addition, ER $\alpha$ 36 gene silencing has been shown to reduce the expression of Cyclin D1, the proliferating cell nuclear antigen (PCNA) and B cell lymphoma-2 (Bcl-2) antiapoptotic protein while increasing the expression of proapoptotic protein Bax. Furthermore, the regulation of some pathways such as MAPK/ERK and PI3K/AKT has been shown to be dependent on the interaction between Caveolin-1 and ER $\alpha$ 36, i.e., on lipid raft function (61).

## Glioblastoma

Glioblastoma (GBM) is a highly aggressive and highly invasive primary brain tumor. Patients with GBM have a poor prognosis with an average survival of approximately 1 year (62). Although several studies have shown that adjuvant treatment with tamoxifen could be capable of sensitizing glioblastoma cells to radiation therapy also inhibiting their proliferation, this approach cannot be used for all types of glioblastoma. In addition, long-term use of tamoxifen can lead to the induction of tamoxifen resistance (63, 64). Therefore, several studies have been conducted to understand the molecular mechanism of tamoxifen resistance and to improve the quality of life of patients with glioblastoma (65). In this regard, an involvement of ER $\alpha$ 36, that is highly expressed in GBM, has been suggested as pivotal in the induction of resistance to tamoxifen treatment. In fact, Qu et al. observed that high levels of ER $\alpha$ 36 could block the tamoxifen-mediated cell growth inhibition and induce autophagy by hindering the AKT/mTOR signaling pathway. However, the effect of autophagy on tumor cell viability is still to be elucidated. Indeed, it seems that in conditions of nutrient and oxygen deficiency, when the tumor size increased, autophagy could promote the survival of cancer cells. Accordingly, *in vitro* studies on glioblastoma cells treated with tamoxifen showed a significant increase in ER $\alpha$ 36 expression level accompanied by an increased cytoprotection by autophagy. These results provide new insights into the mechanism underlying the antiproliferative, cytostatic, properties of tamoxifen and the involvement of ER $\alpha$ 36 in resistance to tamoxifen treatment (65) also suggesting the

contribution of autophagy pathway to the development and progression of glioblastoma (62).

## Breast Cancer

This is the most common form of cancer among women. About 70% of breast cancers express ER $\alpha$ 66, which is involved in the transcriptional regulation of estrogen-sensitive genes (66–68). Among the main therapeutic treatments used, there are anti-estrogens, which tend to block the molecular pathogenic pathways mediated by ER $\alpha$ 66. Unfortunately, many patients develop *de novo* or acquired resistance to these therapeutic agents, which is associated with the onset of metastasis and poor prognosis (69–71). Recently, ER $\alpha$ 36 has been found expressed in ER-positive and ER-negative breast cancer cells (20, 24, 72). ER $\alpha$ 36 expression levels have been associated with some clinicopathological features of breast cancer (tumor size, clinical stage, histological grade, lymph node metastasis) (28). Triple negative breast cancers (TNBC), i.e., lacking of ER $\alpha$ 66, progesterone receptor (PR) and epidermal growth factor receptor (EGFR), are very aggressive tumors with high recurrence, elevated mortality rates and limited therapeutic options. Maczisz et al., observed that ER $\alpha$ 36 is expressed in TNBC and is involved in E2-induced activation of sphingosine kinase 1 (SphK1) and for the production of shingosine-1-phosphate (S1P), which has a role in tumor growth, progression, transformation and metastasis (73). Interestingly, tamoxifen could act as an agonist of ER $\alpha$ 36 and induce proliferation, invasion and metastasis in breast cancer cells. In support of these data, Wang et al. observed that ER $\alpha$ 36, through the tamoxifen agonist activity, can be able to increase the expression of aldehyde dehydrogenase 1A1 (ALDH1A1), a molecule involved in cancer stem cell maintenance and metastasis. In particular, treatment with tamoxifen induces the nuclear translocation of the ER $\alpha$ 36 receptor, which directly regulates the transcription of ALDH1A1, suggesting a genomic-type mechanism of action in stem/progenitor cells of ER-positive breast cancers (29, 74).

