

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



ER-Alpha36 Mediates Non-Genomic Estrogen and Anti-Estrogen Signaling in Breast Cancer Cells

ZhaoYi Wang, XianTian Zhang and LianGuo Kang
*Department of Medical Microbiology & Immunology,
Creighton University Medical School, Omaha, NE,
USA*

1. Introduction

Estrogen signaling is essential in the initiation and development of neoplasia in mammary gland. In the past several decades, extensive efforts have been dedicated to understand the underlying mechanisms of this important signaling pathway in mammary carcinogenesis, which have facilitated the development of anti-estrogen therapy, the first targeted therapy for human cancer.

It has been well documented that the diverse activities of estrogens and anti-estrogens are mediated by specific receptors designated as estrogen receptors (ERs). Currently, there are two identified ERs, ER- α and ER- β , both of which are ligand-activated transcription factors that stimulate target gene transcription. Compelling evidence indicates that estrogens, especially 17 β -estradiol (E2 β), up-regulate the expression and function of c-Myc and cyclin D1, activate cyclin E-Cdk2 complexes and promotes cell cycle progression from G1 to S phase in mammary epithelial cells. Thus, stimulation of target gene expression by ERs in response to estrogens is prevailingly thought to be responsible for estrogen-stimulated mammary carcinoma initiation and progression.

Despite the clarity with which ERs have been shown to act as ligand-dependent transcription factors, it became apparent now that not all of the activities mediated by ERs are accomplished through a direct effect on gene transcription. Another signaling pathway (also known as a non-classic, non-genomic, extra-nuclear or membrane-initiated signaling pathway) exists that involves cytoplasmic signaling proteins, growth factor receptors and other membrane-initiated signaling pathways. Several intracellular signaling pathways have been shown cross-talking with the membrane-initiated estrogen signaling: the adenylate cyclase pathway, the phospholipase C pathway, the G-protein-coupled receptor-activated pathways, the PI3K/AKT and the MAPK pathways.

Currently, the identity of the membrane-based estrogen receptor that mediates non-genomic estrogen effects has not been fully established. ER- β , two forms of ER- α , full-length (66-kDa) and short form (46-kDa) have been found on or near the plasma membrane and mediates membrane-initiated estrogen signaling. In addition, there is also evidence indicating that GPR30, an orphan G-protein-coupled-receptor, mediates the rapid, non-genomic responses to estrogens. In 2005, our laboratory identified and cloned a novel variant of ER- α , which

has a molecular weight of 36-kDa and thus we have termed it ER- α 36. This ER- α variant differs from the original 66 kDa ER- α (ER- α 66) by lacking both transcriptional activation domains (AF-1 and AF-2) but retaining the DNA-binding domain and partial dimerization and ligand-binding domains. ER- α 36 is predominantly expressed on the plasma membrane of both ER-positive and -negative breast cancer cells, mediates membrane-initiated estrogen and anti-estrogen signaling such as activation of the MAPK/ERK and PI3K/AKT signaling pathways and stimulation of cell proliferation. Thus, ER- α 36 is a novel membrane-associated estrogen receptor that mediates membrane-initiated estrogen and anti-estrogen signaling in both ER-positive and -negative breast cancer cells.

In this chapter, we highlight the historical understanding of non-genomic estrogen signaling and its role in cell survival and proliferation, and specifically illustrate the biological function and the possible underlying mechanisms of ER- α 36 in non-genomic estrogen and anti-estrogen signaling in both ER-positive and -negative breast cancer cells. We also discuss the integral roles of EGFR, Src and STAT5 in the non-genomic estrogen signaling mediated by ER- α 36 and the complex regulatory network among ER- α 66, 46 and 36. In addition, the novel biology of non-genomic estrogen signaling mediated by ER- α 36 also has significance for understanding the physiology of bone remodeling. Thus, ER- α 36-mediated signaling has a broad appeal in physiology of non-classical estrogen targeting tissues and general cancer research.

2. Estrogen receptors and genomic estrogen signaling

Estrogen signaling is pivotal in the establishment and maintenance of reproductive function in men and women. It is also involved in normal development and physiology of bone, liver, cardiovascular and neuronal systems. Inappropriate estrogen signaling is involved in osteoporosis, atherosclerosis and Alzheimer's disease and is essential in the initiation and development of neoplasia in breast and endometrial cancers (Nilsson et al., 2001). Hence, it is important to understand the molecular mechanisms by which this important signaling pathway functions, so its participation in a wide variety of different biological processes can be clarified, including how its dysregulation contributes to different diseases and even neoplastic transformation.

The diverse physiological and pathological functions of estrogens are mediated by specific receptors designated as estrogen receptors (ERs). In 1986, the cloning of the estrogen receptor (ER) was first reported (Green et al., 1986; Greene et al., 1986). Until 1996, it was assumed that there was only one ER responsible for all of the physiological and pharmacological effects of natural and synthetic estrogens and anti-estrogens. However, in 1996, a second ER was cloned (Kuiper et al., 1996). Currently, the first ER discovered is referred to as ER- α , while the second is called ER- β .

ER- α and ER- β share a common structural architecture (Reviewed by Kong et al., 2003; Zheng et al., 2003). Both are composed of three independent but interacting functional domains: the N-terminal A/B domain, the C or DNA-binding domain, and the D/E/F or ligand-binding domain. The N-terminal domain of ERs encodes a ligand-independent activation function (AF-1), a region involved in interaction with co-activators, and transcriptional activation of target genes. The DNA-binding domain or C domain contains a two zinc-finger structure, which is involved in receptor dimerization and binding to specific DNA sequences. The C-terminal E/F domain is a ligand-binding domain that mediates ligand binding, receptor dimerization, nuclear translocation, and a ligand-dependent

transactivation function (AF-2). The relative contributions that both AF-1 and AF-2 exert on transcriptional control vary in a cell-specific and DNA promoter-specific manner (Berry et al., 1990; Tzukerman et al., 1994).

It is well established that estrogen receptors are members of the nuclear receptor superfamily of ligand-activated transcription factors that control various physiological processes. It is prevalently thought that this control often occurs through the regulation of gene transcription (Reviewed by Katzenellenbogen & Katzenellenbogen, 2000; McDonnell & Norris, 2002). The estrogen receptor utilizes multiple mechanisms to either activate or repress transcription of its target genes. These mechanisms include: (a) direct interaction of the ligand-occupied receptor with DNA at a consensus estrogen response elements (ERE, GGTCAnnnTGACC) followed by recruitment of transcriptional coregulator or mediator complexes (Reviewed by Klinge, 2001), (b) interaction of the ligand-occupied ER with other transcription factors such as AP-1 (Kushner et al., 2000), Sp1 (Safe, 2001) or NF- κ B (McKay & Cidlowski, 1999), or (c) indirect modulation of gene transcription via sequestration of general/common transcriptional components (Harnish et al., 2000; Speir et al., 2000). In addition, the ability of an estrogen receptor to regulate transcription through these mechanisms appears to be cell-type specific, perhaps due to differences in the complement of transcriptional co-regulatory factors available in each cell type (Cerillo et al., 1998; Evans et al., 2001; Maret et al., 1999). Also, transcriptional regulation is dependent upon the nature of the ligand, with various natural and synthetic selective estrogen receptor modulators (SERM) acting as either estrogen receptor agonists or antagonists through these various mechanisms (Shang & Brown, 2002; Katzenellenbogen & Katzenellenbogen, 2002; Margeat et al., 2003).

3. Estrogen signaling and breast cancer

Experimental and clinical evidence for the role of endogenous estrogens in normal development of mammary glands and breast cancer etiology has been well documented (Reviewed by Feigelson & Henderson, 1996). Estrogen signaling is involved in mammary epithelial cell proliferation and differentiation. Dysregulated estrogen signaling increases the rate of cell proliferation and thus the risk for development of breast cancer.

ER- α is expressed in approximately 15-30% of luminal epithelial cells and not at all in any other cell types in the normal human breast (Clarke *et al.*, 1997). However, dual label immunofluorescent technique revealed that ER- α expressing cells are separate from those labeled with proliferation markers such as Ki67 and cyclin A in both normal human and rodent mammary glands (Clarke et al., 1997; Zeps et al., 1999). This is in direct contrast to estrogen action in breast cancer cells, where estrogens, especially 17 β -estradiol (E2 β), functions as potent mitogens through promoting cell cycle progression from G1 to S phase (Prall et al., 1998; Altucci et al., 1996 & 1997). Thus, it is thought that estrogens directly interact with ERs in ER-positive human breast cancer cells, where they induce transcriptional activation of "immediate early" and cyclin genes, and promote cell cycle progression. Consistent with its role in mammary carcinogenesis, ER- α expression is increased at the earliest stages of ductal hyperplasia and increases even more with increasing atypia, such that most cells in atypical ductal hyperplasias and in ductal cancer in situ of low and intermediate grade contain ER- α (Khan et al., 1994). As ER- α expression increases during breast cancer development, the inverse relationship between ER- α expression and cell proliferation become dysregulated (Shoker *et al.*, 1999 a and b).

Approximately 70% of invasive breast carcinomas express the ER- α and most of these tumors contain ER- α positive proliferating cells (Clarke *et. al.*, 1997).

Currently, ER- α is the most widely used marker in diagnosis of human breast cancer. Breast cancers are diagnosed either as ER-positive or -negative depending on the existence or absence of ER- α . At the onset, 46%-77% of breast cancers are ER-positive (Robertson *et. al.*, 1996). The ER-positive tumors are histologically well differentiated and diploid, and patients with ER-positive tumors generally have a better prognosis since they respond well to anti-estrogen treatment (Clark *et. al.*, 1984; Osborne *et. al.*, 1980). ER-negative breast cancers that account for about one third of breast cancers diagnosed, however, are more malignant and aggressive through a still unknown mechanism (Sheikh *et. al.*, 1994), and they generally respond poorly to anti-estrogen treatment.

ER-negative breast cancers are often considered to be the result of tumor progression from ER-positive premalignant lesions or ER-positive breast cancers by epigenetic alterations such as promoter methylation (Ferguson *et. al.*, 1995) or ER- α protein degradation by the proteasome system after hypoxia in non-vascularized tumors (Stoner *et. al.*, 2002). However, immunohistochemical studies in human breast cancers showed that some proliferative ductal lesions and many high-grade ductal carcinomas in situ (DCIS) are ER- α -negative (Roger *et. al.*, 2000), suggesting some ER-negative breast cancers may be progressed directly from ER-negative premalignant lesions.

4. Non-genomic estrogen signaling

It became clear now that estrogens elicit two signaling pathways; the first requires hours to days to result in transcriptional changes of target genes and is known as genomic, classic or nuclear signaling pathway while the second occurs in seconds to minutes after estrogen treatment and usually initiates at the plasma membrane. Thus, the second signaling pathway is also known as a non-classic, non-genomic, extra-nuclear or membrane-initiated signaling pathway which is mediated by estrogen binding proteins associated with the plasma membrane and employs various cytoplasmic signaling proteins, growth factor receptors and G-protein-coupled-receptor signaling pathways (Segars & Driggers, 2002; Driggers & Segars, 2002; Kelly & Levin, 2001; Levin, 2002; Hammes & Levin, 2007).

Pietras and Szego first described the rapid estrogen signaling more than 30 years ago (Pietras & Szego, 1975 & 1977), noting immediate calcium fluxes in endometrial cells induced by estrogen and specific binding sites at the outer surfaces of isolated endometrial cells. Quick estrogen responses were also recorded in neuroendocrine tissue such as a rapid rise of intracellular calcium in pituitary cells, which leads to cell depolarization within 1 min (Dufy *et. al.*, 1979). Rapid estrogen signaling also induces prolactin secretion from pituitary tumor cells (Watson *et. al.*, 1999). Such signaling of estrogens has also been described in uterine (Aronica *et. al.*, 1994) and ovarian cells (Morley *et. al.*, 1992; Tesarik & Mendoza, 1997) as well as bone (Endoh *et. al.*, 1997; Longo *et. al.*, 2004; Sylvia *et. al.*, 2001), vascular endothelial (Russell *et. al.*, 2000; Lu *et. al.*, 2004) and neuronal cells (Kelly *et. al.*, 1977; Mermelstein *et. al.*, 1996), indicating that the rapid, non-genomic estrogen signaling is involved in various physiological and pathological estrogen activities.

Non-genomic estrogen signaling has been also documented in breast cancer cells for cell proliferation and survival (Song *et. al.*, 2002; Migliaccio *et. al.*, 1996; Ahmad *et. al.*, 1999; Lobenhofer *et. al.*, 2000; Castoria *et. al.*, 1999 & 2001). These findings strongly suggest that the

rapid, non-genomic estrogen signaling is involved in estrogen-induced proliferation in breast cancer cells.

5. Nature of membrane-based estrogen receptor

While it is clear that membrane-initiated estrogen signaling exists, the identity of the membrane-based estrogen receptor that mediates these rapid estrogen effects, especially the effects in mammary epithelial cell proliferation and survival, has not been fully established. Laboratory evidence indicated that both ER- α and ER- β are involved in the rapid, non-genomic estrogen signaling (Razandi et al., 1999). However, evidence also suggests that more than one membrane-initiated signaling pathway is associated with estrogen action. Data from several laboratories using the membrane-impermeable compound 17 β -estradiol-bovine serum albumin (E2 β -BSA) indicates the existence of two functionally distinct membrane-associated pathways: one sensitive to anti-estrogens and one resistant (Chen et al., 1999; Russell et al., 2000; Watters et al., 1997). For example, ER- α /knockout mice retained rapid estrogen-stimulated membrane effects in neurons, which were not blocked by anti-estrogen ICI-182,780 (Gu, et. al., 1999). These findings suggest that another membrane-based ER may exist since all known ERs are sensitive to anti-estrogen inhibition. As a consequence, an orphan G-protein coupled receptor, GPCR30, and some other unknown proteins have been reported to mediate this anti-estrogen resistant non-genomic estrogen signaling.

6. ER- α

A large body of experimental evidence indicated that ER- α is involved in both genomic and non-genomic estrogen signaling. Immunofluorescent staining of non-permeabilized pituitary tumor cells with anti-ER- α antibodies revealed a punctuated staining pattern on cell surface although most ER- α were localized in cell nuclei (Watson et al., 1999). ER- α associated with the plasma membrane is also detected by a panel of antibodies targeting different domains of ER- α in intact breast cancer cells and in breast cancer specimens (Pietras et al., 2005; Kim et al., 2006). The findings that transfection of the nuclear ER- α cDNA into ER-“null” Chinese hamster ovary (CHO) cells leads to expression of both membrane and nuclear ER- α further support the notion that membrane-associated ER- α is derived from the same transcript as nuclear ER- α (Razandi et al., 1999). However, recent evidence indicates that the CHO-K1, Rat2-fibroblasts and COS7 cells previously considered as ER “null” cells and extensively used to transfect ER cDNAs in order to demonstrate rapid estrogen signaling actually exhibit strong non-genomic estrogen signaling such as activation of the MAPK/ERK before transfection (Nethrapalli et al., 2005), suggesting these cells already possess some unknown proteins other than classical ERs that mediate the rapid estrogen signaling.

It is widely accepted that estrogen signaling promotes cell proliferation in target cells. However, the explanation of this effect of estrogen signaling simply by the function of ER- α is confounding. When ER- α was expressed in ER-“null” CHO cells (Kushner *et. al.*, 1990), and human cervical cancer HeLa cells (Touitou *et. al.*, 1990), E2 β failed to stimulate cell growth. On the contrary, E2 β inhibited cell proliferation and even induced cell apoptosis. Likewise, the ER-negative immortal MCF10A breast epithelial cells (Pilat *et. al.*, 1996), and MDA-MB-231 breast cancer cells (Jiang & Jordan, 1992) were both growth inhibited by

estrogen when stably transfected with ER- α cDNA. These experimental results argue against a positive function of the well-known ER- α alone in mitogenic estrogen signaling.

7. ER- β

ER- β acts as a classical ligand-induced transcription factor. Like ER- α , ligand-bound ER- β functions by regulating downstream target genes. ER- β also heterodimerizes with ER- α and modulates ER- α function (Reviewed by Harris, 2007; Deroo & Buensuceso, 2010). ER- β has been shown to be S-palmitoylated, which facilitates the localization of ER- β at the plasma membrane to mediate the rapid, non-genomic estrogen effects (Marino & Ascenzi, 2008). Indeed, the involvement of ER- β in rapid, non-genomic estrogen signaling has been documented when ER- β cDNA was introduced into CHO cells (Razandi et al., 1999). In colon cancer, pro-apoptotic activities of ER- β have been reported to be mediated by membrane-initiated signaling; induction of ER- β via the MAPK/p38 signaling pathway, which in turn leads to downstream apoptotic events (Caiazza et al., 2007), indicating a potential role of ER- β as a tumor suppressor. In addition, inhibition of ER- β palmitoylation in colon cancer cells abrogated the pro-apoptotic activity of ER- β (Galluzzo et al., 2007), suggesting that non-genomic effects mediated by membrane-associated ER- β is important for its pro-apoptotic function.

The biological significance of ER- β in breast cancer has not been well established. Approximately 70% of breast tumor express ER- β and most tumor co-express both ER- α and - β (Dotzlaw et al., 1997; Fuqua et al., 1999 & 2003). Studies indicated that ER- β expression in human breast cancer is associated with a poorer prognosis, compared with tumors that only express ER- α (Speirs et al., 1999). ER- β expression is associated with elevated levels of proliferation markers, Ki67 and cyclin A, in human breast cancer (Jensen et al., 2001) These studies suggested that ER- β may promote cell proliferation and breast cancer progression.

In contrast, accumulating evidence indicated that ER- β acts as a tumor suppressor in breast cancer. ER- β is expressed in both normal and malignant mammary glands (Warner et al., 2000; Speirs et al., 2002). In the rodent mammary gland, ER- β expressing cells can proliferate but the majority cells that express proliferation markers do not express either ER (Saji et al., 2001). The levels of ER- β expression are highest in normal mammary gland and are decreased as tumors progress from pre-invasive to invasive (Leygue et al., 1998; Iwao et al., 2000; Roger et al., 2001). The presence of ER- β in breast cancer confers a more favorable prognosis and is associated with node-negative, low-grade tumors (Jarvinen et al., 2000) as well as a greater disease-free survival rate (Omoto et al., 2001). Several laboratory studies demonstrated that ER- β inhibited angiogenesis and malignant growth of T47D breast tumor xenograft and malignant growth of MCF7 breast cancer cells *in vitro* and in nude mice (Hartman et al., 2006; Paruthiyil et al., 2004). Intriguingly, ER- β also inhibited proliferation of ER-negative breast cancer MDA-MB-231 cells in a ligand-independent manner whereas ER- α inhibition of MDA-MB-231 cell proliferation is estrogen-dependent (Lazennec et al., 2001). These results indicate that ER- β negatively regulates mitogenic estrogen signaling.

8. ER- α 46

Previously, it was reported that a 46-kDa antigen is tightly associated with ER- α in human breast cancer samples (Diaz-Chico *et. al.*, 1988). This 46-kDa antigen is enriched in the cell

cytosol and could be recognized by an isolated monoclonal antibody, E476, raised against the human ER- α (Diaz-Chico *et. al.*, 1988). Similarly, Jozan (1991) later reported that there are two species of ER with different molecular weight (65 and 47 kDa), and three species of tumors (36% containing the highest form of ER alone, 49% bearing the two forms in variable amounts, and 15% bearing only the minor form). These results strongly suggest that two different forms of ER- α exist at different ratios in human breast cancer.

In 2000, Flouriot *et. al.* cloned a 46-kDa isoform of ER- α and demonstrated that the 46-kDa isoform lacks the first 173 amino acids (A/B or AF-1 domain) and is derived from alternative splicing of the ER- α gene by skipping exon 1. This alternative splicing event generates an mRNA that has an AUG in a favorable Kozak sequence for translation initiation in frame with the remainder of the open reading frame of ER- α (Flouriot *et. al.*, 2000). This new isoform of ER- α is named as ER- α 46 and the original one as ER- α 66. ER- α 46 forms homo-dimers and binds to an ERE, and it can also form heterodimers with ER- α 66 (Flouriot *et. al.*, 2000). Furthermore, the ER- α 46/66 heterodimers form preferentially over the ER- α 66 homodimers and ER- α 46 acts competitively to inhibit transactivation mediated by AF-1 domain of ER- α 66 but without effect on AF-2-dependent transactivation (Floutiot *et. al.*, 2000). Therefore, ER- α 46 is a naturally occurring isoform of ER- α that regulates genomic estrogen signaling mediated by the AF-1 domain of ER- α 66.

Previously, two forms of ER- α , full-length (66-kDa) and short form (46-kDa) were co-purified with 5' nucleotidase, a plasma membrane-marker enzyme (Marquez & Pietras, 2001), suggesting a possible role of ER- α 46 in the rapid, non-genomic estrogen signaling. Recently, ER- α 46 was localized on the plasma membrane, in the cytosol, and nucleus of endothelial cells and mediated rapid estrogen signaling such as activation of the Src/PI3K/AKT signaling and stimulation of NO synthesis (Kim & Bender, 2005; Li *et. al.*, 2003; Reviewed by Moriarty *et al.*, 2006), further confirming that the ER- α 46 isoform functions as a membrane-associated estrogen receptor.

Although it is clear now that ER- α 46 is involved in both genomic and non-genomic estrogen signaling, its function in breast cancer has been less investigated. Forced expression of ER- α 46 inhibited proliferation of MCF-7 breast cancer cells and cyclin D1 promoter activity (Penot *et al.*, 2005). Overexpression of both ER- α 46 and ER- α 66 in ER-negative MDA-MB-231 cells revealed that ER- α 46 inhibited basal transcription of the estrogen responsive gene pS2 while estrogen treatment released this inhibition (Metivier *et al.*, 2004). Recently, expression levels of ER- α 46 was found to be down-regulated in tamoxifen-resistant breast cancer cells and re-introduction of ER- α 46 into these cells inhibited cell proliferation and ER- α 66-regulated gene transcription (Klinge *et al.*, 2010). Thus, these results suggest that ER- α 46 may function as a negative-regulator of mitogenic estrogen signaling in ER-positive breast cancer cells.

9. ER- α 36

In 2005, our laboratory identified a 5.4 kb cDNA clone from a normal human endometrium cDNA library (RZPD clone number: DKFZp686N23123) and later cloned this cDNA from a human placenta cDNA library (Wang *et al.*, 2005). This cDNA clone harbors a 310 amino acid open-reading frame that can produce a protein with a predicted molecular weight of 35.7 kDa. To differentiate it from ER- α 66 and 46, and apply consistent nomenclature, this novel isoform of ER- α was named as ER- α 36.