Previous studies have shown that there are several molecular mechanisms and/or pathways involved in treatment resistance, including EGFR and HER2 (69, 75). Recently, the existence of a positive correlation between the levels of expression of ER $\alpha$ 36 and EGFR/HER2 has been suggested to be involved in the mechanism of resistance to tamoxifen and in the increased proliferative capacity of breast cancer (20, 27, 69, 75–77). The activation of the SphK1/S1P/S1PR axis mediated by E2 through ER $\alpha$ 36 could be one of the mechanisms that lead to the development of *de novo* and acquired resistance to anti-estrogenic therapy of breast cancer (73). In addition, a positive correlation between I-kappa-B-kinase-epsilon IKK $\epsilon$  (an oncogene, member of the IKK family) and ER $\alpha$ 36 has been observed (78). In particular, IKK $\epsilon$  interacts with ER $\alpha$ -36 and increases its expression. IKK $\epsilon$  seems to promote the mitogenic signaling of estrogens through ER $\alpha$ 36 with the consequent induction of proliferation in ER-negative breast cancer cells (78). To note, in ER-negative cancer cells, ER $\alpha$ 36 can inhibit and/or activate the EGFR/signal transducers and activators of transcription 5 (STAT5) pathway through the regulatory function of Src. *In vitro* studies on ER-negative cells show how low

concentrations the tamoxifen are able to induce the activation of MAPK/ERK pathway whereas at higher concentrations the signal is turned off. This could be explained by the fact that different concentrations of anti-estrogens could lead to different conformations and/or functions of ER $\alpha$ 36 (79). Overall, even though several molecular mechanisms are involved in the occurrence of anti-estrogenic resistance, ER $\alpha$ 36 seems to contribute to these mechanisms also playing a role in the maintenance of stem/progenitor cells of breast cancer.

Recently, the presence of serum autoantibodies reacting with ER $\alpha$  (anti-ER $\alpha$  Abs) in a large percentage of patients with breast cancer has been shown (80, 81). *In vitro* studies with anti-ER $\alpha$  Abs purified from sera of patients by affinity with the recombinant ER $\alpha$ 66, allowed us to observe that they bind to and activate ER $\alpha$  expressed at membrane level, located within the lipid rafts triggering rapid MAP kinase activation and inducing tumor cell proliferation (80). Moreover, anti-ER $\alpha$  Abs are also able to interfere with the efficacy of tamoxifen treatment suggesting that they can react and activate also the ER $\alpha$ 36 isoform (81). However, in contrast, anti-ER $\alpha$  Abs showed no reactivity with the ER $\alpha$ 66-negative, ER $\alpha$ 36 positive MDA-MB-231 cells, indicating that ER $\alpha$ 36 epitopes could be not accessible to antibodies, perhaps for conformational modification in these tumor cells. To note, treatment with simvastatin, removing or lowering cellular cholesterol, an integral component of lipid rafts (82, 83), inhibits anti-ER $\alpha$  Abs effect on proliferation and cell cycle progression (81).

Finally, even though it has been observed that cell surface ER could activate intracellular pathways, the precise mechanisms are still unknown. An interaction with receptor tyrosine kinases and/or with G protein-coupled receptors could be involved in downstream signaling pathways (e.g., phosphatidylinositol-3-kinase, Akt/protein kinase B and the mitogen-activated protein kinase cascade). Moreover, we also cannot exclude a cross-reaction of these autoantibodies with

GPR30 leading to a rapid non-genomic signals. In all cases, the identification of autoantibodies reacting with mER $\alpha$  could be considered as a potential prognostic and predictive tool for breast cancer.

## CONCLUSIONS

Summarizing, it can be hypothesized that ER $\alpha$ 36 could play an important role in estrogen signaling during the development and progression of several forms of cancer. However, to date, some open questions remain unanswered.

- (i) The first one regards the potential role of ER $\alpha$ 36 in further forms of human cancers, in particular, those presenting significant sex/gender differences in terms of incidence or progression such as melanoma and colon cancer.
- (ii) The second open question concerns the potential therapeutic usefulness of statins. These drugs, impairing ER $\alpha$ 36 function in patients with estrogen-dependent cancers, could exert some beneficial effect.

Further studies are thus mandatory in order to clarify both these aspects but, also, to better evaluate the role of ER $\alpha$ 36 in the clinical practice as prognostic biomarker and/or as therapeutic target leading to the reduction of tumor growth and progression and/or reducing the occurrence of anti-estrogenic therapy resistance.

## AUTHOR CONTRIBUTIONS

MP study conception and design, manuscript drafting. EO study conception and design, critical revision. MD study conception and design, manuscript drafting, critical revision. All authors contributed to the article and approved the submitted version.

## REFERENCES

1. Nilsson S, Makela S, Treuter E, Tujague M, Thomsen J, Andersson G, et al. Mechanisms of estrogen action. *Physiol Rev.* (2001) 81:1535–65. doi: 10.1152/physrev.2001.81.4.1535
2. Green S, Walter P, Kumar V, Krust A, Bornert JM, Argos P, et al. Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. *Nature.* (1986) 320:134–9. doi: 10.1038/320134a0
3. Greene GL, Gilna P, Waterfield M, Baker A, Hort Y, Shine J. Sequence and expression of human estrogen receptor complementary DNA. *Science.* (1986) 231:1150–4. doi: 10.1126/science.3753802
4. Kuiper GG, Enmark E, Peltö-Huikko M, Nilsson S, Gustafsson JA. Cloning of a novel receptor expressed in rat prostate and ovary. *Proc Natl Acad Sci USA.* (1996) 93:5925–30. doi: 10.1073/pnas.93.12.5925
5. Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Lett.* (1996) 392:49–53. doi: 10.1016/0014-5793(96)00782-X
6. Krust A, Green S, Argos P, Kumar V, Walter P, Bornert JM, et al. The chicken oestrogen receptor sequence: homology with v-erbA and the human oestrogen and glucocorticoid receptors. *EMBO J.* (1986) 5:891–7. doi: 10.1002/j.1460-2075.1986.tb04300.x
7. Schwabe JW, Teichmann SA. Nuclear receptors: the evolution of diversity. *Sci STKE.* (2004) 2004:pe4. doi: 10.1126/stke.2172004pe4
8. Farach-Carson MC, Davis PJ. Steroid hormone interactions with target cells: cross talk between membrane and nuclear pathways. *J Pharmacol Exp Ther.* (2003) 307:839–45. doi: 10.1124/jpet.103.055038
9. Losel R, Wehling M. Nongenomic actions of steroid hormones. *Nat Rev Mol Cell Biol.* (2003) 4:46–56. doi: 10.1038/nrm1009
10. Pedram A, Razandi M, Aitkenhead M, Hughes CC, Levin ER. Integration of the non-genomic and genomic actions of estrogen. Membrane-initiated signaling by steroid to transcription and cell biology. *J Biol Chem.* (2002) 277:50768–75. doi: 10.1074/jbc.M210106200
11. Levin ER, Hammes SR. Nuclear receptors outside the nucleus: extranuclear signalling by steroid receptors. *Nat Rev Mol Cell Biol.* (2016) 17:783–97. doi: 10.1038/nrm.2016.122
12. Marino M, Ascenzi P. Membrane association of estrogen receptor alpha and beta influences 17beta-estradiol-mediated cancer cell proliferation. *Steroids.* (2008) 73:853–8. doi: 10.1016/j.steroids.2007.12.003
13. Gonzalez de Valdivia E, Broselid S, Kahn R, Olde B, Leeb-Lundberg LMF. G protein-coupled estrogen receptor 1 (GPER1)/GPR30 increases ERK1/2 activity through PDZ motif-dependent and -independent mechanisms. *J Biol Chem.* (2017) 292:9932–43. doi: 10.1074/jbc.M116.765875
14. Enmark E, Peltö-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, et al. Human estrogen receptor beta-gene structure, chromosomal localization, and expression pattern. *J Clin Endocrinol Metab.* (1997) 82:4258–65. doi: 10.1210/jcem.82.12.4470