The cDNA sequence of the open-reading frame matches 100% to the DNA sequence of the exons 2 to 6 of the ER- α 66 gene. The 5' untranslated region (5'UTR) of the cDNA showed 100% homology to the DNA sequence of the first intron of the ER- α 66 gene. Thus, the transcript of this ER- α isoform is initiated from a previously unidentified promoter in the first intron of the ER- α 66 gene (Figure 1, Zou et al., 2009). A small, non-coding novel exon in the first intron of the ER- α 66 gene was designated as exon 1'. The exon 1' is then spliced directly into the exon 2 of the ER- α 66 gene and continues from exon 2 to exon 6 of the ER- α 66 gene. Exon 6 is then spliced to an exon located 64,141 bp downstream of the ER- α 66 gene (Figure 1). The cDNA sequence encoding the last 27 amino acids and the 4,293 bp 3' untranslated region (3'UTR) was matched 100% to a continuous sequence from the genomic sequence of clone RP1-1304 on chromosome 6q24.2-25.3 (GeneBank accession number AL78582), indicating the remaining cDNA sequence of this novel ER- α isoform is transcribed from one big exon of 4,374 bp located downstream of the ER- α 66 gene. This exon is thus designated as exon 9 to reflect the extra exon beyond the previous reported eight exons for ER- α 66 gene (Figure 1). All of these splicing events are supported by the identification of perfect splice donors and acceptors at the splice juncture. The protein ER- α 36 can be produced from a perfect Kozak sequence located in the second exon, the same initiation codon used to produce ER- α 46 (Flouriot *et al.*, 2000). ER- α 36 differs from the ER- α 66 by lacking both transcriptional activation domains (AF-1 and AF-2) but retaining the DNA-binding and dimerization domains, and partial ligand-binding domains. It also possesses an extra, unique 27 amino acid domain to replace the last 138 amino acids encoded by exon 7 and 8 of the ER- α 66 (Figure 2). Thus, ER- α 36 is another naturally occurring novel isoform of ER- α that may play an important role in both genomic and non-genomic estrogen signaling. Currently, there are three major ER- α isoforms observed in ER-positive breast cancer MCF7 cells that correspond to three mRNA variants generated from different promoter usage and alternative splicing (Figure 2).

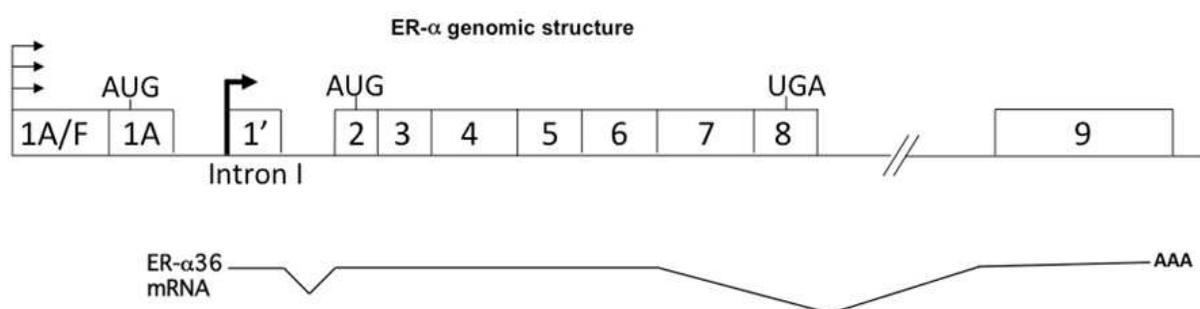


Fig. 1. Genomic organization of the human ER- α 36 gene. The locations of multiple promoters of human ER- α 66 gene are shown as arrows. The translation start and stop codons are indicated as AUG and UGA. The common exons are shown as numbered open boxes. The extra exon that is beyond the 8 exons of the human ER- α 66 gene is numbered as 9 in the open box. The intron 1 is also shown with the exon 1' in the open box. The lower panel shows mRNA structure of human ER- α 36 isoform.

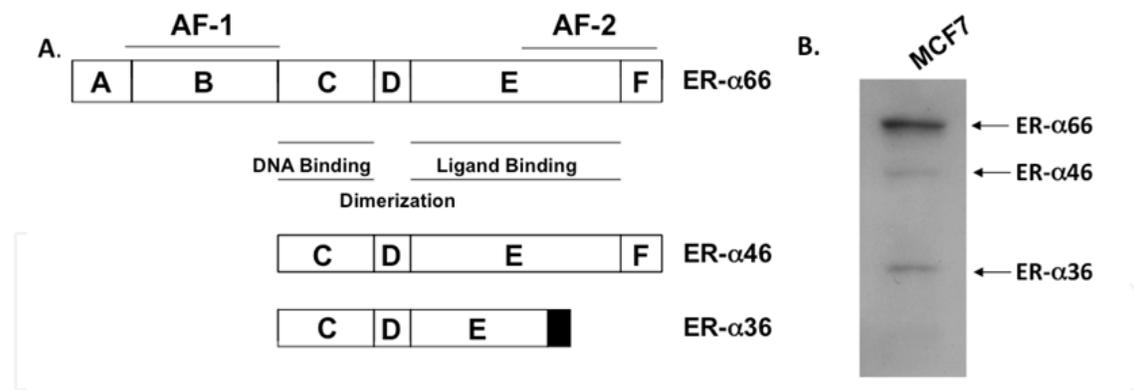


Fig. 2. Domain structure and expression of human estrogen receptor- α variants in ER-positive breast cancer MCF7 cells. (A). Domain structure representation of human ER- α isoforms. Domains (labeled A-F), and activation function domains (AF-1 and -2) are shown. The function of each domain is indicated. The last 27 amino acids of human ER- α 36 are indicated as a filled box. (B). Western blot analysis of three human ER- α isoforms in MCF7 cells with an anti-ER- α 66 antibody (H222).

10. GPR30

Previously, different laboratories demonstrated the existence of two distinct membrane-associated pathways: one sensitive to anti-estrogens and one resistant (Chen et al., 1999; Russell et al., 2000; Watters et al., 1997). These results suggest that another membrane-associated estrogen receptor may exist since both ER- α and - β are sensitive to anti-estrogen inhibition.

An orphan G protein-coupled receptor, GPR30 was reported to mediate the rapid, non-genomic estrogen signaling that was insensitive to ICI 182,780; estrogen stimulates changes of Ca^{2+} currents and cAMP signaling in cells expressing GPR30 (Revankar et al., 2005) and activates the MAPK/ERK phosphorylation and the PI3K/Akt signalling pathways via transactivation of the EGFR pathway in ER-negative but GPR30-positive breast cancer cells (Filardo et al., 2000 & 2007). Thus, GPR30 was considered as a novel type of membrane-associated estrogen receptor that mediates the rapid, non-genomic estrogen signaling.

There are also reports that challenge the role of GPR30 as a novel estrogen receptor. A study showed that introduction of GPR30 anti-sense oligonucleotides failed to block the MAPK/ERK activation and cell growth induced by estrogen in ER-positive breast cancer cells (Ahola et al., 2002). Pedram *et al.* (2006) failed to observe the cAMP or ERK activation in GPR30-positive, ER-negative breast cancer cells. Another study demonstrated that the GPR30 selective agonist G1 failed to exert estrogenic effect in two classical estrogen target organs, the uterus and the mammary gland (Otto et al., 2008). Recently, Otto *et al.* generated GPR30-deficient mice and demonstrated that the development of reproductive organs was unimpaired in these mice and the estrogenic responses in the uterus and the mammary gland were completely maintained in GPR30-deficient animals (Otto et al., 2009).

Recently, our group reported that knockdown of GPR30 expression in ER-negative breast cancer SK-BR-3 cells down-regulated the expression levels of ER- α 36 (Kang et al., 2010b). Introduction of a GPR30 expression vector into GPR30 non-expressing cells induced endogenous ER- α 36 expression and GPR30 activated the promoter activity of ER- α 36 via an AP-1 binding site located in the 5'-flanking region of ER- α 36 (Kang et al., 2010b). Thus, ER-

$\alpha 36$ is a downstream target gene of GPR30-mediated signaling and the previously reported activities of GPR30 as a membrane-based estrogen receptor are through its ability to induce ER- $\alpha 36$ expression.

11. Other putative estrogen receptors

Other unknown proteins that may be involved in the rapid, non-genomic estrogen signaling have been also reported. For an example, a report that E2 β activated the MAPK/ERK signaling in un-transfected CHO-K1, COS7 and Rat2-fibroblasts (Nethrapalli et al., 2005) suggested the existence of an unidentified membrane-associated mER. Recently, we reported that un-transfected COS7 cells express high levels of endogenous ER- $\alpha 36$ (Kang et al., 2010b) and found that CHO-K1 cells also express ER- $\alpha 36$ (Kang et al., unpublished observations), suggesting that ER- $\alpha 36$ may mediate the rapid, non-genomic estrogen signaling observed in these cells.

Another unique membrane-associated ER with an estimated molecular weight of 63-65 kDa, referred to as ER-X, is developmentally regulated differently from both ER- α and - β . Its pharmacological profile was also different from ER- α and - β , even with some features opposite to those shown for these two receptors (Toran-Allerand et al., 2002). For examples, ER-X mediated rapid estrogen signaling was not sensitive to anti-estrogen, and the association of Hsp90 is required for the inactive state of ER- α while ER-X requires to be associated with hsp90 for its activity (Toran-Allerand et al., 2002). Whereas ER-X shares limited similarities with ER- $\alpha 36$, such as reaction with antibodies to the ligand-binding domain of ER- $\alpha 66$ and responding equally to 17 α - and β -estradiol, the molecular similarity of these two receptors awaits for the cloning and sequencing of ER-X.

Additionally, a heterodimeric estrogen-binding protein, referred to as the putative ER (pER), was reported to bind to E2 β at a sub-nanomolar affinity but was unable to bind other estrogens or anti-estrogens. Depending on cell types, pER is expressed on the plasma and/or nuclear membranes or in the cytoplasm and nucleus (Rao et al., 1998). Since the polyclonal anti-pER antibody failed to react with estrogen receptors and was unable to detect pER expression in reproductive organs (Rao et al., 1998), the role of this putative estrogen receptor in non-genomic estrogen signaling of breast cancer cells remains elusive.

12. Negative regulation of genomic estrogen signaling by ER- $\alpha 36$

Comparison of protein structures of ER- $\alpha 66$ and ER- $\alpha 36$ indicated that ER- $\alpha 36$ lacks both AF-1 and -2 transcription activation domains but retains the DNA-binding domain and dimerization domain. Transient co-transfection assays using a luciferase-expressing reporter construct that contains two Estrogen Response Element (ERE) placed upstream of the thymidine kinase promoter (2 X ERE-tk-Luc) revealed that ER- $\alpha 36$ has no intrinsic transcriptional activity in the presence and absence of E2 β (Wang et al., 2006). However, ER- $\alpha 36$ strongly inhibited the transactivation activities mediated by the AF-1 and -2 domains of both ER- $\alpha 66$ and ER- β (Wang et al., 2006). These data indicate that ER- $\alpha 36$ itself is unable to mediate genomic estrogen signaling by regulate target gene expression. However, ER- $\alpha 36$ acts as a naturally occurring dominant-negative regulator of the genomic estrogen signaling mediated by the AF1 and AF2 domains of ER- $\alpha 66$ and ER- β .