15. Gosden JR, Middleton PG, Rout D. Localization of the human oestrogen receptor gene to chromosome 6q24—q27 by in situ hybridization. *Cytogenet Cell Genet.* (1986) 43:218–20. doi: 10.1159/000132325
16. Murphy AJ, Guyre PM, Wira CR, Pioli PA. Estradiol regulates expression of estrogen receptor ERalpha36 in human macrophages. *PLoS ONE.* (2009) 4:e5539. doi: 10.1371/journal.pone.0005539
17. Li L, Haynes MP, Bender JR. Plasma membrane localization and function of the estrogen receptor alpha variant (ER46) in human endothelial cells. *Proc Natl Acad Sci USA.* (2003) 100:4807–12. doi: 10.1073/pnas.0831079100
18. Denger S, Reid G, Kos M, Flouriot G, Parsch D, Brand H, et al. ERalpha gene expression in human primary osteoblasts: evidence for the expression of two receptor proteins. *Mol Endocrinol.* (2001) 15:2064–77. doi: 10.1210/me.15.12.2064
19. Wang Z, Zhang X, Shen P, Loggie BW, Chang Y, Deuel TF. Identification, cloning, and expression of human estrogen receptor-alpha36, a novel variant of human estrogen receptor-alpha66. *Biochem Biophys Res Commun.* (2005) 336:1023–7. doi: 10.1016/j.bbrc.2005.08.226
20. Shi L, Dong B, Li Z, Lu Y, Ouyang T, Li J, et al. Expression of ER-alpha36, a novel variant of estrogen receptor {alpha}, and resistance to tamoxifen treatment in breast cancer. *J Clin Oncol.* (2009) 27:3423–9. doi: 10.1200/JCO.2008.17.2254
21. Zhang J, Ren J, Wei J, Chong CC, Yang D, He Y, et al. Alternative splicing of estrogen receptor alpha in hepatocellular carcinoma. *BMC Cancer.* (2016) 16:926. doi: 10.1186/s12885-016-2928-3
22. Soltysik K, Czekaj P. ERalpha36—Another piece of the estrogen puzzle. *Eur J Cell Biol.* (2015) 94:611–25. doi: 10.1016/j.ejcb.2015.10.001
23. Lin SL, Yan LY, Zhang XT, Yuan J, Li M, Qiao J, et al. ER-alpha36, a variant of ER-alpha, promotes tamoxifen agonist action in endometrial cancer cells via the MAPK/ERK and PI3K/Akt pathways. *PLoS ONE.* (2010) 5:e9013. doi: 10.1371/journal.pone.0009013
24. Omarjee S, Jacquemeton J, Poulard C, Rochel N, Dejaegere A, Chebaro Y, et al. The molecular mechanisms underlying the ERalpha-36-mediated signaling in breast cancer. *Oncogene.* (2017) 36:2503–14. doi: 10.1038/ncr.2016.415
25. Tong JS, Zhang QH, Wang ZB, Chen B. ER-alpha36, a novel variant of ER-alpha, mediates estrogen-stimulated proliferation of endometrial carcinoma cells via the PKCdelta/ERK pathway. *PLoS ONE.* (2010) 5:e15408. doi: 10.1371/journal.pone.0015408
26. Chaudhri RA, Olivares-Navarrete R, Cuenca N, Hadadi A, Boyan BD, Schwartz Z. Membrane estrogen signaling enhances tumorigenesis and metastatic potential of breast cancer cells via estrogen receptor-alpha36 (ERalpha36). *J Biol Chem.* (2012) 287:7169–81. doi: 10.1074/jbc.M111.292946
27. Li G, Zhang J, Jin K, He K, Zheng Y, Xu X, et al. Estrogen receptor-alpha36 is involved in development of acquired tamoxifen resistance via regulating the growth status switch in breast cancer cells. *Mol Oncol.* (2013) 7:611–24. doi: 10.1016/j.molonc.2013.02.001
28. Wang ZY, Yin L. Estrogen receptor alpha-36 (ER-alpha36): A new player in human breast cancer. *Mol Cell Endocrinol.* (2015) 418 (Pt 3):193–206. doi: 10.1016/j.mce.2015.04.017
29. Su X, Xu X, Li G, Lin B, Cao J, Teng L. ER-alpha36: a novel biomarker and potential therapeutic target in breast cancer. *Onco Targets Ther.* (2014) 7:1525–33. doi: 10.2147/OTT.S65345
30. Kabat GC, Silvera SA, Miller AB, Rohan TE. A cohort study of reproductive and hormonal factors and renal cell cancer risk in women. *Br J Cancer.* (2007) 96:845–9. doi: 10.1038/sj.bjc.6603629
31. Wang Q, Zhang W, Yang J, Liu YL, Yan ZX, Guo ZJ, et al. High ERalpha36 expression level and membrane location predict poor prognosis in renal cell carcinoma. *Medicine.* (2015) 94:e1048. doi: 10.1097/MD.0000000000001048
32. Hima S, Sreeja S. Modulatory role of 17beta-estradiol in the tumor microenvironment of thyroid cancer. *IUBMB Life.* (2016) 68:85–96. doi: 10.1002/iub.1462
33. Li H, Li J. Thyroid disorders in women. *Minerva Med.* (2015) 106:109–14.
34. Przybylik-Mazurek E, Hubalewska-Dydejczyk A, Fedorowicz A, Pach D. Factors connected with the female sex seem to play an important role in differentiated thyroid cancer. *Gynecol Endocrinol.* (2012) 28:150–5. doi: 10.3109/09513590.2011.563909