13. Membrane-association and mitogenic signaling of ER- α 36

The fact that ER- α 36 possesses no intrinsic transcription regulatory activity suggests that ER- α 36 may be a membrane-associated estrogen receptor. The sub-cellular fractionation assay in ER- α 36 transfected HEK293 cells revealed that a high percentage of ER- α 36 (~50%) is localized on or near the plasma membrane and a low percentage of it in cytosol (~40%) and nucleus (~10%) (Wang *et al.*, 2006). Immuno-fluorescence staining of intact breast cancer MCF7 cells and endometrial cancer Hec1A cells using the ER- α 36 specific antibody recognizing the last 20 amino acids that are unique to ER- α 36 exhibits a membrane expression pattern that is co-localized with cavin-1, a typical cell-surface protein (Lin *et al.*, 2010). Immunohistochemistry analysis of specimens from breast cancer patients also demonstrated an expression pattern of ER- α 36 predominantly on the breast cancer cell surface (Lee *et al.*, 2008; Vranic *et al.*, 2011). This anti-ER- α 36 specific antibody also blocked ER- α 36-mediated non-genomic estrogen signaling such as activation of the MAPK/ERK signaling in breast cancer cells presumably through steric hindrance of estrogen accesses to its binding pocket (Kang *et al.*, 2010b), indicating that the antibody is accessible to the C-terminal region of the plasma membrane-associated ER- α 36 in intact cells. These results thus demonstrated that ER- α 36 is expressed on or near the plasma membrane and suggested that ER- α 36 may be also shuttled to the cell cytoplasm and nucleus depending on cell context and extracellular signals.

Both 17 β -estradiol (E2 β) and BSA-conjugated E2 β elicit the rapid, membrane-initiated estrogen signaling such as activation of the MAPK/ERK signaling pathway and stimulation of cell proliferation in ER- α 36 transfected HEK293 cells, which is not blocked by anti-estrogens such as tamoxifen, 4-hydroxy-tamoxifen and ICI 182, 780 (Wang *et al.*, 2006). In addition, other estrogens including estrone (E1), 17 α -estradiol (E2 α), estriol (E3) and estetrol (E4) all activate the ERK1/2 phosphorylation at a very similar level (Wang *et al.*, 2006). Recently, it was reported that ER- α 36 even mediated testosterone-stimulated activation of the MAPK/ERK and PI3K/Akt signaling pathways in endometrial cancer Hec1A cells (Lin *et al.*, 2009). ER- α 36 also mediates estrogen activation of the PKC δ /ERK signaling pathway (Tong *et al.*, 2010). The finding that the non-genomic estrogen signaling mediated by ER- α 36 was insensitive to anti-estrogens suggests that ER- α 36 may be a receptor involved in the anti-estrogen-insensitive estrogen signaling described in different systems before. Taken together, ER- α 36 is a membrane-associated estrogen receptor that mediates rapid and mitogenic estrogen signaling.

14. ER- α 36 in anti-estrogen signaling and anti-estrogen resistance

Since mitogenic estrogen signaling plays a pivotal role in development and maintenance of ER-positive breast cancer, treatment with anti-estrogens such as tamoxifen (TAM) has become a first-line therapy for advanced ER-positive breast cancer. However, laboratory and clinical evidence indicated that TAM and its metabolites such as 4-hydroxytamoxifen (4-OHT) have mixed agonist/antagonist or estrogenic/anti-estrogenic actions depending on cell and tissue context, and the agonist activity of tamoxifen may contribute to tamoxifen resistance observed in almost all patients treated with tamoxifen. As a consequence, a more potent and "pure" anti-estrogen, ICI 182, 780 (Fulvestrant, Faslodex) has been developed (Reviewed by Howell *et al.*, 2000).

TAM and 4-OHT are thought to function as antagonists by competing with E2 β and other estrogens for binding to ERs. Further structural studies revealed that TAM induces an ER- α conformation that does not recruit coactivators to trans-activate target genes but recruits corepressors (Shang *et al.*, 2000), suggesting that TAM- and 4-OHT-bound ER- α 66 is unable to effectively activate genes involved in cell growth and breast cancer development. On the other hand, ICI 182, 780, a 'pure' antiestrogen without estrogenic activity, works in a different mechanism. ICI 182, 780 binds to ERs, impairs receptor dimerization and inhibits nuclear localization of receptor (Fawell, *et al.*, 1990; Dauvois *et al.*, 1992). Furthermore, ICI 182, 780 also accelerates degradation of the ER- α 66 protein without a reduction of ER- α 66 mRNA (Nicholson *et al.*, 1995). Thus, ICI 182, 780 binds ER- α 66 and accelerates degradation of ER- α 66 protein, resulting in a complete inhibition of estrogen signaling mediated by ER- α 66.

Although ICI 182, 780 has been depicted as a non-agonist or 'full' or 'pure' anti-estrogen, different laboratories documented estrogenic agonist activities of ICI 182, 780 in different systems. Estrogenic agonist activity of ICI 182, 780 has been found in hippocampal neurons and in bone cells where ICI 182, 780 promoted bone growth (Zhao *et al.*, 2006; Sibonga *et al.*, 1998). Agonist-like activities of ICI 182, 780 have also been reported in tamoxifen-resistant KPL-1 breast cancer cells (Kurebayashi *et al.*, 1998) and Yeast (Dudley *et al.*, 2000). Both tamoxifen and ICI 182, 780 were also reported to induce phosphorylation of the adhesion molecules p130Cas/BCR1, FAK and Src in ER-positive breast cancer MCF7 cells (Cowell *et al.*, 2006). The molecular mechanisms by which ICI 182, 780 acts as an estrogenic agonist have never been elucidated. Studies from a number of laboratories suggested that a membrane associated estrogen-binding receptor mediates the agonist actions of ICI 182, 780 in neurons (Reviewed by Brinton 2001; Zhao *et al.*, 2005; McEwen, 2002).

As described above, ER- α 36 mediated non-genomic estrogen signaling is insensitive to anti-estrogens such as TAM and ICI 182, 780 (Wang *et al.*, 2006). ER- α 36 also mediates agonist activities of tamoxifen and ICI 182, 780 such as activation of the MAPK/ERK and the PI3K/AKT signaling pathways in breast and endometrial cancer cells (Wang *et al.*, 2006; Lin *et al.*, 2010). ICI 182, 780 failed to induce degradation of ER- α 36 (Kang *et al.*, 2010a) presumably because ER- α 36 has a truncated ligand-binding domain that lacks the last 4 helices (helix 9-12) of ER- α 66 (Wang *et al.*, 2005). The helix-12 domain is critical in protein degradation induced by ICI 182, 780 and different positioning of the helix 12 and the F domain of ER- α 66 regulates functional differences between agonists and antagonists (Mahfoudi *et al.*, 1995; Pearce *et al.*, 2003). Thus, it is possible that ER- α 36 is a receptor that mediates agonist activities of TAM and ICI 182, 780 recorded in different systems.

Despite the significant anti-neoplastic activities of anti-estrogens, most breast tumors are eventually resistant to anti-estrogen therapy. Essentially, two forms of anti-estrogen resistance occur: *de novo* and acquired resistance (Reviewed by Clarke *et al.*, 2001 & 2003; Ring & Dowestt, 2004). Although absence of ER- α 66 expression is the most common *de novo* resistance mechanism, about 40-50% ER-positive tumors are already resistant to anti-estrogens by the time of diagnosis; this *de novo* resistance mechanism in these ER-positive tumors is largely unknown (Reviewed by Clarke *et al.*, 2001 & 2003; Ring & Dowestt, 2004). Furthermore, most initially responsive breast tumors gradually acquire anti-estrogen resistance by loss of anti-estrogen responsiveness. The underlying mechanism of breast tumors loss their anti-estrogen responsiveness remains unknown. Breast tumors with acquired TAM resistance frequently but not always retain levels of ER- α 66 expression that

would still define them as ER-positive tumors (Reviewed by Clarke *et al.*, 2001 & 2003). Therefore, a loss of ER- α 66 expression is not the major mechanism driving acquired anti-estrogen resistance. Up-regulation of Erb-B2 (Her2/Neu) and activation of the MAPK signaling pathways are usually associated with development of anti-estrogen resistance (Reviewed by Clarke *et al.*, 2001 & 2003; Ring & Dowsett, 2004).

A recent retrospective study of 896 cases of breast cancer patients revealed that about 40% cases of human breast cancer patients were positive for ER- α 36; about 40% cases ER-positive breast cancer co-expressed ER- α 66 and ER- α 36 and about 40% cases of ER-negative breast cancer that lacked ER- α 66 expression were positive for ER- α 36 (Shi *et al.*, 2009). The breast cancer patients with tumors expressing high levels of ER- α 36 were less benefited from tamoxifen therapy than those with low levels of ER- α 36 expression did, and ER- α 36 expression is significantly associated with Her2/Neu expression (Shi *et al.*, 2009). These data suggest that high levels of ER- α 36 expression in breast cancer cells is one of the underlying mechanisms of *de novo* tamoxifen resistance found in ER-positive breast cancer patients.

The aromatase inhibitors (AIs), on the other hand, inhibit the action of the enzyme aromatase, which converts testosterone to E2 (estradiol) and androstenedione to E1 (estrone). The third generation AIs exemplified by anastrozole and letrozole, provides a second line therapeutic strategy in advanced ER-positive patients (Reviewed by Santen, 2003). However, breast cancer cells that express high levels of ER- α 36 are super sensitive to estrogen; activation of the MAPK/ERK in response to an extreme low concentration of estrogen, 1×10^{-16} M/L (Zhang *et al.*, 2011). The anastrozole usually suppresses a plasma level of E2 β to a mean of 2.6 pmol/L and letrozole to a mean of 2.1 pmol/L (Geisler *et al.*, 2002). These data suggest that breast cancer patients with tumors expressing high levels of ER- α 36 may also be refractory to the third generation of AIs.

15. ER- α 36 in ER-negative breast cancer

Because of the lack of ER- α 66 expression, it is prevalently thought that estrogen signaling is not involved in development and progression of ER-negative breast cancer. However, early study showed that ovariectomy prevents formation of both ER-positive and -negative breast cancers (Nissen-Meyer, 1964). In addition, BRCA1 mutation related tumors, the vast majority of which are ER-negative, are also effectively prevented by prophylactic ovariectomy (Rebbeck *et al.*, 1999). The increased risk developing breast cancer following pregnancy is assumed due to the ability of pregnancy-associated hormones such as estrogen to stimulate mammary epithelial cell proliferation. Surprisingly, however, the majority of breast cancers that develop following pregnancy are negative for either estrogen and progesterone receptors. To explain this contradictory findings, Gupta *et al.*, (2007) proposed that estrogen may promote the growth of ER-negative breast cancer via a systemic increase in host angiogenesis.