35. Dai YJ, Qiu YB, Jiang R, Xu M, Zhao L, Chen GG, et al. Concomitant high expression of ERalpha36, EGFR and HER2 is associated with aggressive behaviors of papillary thyroid carcinomas. *Sci Rep.* (2017) 7:12279. doi: 10.1038/s41598-017-12478-1
36. Cook MB, Dawsey SM, Freedman ND, Inskip PD, Wichner SM, Quraishi SM, et al. Sex disparities in cancer incidence by period and age. *Cancer Epidemiol Biomarkers Prev.* (2009) 18:1174–82. doi: 10.1158/1055-9965.EPI-08-1118
37. Dorsey M, Benghuzzi H, Tucci M, Cason Z. Growth and cell viability of estradiol and IP-6 treated Hep-2 laryngeal carcinoma cells. *Biomed Sci Instrum.* (2005) 41:205–10.
38. Hagedorn HG, Nerlich AG. Analysis of sex-hormone-receptor expression in laryngeal carcinoma. *Eur Arch Otorhinolaryngol.* (2002) 259:205–10. doi: 10.1007/s004050100400
39. Schwartz N, Chaudhri RA, Hadadi A, Schwartz Z, Boyan BD. 17Beta-estradiol promotes aggressive laryngeal cancer through membrane-associated estrogen receptor-alpha 36. *Horm Cancer.* (2014) 5:22–32. doi: 10.1007/s12672-013-0161-y
40. Shang Y. Molecular mechanisms of oestrogen and SERMs in endometrial carcinogenesis. *Nat Rev Cancer.* (2006) 6:360–8. doi: 10.1038/nrc1879
41. Chaudhry P, Asselin E. Resistance to chemotherapy and hormone therapy in endometrial cancer. *Endocr Relat Cancer.* (2009) 16:363–80. doi: 10.1677/ERC-08-0266
42. Kaaks R, Lukanova A, Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. *Cancer Epidemiol Biomarkers Prev.* (2002) 11:1531–43.
43. Navaratnarajah R, Pillay OC, Hardiman P. Polycystic ovary syndrome and endometrial cancer. *Semin Reprod Med.* (2008) 26:62–71. doi: 10.1055/s-2007-992926
44. Bulun SE, Simpson ER. Aromatase expression in women's cancers. *Adv Exp Med Biol.* (2008) 630:112–32. doi: 10.1007/978-0-387-78818-0\_8
45. Tu BB, Lin SL, Yan LY, Wang ZY, Sun QY, Qiao J. ER-alpha36, a novel variant of estrogen receptor alpha, is involved in EGFR-related carcinogenesis in endometrial cancer. *Am J Obstet Gynecol.* (2011) 205:227 e1–6. doi: 10.1016/j.ajog.2011.04.015
46. Sun L, Wang J, Zhang L, Li X, Shen D. Expression of ER-alpha36, a novel variant of estrogen receptor in endometrial carcinoma and its clinical significance. *Gynecol Obstet Invest.* (2012) 75:68–72. doi: 10.1159/000339933
47. Shi L, Feng Y, Lin H, Ma R, Cai X. Role of estrogen in hepatocellular carcinoma: is inflammation the key? *J Transl Med.* (2014) 12:93. doi: 10.1186/1479-5876-12-93
48. Villa E. Role of estrogen in liver cancer. *Womens Health.* (2008) 4:41–50. doi: 10.2217/17455057.4.1.41
49. Shimizu I. Impact of oestrogens on the progression of liver disease. *Liver Int.* (2003) 23:63–9. doi: 10.1034/j.1600-0676.2003.00811.x
50. Miceli V, Cocciaferro L, Fregapane M, Zarcone M, Montalto G, Polito LM, et al. Expression of wild-type and variant estrogen receptor alpha in liver carcinogenesis and tumor progression. *OMICS.* (2011) 15:313–7. doi: 10.1089/omi.2010.0108
51. Chen J, Chen L, Lu T, Xie Y, Li C, Jia Z, et al. ERalpha36 is an effective target of epigallocatechin-3-gallate in hepatocellular carcinoma. *Int J Clin Exp Pathol.* (2019) 12:3222–34.
52. La Vecchia C, D'Avanzo B, Franceschi S, Negri E, Parazzini F, Decarli A. Menstrual and reproductive factors and gastric-cancer risk in women. *Int J Cancer.* (1994) 59:761–4. doi: 10.1002/ijc.2910590609
53. Palli D, Cipriani F, Decarli A, Galli M, Saieva C, Fraumeni JF, et al. Reproductive history and gastric cancer among post-menopausal women. *Int J Cancer.* (1994) 56:812–5. doi: 10.1002/ijc.2910560609
54. Tokunaga A, Nishi K, Matsukura N, Tanaka N, Onda M, Shirota A, et al. Estrogen and progesterone receptors in gastric cancer. *Cancer.* (1986) 57:1376–9. doi: 10.1002/1097-0142(19860401)57:7<1376::AID-CNCR2820570722>3.0.CO;2-Z
55. Freedman ND, Ahn J, Hou L, Lissowska J, Zatonski W, Yeager M, et al. Polymorphisms in estrogen- and androgen-metabolizing genes and the risk of gastric cancer. *Carcinogenesis.* (2009) 30:71–7. doi: 10.1093/carcin/bgn258
56. Deng H, Huang X, Fan J, Wang L, Xia Q, Yang X, et al. A variant of estrogen receptor-alpha, ER-alpha36 is expressed in human gastric cancer and is highly correlated with lymph node metastasis. *Oncol Rep.* (2010) 24:171–6. doi: 10.3892/or\_00000842