Rapid activation of the PI3K/Akt pathway in ER-negative breast cancer MDA-MB-231 cells that could not be blocked by estrogen antagonists was reported (Tsai *et al.*, 2001), which was explained as estrogen signaling through an ER-independent pathway. Taken together, these data suggest that subsets of ER-negative breast cancer cells may retain non-genomic estrogen signaling, which may contribute to development and progression of ER-negative breast cancers. Several studies demonstrated that ER- α 36 variant is expressed in established

ER-negative breast cancer cell lines such as MDA-MB-231 and MDA-MB-436 and about 40% specimens from ER-negative breast cancer patients (Wang et al., 2006; Lee et al., 2008; Shi et al., 2009). Our group recently reported that E2 β treatment activated the MAPK/ERK signaling pathway, induced expression of growth-promoting genes, c-Myc and cyclin D1, stimulated cell proliferation and accelerated tumor growth *in vivo* in ER-negative breast cancer MDA-MB-231 and MDA-MB-436 cells, all of which could be abrogated by knock-down of ER- α 36 expression using ER- α 36 specific shRNA (Zhang et al., 2011). Thus, ER- α 36-mediated mitogenic estrogen signaling contributes malignant growth of ER-negative breast cancer cells.

16. Underlying mechanism of mitogenic estrogen signaling mediated by ER- α 36

Compelling evidence demonstrated that estrogens up-regulate the expression and function of c-Myc and cyclin D1, and activate cyclin E-Cdk2 complexes, all of which are rate limiting factors for cell cycle progression from G1 to S phase (Prall *et. al.*, 1998; Altucci *et. al.*, 1996 & 1997). Based on these findings, it is believed that estrogens directly interact with ERs in ER-positive human breast cancer cells, where they induce transcriptional activation of “immediate early” and cyclin genes and promote cell cycle progression. However, membrane-initiated estrogen signaling, including rapid changes of the signal transduction cascades, has been proposed to be also essential for the mitogenic action of estrogen signaling. Castoria *et. al.*, (1999) reported that NIH3T3 fibroblasts are made equally E2 β -responsive in terms of DNA synthesis by transient transfection with either the wild-type or the transcriptionally inactive ER- α 66 mutant. Castoria *et. al.*, (2001) later demonstrated that the PI3K/Akt signaling pathway and Src together mediates activation of cyclin D1 promoter activity and promotion of the S-phase entry in estrogen-stimulated ER-positive breast cancer MCF7 cells. These findings together with other reports (Song et al., 2002; Migliaccio et al., 1996; Ahmad et al., 1999; Lobenhofer et al., 2000) highlight the importance of the non-genomic action of estrogen signaling in estrogen-stimulated cell proliferation and mammary tumorigenesis.

The epidermal growth factor receptor (EGFR) is essential for ductal morphogenesis during normal mammary gland development and its overexpression either at the gene or protein levels is well documented in human breast cancer (Reviewed by Troyer & Lee, 2001). Overexpression of EGFR alone usually does not constitute efficient transformation and tumorigenesis in breast cancer models. However, co-expression with the non-receptor kinase c-Src dramatically increases tumorigenesis (Maa et al., 1995; Tice et al., 1999; Biscardi et al., 1998). Co-expression of EGFR and c-Src in breast cancer cell lines results in their association and c-Src-mediated phosphorylation of the EGFR at tyrosine 845 (Tyr845) within its catalytic domain, which contributes to enhanced proliferation *in vitro* and tumor formation *in vivo* (Biscardi et al., 1998; Maa et al., 1995; Tice et al., 1999; Biscardi et al., 2000). Accumulating evidence indicated that c-Src is involved in non-genomic estrogen signaling by interacting with ER- α 66 in ER-positive breast cancer cells (Shupnik, 2004). In ER-negative breast cancer cells, E2 β induced the MAPK/ERK activation through a mechanism that involves the interaction between ER- α 36 and the EGFR/Src/Shc complex (Zhang et al., 2011). Intriguingly, ER- α 36 interacts strongly with EGFR in the absence of estrogen and is progressively dissociated from EGFR after estrogen treatment (Zhang et al., 2011), which is

in contradictory to the finding that estrogen stimulates recruitment of ER- α 66 to the EGFR complex (Reviewed by Levin, 2003). Like ER- α 66 (Song et al., 2002), interaction between ER- α 36 and the Src/Shc was estrogen-dependent (Zhang et al., 2011), which results in Src-Tyr-416 phosphorylation and phosphorylation of the EGFR-Tyr-845 but not the major auto-phosphorylation sites of EGFR such as Tyr-992, -1068 and -1073. Tyr-845 in the EGFR is not an auto-phosphorylation site and is not required for EGFR kinase activity but is phosphorylated by Src (Biscardi et al., 1998).

Signal transducer and activator of transcription 5b (STAT5b), c-Src and EGFR play important roles in estrogen-stimulated proliferation of ER-positive breast cancer cells (Fox et al., 2008). Estrogen-induced Src activation and Src-dependent phosphorylation of EGFR-Tyr-845 recruit STAT5b as a downstream effector of phosphorylated EGFR-Tyr-845 to induce c-Myc and cyclin D1 expression (Fox et al., 2008). Introduction of a dominant-negative STAT5a into ER-positive T47D breast cancer cells inhibits E2 β -stimulated cell proliferation and induces apoptosis (Yamashita et al., 2003). The involvement of STAT5 in ER- α 36-mediated estrogen activation of the cyclin D1 promoter activity has been established (Zhang et al., unpublished data). Thus, it is possible that ER- α 36 mediates mitogenic estrogen signaling through the EGFR/Src/STAT5 pathway in breast cancer cells.

17. Transcriptional regulation of ER- α 36

Transcription of ER- α 36 is initiated from a previously unidentified promoter in the first intron of the ER- α 66 gene (Figure 1). The putative 5'-flanking region of the ER- α 36 has been cloned and sequenced (Zou et al., 2009). Computer analysis revealed a TATA binding protein (TBP) recognition sequence upstream of the cDNA start site and several Sp1, NF- κ B and Ap1 binding sites in the 5'-flanking region of ER- α 36. A perfect half ERE site was identified at the promoter region of ER- α 36 that is involved in suppression of ER- α 36 promoter activity by ER- α 66, indicating that ER- α 36 expression is subjected to negative regulation of ER- α 66 (Zou et al., 2009). This is consistent with the finding that ER-positive breast cancer cells tend to express lower levels of ER- α 36 compared to ER-negative breast cancer cells (Wang et al., 2006; Zou et al., 2009). ER- α 46 that lacks the AF-1 domain, however, had no effect on ER- α 36 promoter activity while ER- α 46 released the suppression activity of ER- α 66 when co-expressed with ER- α 66 (Zou et al., 2009). Thus ER- α 66 suppresses the promoter activity of ER- α 36 presumably through ligand-independent activity mediated by its AF1 domain, which can be blocked by ER- α 46. In addition, co-expression of ER- α 36 also released the suppression activity mediated by ER- α 66 (Zou et al., 2009), suggesting that ER- α 36 may be regulated by a positive-feedback mechanism.

BRCA1 mutations and downregulation are found in familial and sporadic breast cancers and BRCA1-related tumors are more likely to be ER-negative than are non-BRCA1 related breast cancers. It is still unknown why dysfunctional BRCA1 only predispose to cancers of estrogen responsive tissues and why these BRCA1-related breast tumors are often ER-negative. BRCA1 mediates the ligand-independent transcriptional repression activity of the ER- α 66 through its AF-1 domain (Zheng *et. al.*, 2003). Since ER- α 66 represses the promoter activity of ER- α 36 through its AF-1 domain, it is reasonable to postulate that loss of BRCA1 function either by mutations or downregulation may activate ER- α 36 expression, which then activates ER- α 36-mediated mitogenic estrogen signaling that eventually leads to development of breast cancers characterized as ER-negative since they lack ER- α 66 expression.

The analysis of ER- α 36 promoter revealed several important features of transcriptional regulation of this potentially important player in estrogen signaling. The finding of several NF- κ B binding sites indicates that ER- α 36 is subject to regulation by the NF- κ B signaling pathway and by different cytokines that activate the NF- κ B signaling pathway. The existence of Ap-1 binding sites in the ER- α 36 promoter region raised the possibility that growth factor signaling may regulate ER- α 36 expression, which was confirmed by our recent report that EGFR-mediated signaling induces ER- α 36 expression via one of the Ap-1 binding sites (Zhang et al., 2011). Thus, further study of the transcription regulation of ER- α 36 will provide more information about the mechanisms underlying regulation of ER- α 36 mediated non-genomic estrogen signaling in cells and tissues other than mammary gland.

18. Cross-regulation of ER- α 36 and members of the EGFR family

In ER-positive breast cancer cells, it has been well documented that the cross-communication between EGFR and ER- α 66 leads to serine phosphorylation of ER- α 66 and ligand-independent activation of the ER- α 66-mediated transcription (Kato et al., 1995; Bunone et al., 1996). Conversely, EGF signaling is strongly enhanced by the ER- α 66 in ER-positive MCF7 cells and anti-estrogen ICI 182, 780 was able to block the EGF signaling (Migliaccio et al., 2006). In addition, our group has reported another level of cross-talk mechanism by which EGFR and ER- α 36 positively regulate each other's expression in triple-negative breast cancer cells (Zhang et al., 2011); EGFR signaling activates the promoter activity of ER- α 36 and ER- α 36 stabilizes the steady state levels of EGFR protein. This positive feedback loop provides a molecular explanation to the aggressiveness of triple-negative breast cancer. A similar cross-regulation between Her2/Neu and ER- α 36 was also found (Kang et al., unpublished observations). However, unlike EGFR, ER- α 36-mediated estrogen signaling up-regulates the promoter activity of Her2/Neu gene, suggesting that ER- α 36 positively regulates Her2/Neu expression. This data is consistent with the finding that ER- α 36 expression is significantly correlated with Her-2/Neu expression in specimens from breast cancer patients (Shi et al., 2009). Thus, the interplay between growth factor receptors and ER- α 36 may play an important role in development and progression of subsets of breast cancer that highly express ER- α 36.

19. ER- α 36 in osteoporosis

Non-genomic estrogen signaling plays an important role in bone protection; estrogen is able to protect the adult skeleton against bone loss by maintaining a focal balance between bone formation and resorption, which mainly results from the opposite effects of osteoblasts (OBs) and osteoclasts (OCs). Estrogen has anti-apoptotic effects on OBs and pro-apoptotic effects on OCs through an extra-nuclear signaling that leads to activation of the MAPK/ERK signaling pathway and kinase-dependent changes in transcription activities (Reviewed by Manolagas, 2000; Manolagas et al., 2004). However, the underlying mechanisms of this opposite effects of estrogen signaling have not been established. Xie et al., (2011) recently reported that the postmenopausal level of E2 β induces mitogenic, anti-apoptotic and anti-osteogenic effects in postmenopausal OBs and pro-apoptotic effects in postmenopausal OCs, respectively. ER- α 36 mediates the effects of post-menopausal-level E2 β on proliferation, apoptosis, and differentiation of OBs through transient activation of the MAPK/ERK

pathway, whereas ER- α 36 mediates post-menopausal-level E2 β induced apoptosis of OCs through prolonged or sustained activation of the MAPK/ERK pathway. The levels of ER- α 36 expression in bone are positively associated with bone mineral density in post-menopausal women. Thus, the high levels of ER- α 36 expression are required for preserving bone mass in post-menopausal and menopausal women.

20. Conclusions

Most previous studies of estrogen signaling in human breast cancer were focused on the ER- α 66, the only known estrogen receptor for many years. The discovery of ER- α 36 in our laboratory, combined with the previous reports of membrane-based ER- α 46, raised the intriguing possibility that ER- α 36 and ER- α 46 are also involved in estrogen signaling. Net estrogen response in a specific target cell thus depends on absolute and relative levels of the regulated expression of full-length and alternatively processed estrogen receptor- α isoforms.