57. Wang J, Li J, Fang R, Xie S, Wang L, Xu C. Expression of ERalpha36 in gastric cancer samples and their matched normal tissues. *Oncol Lett.* (2011) 3:172–5. doi: 10.3892/ol.2011.437
58. Tsubota S, Kadamatsu K. Origin and initiation mechanisms of neuroblastoma. *Cell Tissue Res.* (2018) 372:211–21. doi: 10.1007/s00441-018-2796-z
59. Cao P, Feng F, Dong G, Yu C, Feng S, Song E, et al. Estrogen receptor alpha enhances the transcriptional activity of ETS-1 and promotes the proliferation, migration and invasion of neuroblastoma cell in a ligand dependent manner. *BMC Cancer.* (2015) 15:491. doi: 10.1186/s12885-015-1495-3
60. Loven J, Zinin N, Wahlstrom T, Muller I, Brodin P, Fredlund E, et al. MYCN-regulated microRNAs repress estrogen receptor-alpha (ESR1) expression and neuronal differentiation in human neuroblastoma. *Proc Natl Acad Sci USA.* (2010) 107:1553–8. doi: 10.1073/pnas.0913517107
61. Wang HB, Li T, Ma DZ, Zhi H. ERalpha36 gene silencing promotes tau protein phosphorylation, inhibits cell proliferation, and induces apoptosis in human neuroblastoma SH-SY5Y cells. *FASEB J.* (2018) 32:fj201701386. doi: 10.1096/fj.201701386
62. Qu C, Ma J, Zhang Y, Han C, Huang L, Shen L, et al. Estrogen receptor variant ER-alpha36 promotes tamoxifen agonist activity in glioblastoma cells. *Cancer Sci.* (2019) 110:221–34. doi: 10.1111/cas.13868
63. Parney IF, Chang SM. Current chemotherapy for glioblastoma. *Cancer J.* (2003) 9:149–56. doi: 10.1097/00130404-200305000-00003
64. Puchner MJ, Giese A. Tamoxifen-resistant glioma-cell sub-populations are characterized by increased migration and proliferation. *Int J Cancer.* (2000) 86:468–73. doi: 10.1002/SIC11097-02152000051586:4468::AID-IJC43.0.CO;2-R
65. Liu Y, Huang L, Guan X, Li H, Zhang QQ, Han C, et al. ER-alpha36, a novel variant of ERalpha, is involved in the regulation of Tamoxifen-sensitivity of glioblastoma cells. *Steroids.* (2016) 111:127–33. doi: 10.1016/j.steroids.2016.02.009
66. Davies C, Godwin J, Gray R, Clarke M, Cutter D, Darby S, et al. Relevance of breast cancer hormone receptors and other factors to the efficacy of adjuvant tamoxifen: patient-level meta-analysis of randomised trials. *Lancet.* (2011) 378:771–84. doi: 10.1016/S0140-6736(11)60993-8
67. Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet.* (2005) 365:1687–717. doi: 10.1016/S0140-6736(05)66544-0
68. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin.* (2009) 59:225–49. doi: 10.3322/caac.20006
69. Musgrove EA, Sutherland RL. Biological determinants of endocrine resistance in breast cancer. *Nat Rev Cancer.* (2009) 9:631–43. doi: 10.1038/nrc2713
70. Ali S, Coombes RC. Endocrine-responsive breast cancer and strategies for combating resistance. *Nat Rev Cancer.* (2002) 2:101–12. doi: 10.1038/nrc721