It is well established that estrogen stimulates mammary epithelial cell proliferation. However, the function of ER- α 66, ER- β and ER- α 46 in mitogenic estrogen signaling is confounding. A plethora of experimental evidence indicates that ER- α 66, ER- α 46, and ER- β negatively regulate mitogenic estrogen signaling in transfected cells, which argue against the positive role of these receptors in estrogen-stimulated cell proliferation. Thus far, ER- α 36 is the only reported estrogen receptor that mediates mitogenic estrogen signaling and stimulates cell proliferation in transfected cells and in ER-negative breast cancer cells that lack expression of ER- α 66, ER- α 46 and ER- β but express high level of endogenous ER- α 36. In addition, the finding that ER- α 36 functions as a potent dominant-negative regulator of the genomic estrogen signaling mediated by ER- α 66 and ER- β implicates that when ER- α 36 mediates mitogenic estrogen signalling, it may require the silence of genomic estrogen signaling. This again challenges the well-known role of genomic estrogen signaling in estrogen-stimulated cell proliferation.

Accumulating evidence highlights the importance of ER- α 36-mediated non-genomic estrogen signaling in malignant growth of breast cancer and endometrial cancer cells. The expression of ER- α 36 has also been detected in other types of human cancer such as human colon cancer and liver cancer (Jiang et al., 2008; Miceli et al., 2011), suggesting that ER- α 36 may also involved in initiation and development of human malignancy in non-classical estrogen targeting organs. Elucidating the functions of ER- α 36-mediated non-genomic estrogen signaling in different types of human malignancy could provide more informed approaches to better understand the underlying mechanisms of mitogenic estrogen signaling in mammary carcinogenesis. We hope that further investigation of the function and underlying mechanisms of this ER variant in different subtypes of human breast cancer and even in the putative breast cancer stem/progenitor cells will lead to development of high efficacy, less toxic therapeutic agents for patients affected with mammary malignancies.

21. Acknowledgments

We regret the inability to cite many fine papers that contribute to this field due to space limitations. This work was supported by grants from the National Institute of Health, Susan G. Komen for the Cure and the Nebraska Tobacco Settlement Biomedical Research Program.

22. References

- Ahmad, S., Singh, N. & Glazer, RI. (1999). Role of AKT1 in 17beta-estradiol- and insulin-like growth factor I (IGF-I)-dependent proliferation and prevention of apoptosis in MCF-7 breast carcinoma cells. *Biochem Pharmacol* 58:425-430.
- Ahola, TM., Alkio, N., Manninen, T. & Ylikomi, T. (2002). Progesterin and G protein-coupled receptor 30 inhibit mitogen-activated protein kinase activity in MCF-7 breast cancer cells. *Endocrinology*. 143:4620-4626.
- Altucci, L., Addeo, R., Cicatiello, L., Dauvois, S., Parker, MG., Truss, M., Beato, M., Sica, V., Bresciani, F. & Weisz, A. (1996). 17beta-Estradiol induces cyclin D1 gene transcription, p36D1-p34cdk4 complex activation and p105Rb phosphorylation during mitogenic stimulation of G1-arrested human breast cancer cells. *Oncogene*. 12:2315-2324.
- Altucci, L., Addeo, R., Cicatiello, L., Germano, D., Pacilio, C., Battista, T., Cancemi, M., Petrizzi, VB., Bresciani, F. & Weisz, A. (1997). Estrogen induces early and timed activation of cyclin-dependent kinases 4, 5, and 6 and increases cyclin messenger ribonucleic acid expression in rat uterus. *Endocrinology*. 138:978-984.
- Aronica, SM., Kraus, WL. & Katzenellenbogen, BS. (1994). Estrogen action via the cAMP signaling pathway: stimulation of adenylate cyclase and cAMP-regulated gene transcription. *Proc Natl Acad Sci U S A*. 91:8517-8521.
- Berry, M., Metzger, D. & Chambon, P. (1990). Role of the two activating domains of the oestrogen receptor in the cell-type and promoter-context dependent agonistic activity of the anti-oestrogen 4-hydroxytamoxifen. *EMBO J*. 9:2811-2818.
- Biscardi, JS., Belsches, AP. & Parsons, SJ. (1998). Characterization of human epidermal growth factor receptor and c-Src interactions in human breast tumor cells. *Mol. Carcinog*. 21:261-272.
- Biscardi, JS., Ishizawar, RC., Silva, CM. & Parsons, SJ. (2000). Tyrosine kinase signalling in breast cancer: epidermal growth factor receptor and c-Src interactions in breast cancer. *Breast Cancer. Res*. 2:203-210.
- Brinton, RD. (2001). Cellular and molecular mechanisms of estrogen regulation of memory function and neuroprotection against Alzheimer's disease: recent insights and remaining challenges. *Learn Mem*. 8:121-133.
- Bunone, G., Briand, PA., Miksicek, RJ. & Picard, D. (1996). Activation of the unliganded estrogen receptor by EGF involves the MAP kinase pathway and direct phosphorylation. *EMBO J*. 15:2174-2183.
- Caiazza F, Galluzzo P, Lorenzetti S, Marino M (2007) 17Beta-estradiol induces ERbeta up-regulation via p38/MAPK activation in colon cancer cells. *Biochem Biophys Res Commun* 359:102-107.
- Castoria, G., Barone, M.V, Di Domenico, M., Bilancio, A., Ametrano, D., Migliaccio, A. & Auricchio, F. (1999). Non-transcriptional action of oestradiol and progesterin triggers DNA synthesis. *EMBO J*. 18:2500-2510.
- Castoria, G., Migliaccio, A., Bilancio, A., Di Domenico, M., de Falco, A., Lombardi, M., Fiorentino, R., Varricchio, L., Barone, MV. & Auricchio, F. (2001). PI3-kinase in concert with Src promotes the S-phase entry of oestradiol-stimulated MCF-7 cells. *EMBO J*. 20:6050-6059.

- Cerillo, G., Rees, A., Manchanda, N., Reilly, C., Brogan, I., White, A. & Needham, M. (1998). The oestrogen receptor regulates NFkappaB and AP-1 activity in a cell-specific manner. *J Steroid Biochem. Mol. Biol.* 67:79-88.
- Chen, Z., Yuhanna, IS., Galcheva-Gargova, Z., Karas, RH., Mendelsohn, ME. & Shaul, PW. (1999.) Estrogen receptor alpha mediates the nongenomic activation of endothelial nitric oxide synthase by estrogen. *J Clin. Invest.* 103:401-406.
- Clark, GM., Osborne, CK. & McGuire, WL. (1984). Correlations between estrogen receptor, progesterone receptor, and patient characteristics in human breast cancer. *J Clin Oncol.* 2:1102-1109.
- Clark, GM. (2000). Prognostic and predictive factors. *Diseases of the breast*, Lippincott Williams & Wilkins, Vol. 2:489-514.
- Clarke, R., Leonessa, F., Welch, JN. & Skaar, TC. (2001). Cellular and molecular pharmacology of antiestrogen action and resistance. *Pharmacol. Rev.* 53:25-71.
- Clarke, R., Liu, MC., Bouker, KB., Gu, Z., Lee, RY., Zhu, Y., Skaar, TC., Gomez, B., O'Brien, K., Wang, Y. & Hilakivi-Clarke, LA. (2003). Antiestrogen resistance in breast cancer and the role of estrogen receptor signaling. *Oncogene.* 22:7316-7339.
- Clarke, R., Howell, A., Potten, CS. & Anderson, E. (1997). Dissociation between steroid receptor expression and cell proliferation in the human breast. *Cancer Res.* 57:4987-4991.
- Cowell, LN., Graham, JD., Bouton, AH., Clarke, CL. & O'Neill, GM. (2006). Tamoxifen treatment promotes phosphorylation of the adhesion molecules, p130Cas/BCAR1, FAK and Src, via an adhesion-dependent pathway. *Oncogene.* 25:7597-7607.
- Dauvois, S., Danielian, PS., White, R. & Parker, MG. (1992.) Antiestrogen ICI 164,384 reduces cellular estrogen receptor content by increasing its turnover. *Proc Natl Acad Sci U S A.* 89:4037-4041.
- Deroo, BJ. & Buensuceso, AV. (2010.) Minireview: Estrogen receptor-beta: mechanistic insights from recent studies. *Mol. Endocrinol.* 24:1703-1714.
- Diaz-Chico, BN., Ogasawara, Y., Chamness, GC., Salman, M. & McGuire, WL. (1988). A 46-kDa antigen associated with estrogen receptor in human breast cancer. *J Steroid Biochem.* 30:315-320.
- Dotzlaw, H., Leygue, E., Watson, PH. & Murphy, LC. (1997). Expression of estrogen receptor-beta in human breast tumors. *J Clin. Endocrinol. Metab.* 82:2371-2374.
- Driggers, PH. & Segars, JH. (2002). Estrogen action and cytoplasmic signaling pathways. Part II: the role of growth factors and phosphorylation in estrogen signaling. *Trends Endocrinol. Metab.* 13:422-427.
- Dudley, MW., Sheeler, CQ., Wang, H. & Khan, S. (2000). Activation of the human estrogen receptor by the antiestrogens ICI 182,780 and tamoxifen in yeast genetic systems: implications for their mechanism of action. *Proc Natl Acad Sci U S A.* 97:3696-3701.
- Dufy, B., Vincent, JD., Fleury, H., Du Pasquier, P., Gourdj, D & Tixier-Vidal, A. (1979). Membrane effects of thyrotropin-releasing hormone and estrogen shown by intracellular recording from pituitary cells. *Science.* 204:509-511.
- Endoh, H., Sasaki, H., Maruyama, K., Takeyama, K., Waga, I., Shimizu, T., Kato, S. & Kawashima, H. (1997). Rapid activation of MAP kinase by estrogen in the bone cell line. *Biochem. Biophys. Res. Commun.* 235:99-102.
- Evans, MJ., Eckert, A., Lai, K., Adelman, SJ. & Harnish, DC. (2001). Reciprocal antagonism between estrogen receptor and NF-kappaB activity in vivo. *Circ. Res.* 89:823-830.

- Fawell, SE., White, R., Hoare, S., Sydenham, M., Page, M. & Parker, MG. (1990). Inhibition of estrogen receptor-DNA binding by the "pure" antiestrogen ICI 164,384 appears to be mediated by impaired receptor dimerization. *Proc Natl Acad Sci U S A*. 87:6883-6887.
- Feigelson, HS. & Henderson, BE. (1996). Estrogens and breast cancer. *Carcinogenesis*. 17:2279-2284.
- Ferguson, AT., Lapidus, RG., Baylin, SB. & Davidson, NE. (1995). Demethylation of the estrogen receptor gene in estrogen receptor-negative breast cancer cells can reactivate estrogen receptor gene expression. *Cancer Res*. 55:2279-2283.
- Filardo, EJ., Quinn, JA., Bland, KI. & Frackelton, AR. Jr. (2000). Estrogen-induced activation of Erk-1 and Erk-2 requires the G protein-coupled receptor homolog, GPR30, and occurs via trans-activation of the epidermal growth factor receptor through release of HB-EGF. *Mol. Endocrinol*. 14:1649-1660.
- Filardo, E., Quinn, J., Pang, Y., Graeber, C., Shaw, S., Dong, J. & Thomas, P. (2007). Activation of the novel estrogen receptor G protein-coupled receptor 30 (GPR30) at the plasma membrane. *Endocrinology* 148:3236-3245.
- Flouriou, G., Brand, H., Denger, S., Metivier, R., Kos, M., Reid, G., Sonntag-Buck, V. & Gannon, F. (2000). Identification of a new isoform of the human estrogen receptor-alpha (hER-alpha) that is encoded by distinct transcripts and that is able to repress hER-alpha activation function 1. *EMBO J*. 19:4688-4700.
- Fox, EM., Bernaciak, TM., Wen, J., Weaver, AM., Shupnik, MA. & Silva, CM. (2008). Signal transducer and activator of transcription 5b, c-Src, and epidermal growth factor receptor signaling play integral roles in estrogen-stimulated proliferation of estrogen receptor-positive breast cancer cells. *Mol. Endocrinol*. 22:1781-1796.
- Fuqua, SA., Schiff, R., Parra, I., Friedrichs, WE., Su, JL., McKee, DD., Slentz-Kesler, K., Moore, LB., Willson, TM. & Moore, JT. (1999). Expression of wild-type estrogen receptor beta and variant isoforms in human breast cancer. *Cancer Res*. 59:5425-5428.
- Fuqua, SA., Schiff, R., Parra, I., Moore, JT., Mohsin, SK., Osborne, CK., Clark, GM & Allred, DC. (2003). Estrogen receptor beta protein in human breast cancer: correlation with clinical tumor parameters. *Cancer Res*. 63:2434-2439.
- Galluzzo, P., Caiazza, F., Moreno, S. & Marino, M. (2007). Role of ER-beta palmitoylation in the inhibition of human colon cancer cell proliferation. *Endocr. Relat. Cancer*. 14:153-167.
- Geisler, J., Haynes, B., Anker, G., Dowsett, M. & Lonning, PE. (2002). Influence of letrozole and anastrozole on total body aromatization and plasma estrogen levels in postmenopausal breast cancer patients evaluated in a randomized, cross-over study. *J Clin. Oncol*. 20:751-757.
- Green, S., Walter, P., Kumar, V., Krust, A., Bornert, JM., Argos, P. & Chambon, P. (1986). Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. *Nature*. 320:134-139.
- Greene, GL., Gilna, P., Waterfield, M., Baker, A, Hort, Y. & Shine, J. (1986). Sequence and expression of human estrogen receptor complementary DNA. *Science*. 231:1150-1154.
- Gu, Q., Korach, KS. & Moss, RL. (1999). Rapid action of 17beta-estradiol on kainate-induced currents in hippocampal neurons lacking intracellular estrogen receptors. *Endocrinology*. 140:660-666.

- Gupta, PB., Proia, D., Cingoz, O., Weremowicz, J., Naber, SP., Weinberg, RA. & Kuperwasser, C. (2007). Systemic stromal effects of estrogen promote the growth of estrogen receptor-negative cancers. *Cancer Res.* 67:2062-2071.
- Hammes, SR. & Levin, ER. (2007). Extranuclear steroid receptors: nature and actions. *Endocr. Rev.* 28:726-741.
- Harnish, DC., Scicchitano, MS., Adelman, SJ., Lyttle, CR. & Karathanasis, SK. (2000). The role of CBP in estrogen receptor cross-talk with nuclear factor-kappaB in HepG2 cells. *Endocrinology.* 141:3403-3411.
- Harris, HA. (2007). Estrogen receptor-beta: recent lessons from in vivo studies. *Mol. Endocrinol.* 21:1-13.
- Hartman, J., Lindberg, K., Morani, A., Inzunza, J., Strom, A & Gustafsson, JA. (2006). Estrogen receptor beta inhibits angiogenesis and growth of T47D breast cancer xenografts. *Cancer Res.* 66:11207-11213.
- Howell, A., Osborne, CK., Morris, C. & Wakeling, AE. (2000). ICI 182,780 (Faslodex): development of a novel, "pure" antiestrogen. *Cancer.* 89:817-825.
- Iwao, K., Miyoshi, Y., Egawa, C., Ikeda, N. & Noguchi, S. (2000). Quantitative analysis of estrogen receptor-beta mRNA and its variants in human breast cancers. *Int. J Cancer.* 88:733-736.
- Jarvinen, TA., Pelto-Huikko, M., Holli, K. & Isola, J. (2000). Estrogen receptor beta is coexpressed with ER-alpha and PR and associated with nodal status, grade, and proliferation rate in breast cancer. *Am. J Pathol.* 156:29-35.
- Jensen, EV., Cheng, G., Palmieri, C., Saji, S., Makela, S., Van Noorden, S., Wahlstrom, T., Warner, M., Coombes, RC & Gustafsson, JA. (2001). Estrogen receptors and proliferation markers in primary and recurrent breast cancer. *Proc Natl Acad Sci U S A.* 98:15197-15202.
- Jiang, H., Teng, R., Wang, Q., Zhang, XT., Wang, H., Wang, ZY., Cao, J. & Teng, L. (2008). Transcriptional analysis of estrogen receptor alpha variant mRNAs in colorectal cancers and their matched normal colorectal tissues. *J Steroid Biochem. Mol. Biol.* 112:20-24.
- Jiang, SY. & Jordan, VC. (1992). Growth regulation of estrogen receptor-negative breast cancer cells transfected with complementary DNAs for estrogen receptor. *J Natl. Cancer Inst.* 84:580-591.
- Jozan, S., Julia, AM., Carretie, A., Eche, N., Maisongrosse, V., Fouet, B., Marques, B. & David, JF. (1991). 65 and 47 kDa forms of estrogen receptor in human breast cancer: relation with estrogen responsiveness. *Breast Cancer Res. Treat.* 19:103-109.
- Kang, LG. & Wang, ZY. (2010a). Breast cancer cell growth inhibition by phenethyl isothiocyanate is associated with down-regulation of oestrogen receptor-alpha36. *J Cell Mol. Med.* 14:1485-1493.
- Kang, LG., Zhang, XT., Xie, Y., Tu, YP., Wang, D., Liu, ZM. & Wang, ZY. (2010b). Involvement of estrogen receptor variant ER-alpha36, not GPR30, in nongenomic estrogen signaling. *Mol. Endocrinol.* 24:709-721.
- Kato, S., Endoh, H., Masuhiro, Y., Kitamoto, T., Uchiyama, S., Sasaki, H., Masushige, S., Gotoh, Y., Nishida, E., Kawashima, H., Metzger, D. & Chambon, P. (1995). Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. *Science.* 270:1491-1494.
- Katzenellenbogen, BS. & Katzenellenbogen, JA. (2000). Estrogen receptor transcription and transactivation: Estrogen receptor alpha and estrogen receptor beta: regulation by

- selective estrogen receptor modulators and importance in breast cancer. *Breast Cancer Res.* 2:335-344.
- Katzenellenbogen, BS. & Katzenellenbogen, JA. (2002). Biomedicine. Defining the "S" in SERMs. *Science.* 295:2380-2381.
- Kelly, MJ. & Levin, ER. (2001). Rapid actions of plasma membrane estrogen receptors. *Trends Endocrinol. Metab.* 12:152-156.
- Kelly, MJ., Moss, RL., & Dudley, CA. (1976) Differential sensitivity of preoptic-septal neurons to microelectrophoresed estrogen during the estrous cycle. *Brain Res* 114:152-157.
- Kelly, MJ., Moss, RL., Dudley, CA. & Fawcett, CP. (1977). The specificity of the response of preoptic-septal area neurons to estrogen: 17alpha-estradiol versus 17beta-estradiol and the response of extrahypothalamic neurons. *Exp. Brain Res.* 30:43-52.
- Khan, SA., Rogers, MA., Obando, JA. & Tamsen, A. (1994). Estrogen receptor expression of benign breast epithelium and its association with breast cancer. *Cancer Res.* 54:993-997.
- Kim, KH. & Bender, JR. (2005). Rapid, estrogen receptor-mediated signaling: why is the endothelium so special? *Sci. STKE.* 2005:pe28.
- Kim, R., Kaneko, M., Arihiro, K., Emi, M., Tanabe, K., Murakami, S., Osaki, A. & Inai, K. (2006). Extranuclear expression of hormone receptors in primary breast cancer. *Ann Oncol* 17:1213-1220.
- Klinge, CM. (2001). Estrogen receptor interaction with estrogen response elements. *Nucleic Acids Res.* 29:2905-2919.
- Klinge, CM., Riggs, KA., Wickramasinghe, NS., Emberts, CG., McConda, DB., Barry, PN. & Magnusen, JE. (2010). Estrogen receptor alpha 46 is reduced in tamoxifen resistant breast cancer cells and re-expression inhibits cell proliferation and estrogen receptor alpha 66-regulated target gene transcription. *Mol. Cell Endocrinol.* 323:268-276.
- Kong, EH., Pike, AC. & Hubbard, RE. (2003). Structure and mechanism of the oestrogen receptor. *Biochem. Soc. Trans.* 31:56-59.
- Kuiper, GG., Enmark, E., Peltö-Huikko, M., Nilsson, S. & Gustafsson, J.A. (1996). Cloning of a novel receptor expressed in rat prostate and ovary. *Proc. Natl. Acad. Sci. USA.* 93: 5925-5930.
- Kurebayashi J, Otsuki T, Yamamoto S, Kurosumi M, Nakata T, Akinaga S, Sonoo H (1998) A pure antiestrogen, ICI 182,780, stimulates the growth of tamoxifen-resistant KPL-1 human breast cancer cells in vivo but not in vitro. *Oncology* 55 Suppl 1:23-34.
- Kushner, PJ., Agard, DA., Greene, GL., Scanlan, TS., Shiau, AK., Uht, RM. & Webb, P. (2000). Estrogen receptor pathways to AP-1. *J Steroid Biochem. Mol. Biol.* 74:311-317.
- Kushner, PJ., Hort, E., Shine, J., Baxter, JD. & Greene, GL. (1990). Construction of cell lines that express high levels of the human estrogen receptor and are killed by estrogens. *Mol. Endocrinol.* 4:1465-1473.
- Lazennec, G., Bresson, D., Lucas, A., Chauveau, C. & Vignon, F. (2001). ER beta inhibits proliferation and invasion of breast cancer cells. *Endocrinology.* 142:4120-4130.
- Lee, LM., Cao, J., Deng, H., Chen, P., Gatalica, Z. & Wang, ZY. (2008). ER-alpha36, a novel variant of ER-alpha, is expressed in ER-positive and -negative human breast carcinomas. *Anticancer Res.* 28:479-483.
- Levin, ER. (2002). Cellular functions of plasma membrane estrogen receptors. *Steroids.* 67:471-475.