71. Osborne CK, Schiff R. Mechanisms of endocrine resistance in breast cancer. *Annu Rev Med.* (2011) 62:233–47. doi: 10.1146/annurev-med-070909-182917
72. Lee LM, Cao J, Deng H, Chen P, Gatalica Z, Wang ZY. ER-alpha36, a novel variant of ER-alpha, is expressed in ER-positive and -negative human breast carcinomas. *Anticancer Res.* (2008) 28:479–83. 74.
73. Maczys MA, Maceyka M, Waters MR, Newton J, Singh M, Rigsby MF, et al. Sphingosine kinase 1 activation by estrogen receptor alpha36 contributes to tamoxifen resistance in breast cancer. *J Lipid Res.* (2018) 59:2297–307. doi: 10.1194/jlr.M085191
74. Wang Q, Jiang J, Ying G, Xie XQ, Zhang X, Xu W, et al. Tamoxifen enhances stemness and promotes metastasis of ERalpha36(+) breast cancer by upregulating ALDH1A1 in cancer cells. *Cell Res.* (2018) 28:336–58. doi: 10.1038/cr.2018.15
75. Montaser RZ, Coley HM. Crosstalk between ER $\alpha$  and receptor tyrosine kinase signalling and implications for the development of anti-endocrine resistance. *Cancers.* (2018) 10:209. doi: 10.3390/cancers10060209
76. Teymourzadeh A, Mansouri S, Farahmand L, Hosseinzade A, Majidzadeh AK. ER-alpha36 interactions with cytosolic molecular network in acquired tamoxifen resistance. *Clin Breast Cancer.* (2017) 17:403–7. doi: 10.1016/j.clbc.2017.03.013
77. Li G, Zhang J, Xu Z, Li Z. ER $\alpha$ 36 as a potential therapeutic target for tamoxifen-resistant breast cancer cell line through EGFR/ERK signaling pathway. *Cancer Manag Res.* (2020) 12:265–75. doi: 10.2147/CMAR.S226410
78. Li Q, Sun H, Zou J, Ge C, Yu K, Cao Y, et al. Increased expression of estrogen receptor alpha-36 by breast cancer oncogene IKKepsilon promotes growth of ER-negative breast cancer cells. *Cell Physiol Biochem.* (2013) 31:833–41. doi: 10.1159/000350101
79. Zhang X, Ding L, Kang L, Wang ZY. Estrogen receptor-alpha 36 mediates mitogenic antiestrogen signaling in ER-negative breast cancer cells. *PLoS ONE.* (2012) 7:e30174. doi: 10.1371/journal.pone.0030174
80. Maselli A, Capoccia S, Pugliese P, Raggi C, Cirulli F, Fabi A, et al. Autoantibodies specific to estrogen receptor alpha act as estrogen agonists and their levels correlate with breast cancer cell proliferation. *Oncimmunology.* (2015) 5:e1074375. doi: 10.1080/2162402X.2015.1074375
81. Maselli A, Parlato S, Puglisi R, Raggi C, Spada M, Macchia D, et al. Autoantibodies specific to ERalpha are involved in tamoxifen resistance in hormone receptor positive breast cancer. *Cells.* (2019) 8:750. doi: 10.3390/cells8070750
82. Pajak B, Wojewodzka U, Gajkowska B, Orzechowski A. Lipid rafts in anticancer therapy: theory and practice (review). *Mol Med Rep.* (2008) 1:167–72.
83. Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol.* (2000) 1:31–9. doi: 10.1038/35036052

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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