- Levin, ER. (2003). Bidirectional signaling between the estrogen receptor and the epidermal growth factor receptor. *Mol. Endocrinol.* 17:309-317.
- Leygue, E., Dotzlaw, H., Watson, PH. & Murphy, LC. (1998). Altered estrogen receptor alpha and beta messenger RNA expression during human breast tumorigenesis. *Cancer Res.* 58:3197-3201.
- Li, L., Haynes, MP. & Bender, JR. (2003.) Plasma membrane localization and function of the estrogen receptor alpha variant (ER46) in human endothelial cells. *Proc Natl Acad Sci U S A.* 100:4807-4812.
- Lin, SL., Yan, LY., Liang, XW., Wang, ZB., Wang, ZY., Qiao, J., Schatten, H. & Sun, QY. (2009). A novel variant of ER-alpha, ER-alpha36 mediates testosterone-stimulated ERK and Akt activation in endometrial cancer Hec1A cells. *Reprod. Biol. Endocrinol.* 7:102.
- Lin, SL., Yan, LY., Zhang, XT., Yuan, J., Li, M., Qiao, J., Wang, ZY. & Sun, QY. (2010). 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.* 5:e9013.
- Lobenhofer, EK., Huper, G., Iglehart, JD. & Marks, JR. (2000). Inhibition of mitogen-activated protein kinase and phosphatidylinositol 3-kinase activity in MCF-7 cells prevents estrogen-induced mitogenesis. *Cell Growth Differ.* 11:99-110.
- Longo, M., Brama, M., Marino, M., Bernardini, S., Korach, KS., Wetsel, WC., Scandurra, R., Faraggiana, T., Spera, G., Baron, R., Teti, A. & Migliaccio, S. (2004). Interaction of estrogen receptor alpha with protein kinase C alpha and c-Src in osteoblasts during differentiation. *Bone.* 34:100-111.
- Lu Q, Pallas DC, Surks HK, Baur WE, Mendelsohn ME, Karas RH (2004) Striatin assembles a membrane signaling complex necessary for rapid, nongenomic activation of endothelial NO synthase by estrogen receptor alpha. *Proc Natl Acad Sci U S A* 101:17126-17131.
- Maa, MC., Leu, TH., McCarley, DJ., Schatzman, RC. & Parsons, SJ. (1995). Potentiation of epidermal growth factor receptor-mediated oncogenesis by c-Src: implications for the etiology of multiple human cancers. *Proc Natl Acad Sci U S A.* 92:6981-6985.
- Mahfoudi, A., Roulet, E., Dauvois, S., Parker, MG. & Wahli, W. (1995). Specific mutations in the estrogen receptor change the properties of antiestrogens to full agonists. *Proc Natl Acad Sci U S A.* 92:4206-4210.
- Manolagas, SC. (2000). Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr. Rev.* 21:115-137.
- Manolagas, SC., Kousteni, S., Chen, JR., Schuller, M., Plotkin, L. & Bellido, T. (2004). Kinase-mediated transcription, activators of nongenotropic estrogen-like signaling (ANGELS), and osteoporosis: a different perspective on the HRT dilemma. *Kidney Int. Suppl.* S41-49.
- Maret, A., Clamens, S., Delrieu, I., Elhage, R., Arnal, JF. & Bayard, F. (1999). Expression of the interleukin-6 gene is constitutive and not regulated by estrogen in rat vascular smooth muscle cells in culture. *Endocrinology.* 140:2876-2882.
- Margeat, E., Bourdoncle, A., Margueron, R., Poujol, N., Cavailles, V. & Royer, C. (2003). Ligands differentially modulate the protein interactions of the human estrogen receptors alpha and beta. *J Mol. Biol.* 326:77-92.
- Marino, M. & Ascenzi, P. (2008). Membrane association of estrogen receptor alpha and beta influences 17beta-estradiol-mediated cancer cell proliferation. *Steroids.* 73:853-858.

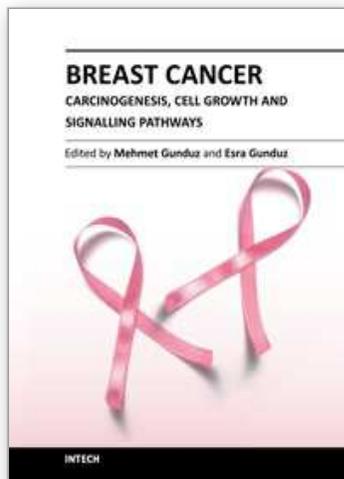
- Marquez, DC. & Pietras, RJ. (2001). Membrane-associated binding sites for estrogen contribute to growth regulation of human breast cancer cells. *Oncogene*. 20:5420-5430.
- McDonnell, DP. & Norris, JD. (2002). Connections and regulation of the human estrogen receptor. *Science*. 296:1642-1644.
- McEwen, B. (2002). Estrogen actions throughout the brain. *Recent Prog. Horm. Res.* 57:357-384.
- McKay LI, Cidlowski JA (1999) Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. *Endocr Rev* 20:435-459.
- Mermelstein, PG., Becker, JB. & Surmeier, DJ. (1996). Estradiol reduces calcium currents in rat neostriatal neurons via a membrane receptor. *J Neurosci*. 16:595-604.
- Metivier, R., Penot, G., Carmouche, RP., Hubner, MR., Reid, G., Denger, S., Manu, D., Brand, H., Kos, M., Benes, V. & Gannon, F. (2004). Transcriptional complexes engaged by apo-estrogen receptor-alpha isoforms have divergent outcomes. *EMBO J*. 23:3653-3666.
- Miceli, V., Cocciadiferro, L., Fregapane, M., Zarccone, M., Montalto, G., Polito, LM., Agostara, B., Granata, OM. & Carruba, G. (2011). Expression of Wild-Type and Variant Estrogen Receptor Alpha in Liver Carcinogenesis and Tumor Progression. *OMICS*. [Epub ahead of print].
- Migliaccio, A., Castoria, G., Di Domenico, M., Ciociola, A., Lombardi, M., De Falco, A., Nanayakkara, M., Bottero, D., De Stasio, R., Varricchio, L. & Auricchio, F (2006). Crosstalk between EGFR and extranuclear steroid receptors. *Ann N Y Acad Sci* 1089:194-200.
- Migliaccio, A., Di Domenico, M., Castoria, G., de Falco, A., Bontempo, P., Nola, E. & Auricchio, F. (1996). Tyrosine kinase/p21ras/MAP-kinase pathway activation by estradiol-receptor complex in MCF-7 cells. *EMBO J* 15:1292-1300.
- Moriarty, K., Kim, KH. & Bender, JR (2006). Minireview: estrogen receptor-mediated rapid signaling. *Endocrinology*. 147:5557-5563.
- Morley, P., Whitfield, JF., Vanderhyden, BC., Tsang, BK. & Schwartz, JL. (1992). A new, nongenomic estrogen action: the rapid release of intracellular calcium. *Endocrinology*. 131:1305-1312.
- Nethrapalli, IS., Tinnikov, AA., Krishnan, V., Lei, CD. & Toran-Allerand, CD. (2005). Estrogen activates mitogen-activated protein kinase in native, nontransfected CHO-K1, COS-7, and RAT2 fibroblast cell lines. *Endocrinology*. 146:56-63.
- Nicholson, RI., Gee, JM., Manning, DL., Wakeling, AE., Montano, MM. & Katzenellenbogen, BS. (1995). Responses to pure antiestrogens (ICI 164384, ICI 182780) in estrogen-sensitive and -resistant experimental and clinical breast cancer. *Ann. N Y Acad. Sci.* 761:148-163.
- Nilsson, S., Makela, S., Treuter, E., Tujague, M., Thomsen, J., Andersson, G., Enmark, E., Pettersson, K., Warner, M. & Gustafsson, JA. (2001). Mechanisms of estrogen action. *Physiol. Rev.* 81:1535-1565.
- Nissen-Meyer, R. (1964). "Prophylactic" ovariectomy and ovarian irradiation in breast cancer. *Acta. Unio. Int. Contra. Cancrum.* 20:527-530.
- Omoto, Y., Inoue, S., Ogawa, S., Toyama, T., Yamashita, H., Muramatsu, M., Kobayashi, S. & Iwase, H. (2001). Clinical value of the wild-type estrogen receptor beta expression in breast cancer. *Cancer Lett.* 163:207-212.

- Osborne, CK., Yochmowitz, MG., Knight, WA., 3rd. & McGuire, WL. (1980). The value of estrogen and progesterone receptors in the treatment of breast cancer. *Cancer*. 46:2884-2888.
- Otto, C, Fuchs, I., Kauselmann, G., Kern, H., Zevnik, B., Andreassen, P., Schwarz, G., Altmann, H., Klewer, M., Schoor M., Vonk, R. & Fritzscheier, KH. (2009). GPR30 does not mediate estrogenic responses in reproductive organs in mice. *Biol. Reprod.* 80:34-41.
- Otto, C., Rohde-Schulz, B, Schwarz, G., Fuchs, I., Klewer, M., Brittain, D., Langer, G., Bader, B., Prella, K., Nubbemeyer, R. & Fritzscheier, KH. (2008). G protein-coupled receptor 30 localizes to the endoplasmic reticulum and is not activated by estradiol. *Endocrinology*. 149:4846-4856.
- Paruthiyil, S., Parmar, H., Kerekatte, V., Cunha, GR., Firestone, GL. & Leitman, DC. (2004). Estrogen receptor beta inhibits human breast cancer cell proliferation and tumor formation by causing a G2 cell cycle arrest. *Cancer Res.* 64:423-428.
- Pearce, ST., Liu, H. & Jordan, VC. (2003). Modulation of estrogen receptor alpha function and stability by tamoxifen and a critical amino acid (Asp-538) in helix 12. *J Biol. Chem.* 278:7630-7638.
- Pedram, A., Razandi, M. & Levin, ER. (2006). Nature of functional estrogen receptors at the plasma membrane. *Mol. Endocrinol.* 20:1996-2009.
- Penot, G., Le Peron, C., Merot, Y., Grimaud-Fanouillere, E., Ferriere, F., Boujrad, N., Kah, O., Saligaut, C., Ducouret, B., Metivier, R. & Flouriot, G. (2005). The human estrogen receptor-alpha isoform hERalpha46 antagonizes the proliferative influence of hERalpha66 in MCF7 breast cancer cells. *Endocrinology*. 146:5474-5484.
- Pietras, RJ., Marquez, DC., Chen, HW., Tsai, E., Weinberg, O. & Fishbein, M. (2005). Estrogen and growth factor receptor interactions in human breast and non-small cell lung cancer cells. *Steroids*. 70:372-381.
- Pietras, RJ. & Szego, CM. (1975). Endometrial cell calcium and oestrogen action. *Nature*. 253:357-359.
- Pietras, RJ. & Szego, CM. (1977). Specific binding sites for oestrogen at the outer surfaces of isolated endometrial cells. *Nature*. 265:69-72.
- Pilat, MJ., Christman, JK. & Brooks, SC. (1996). Characterization of the estrogen receptor transfected MCF10A breast cell line 139B6. *Breast Cancer Res. Treat.* 37:253-266.
- Prall, OW., Rogan, EM. & Sutherland, RL. (1998). Estrogen regulation of cell cycle progression in breast cancer cells. *J Steroid Biochem. Mol. Biol.* 65:169-174.
- Rao, BR. (1998). Isolation and characterization of an estrogen binding protein which may integrate the plethora of estrogenic actions in non-reproductive organs. *J Steroid Biochem, Mol Biol.* 65:3-41.
- Razandi, M., Pedram, A., Greene GL & Levin, ER. (1999). Cell membrane and nuclear estrogen receptors (ERs) originate from a single transcript: studies of ER-alpha and ER-beta expressed in Chinese hamster ovary cells. *Mol Endocrinol.* 13:307-319.
- Rebeck, TR., Levin, AM., Eisen, A., Snyder, C., Watson, P., Cannon-Albright, L., Isaacs, C., Olopade, O., Garber, JE., Godwin, AK., Daly, MB., Narod, SA., Neuhausen, SL., Lynch, HT & Weber, BL. (1999). Breast cancer risk after bilateral prophylactic oophorectomy in BRCA1 mutation carriers. *J Natl. Cancer Inst.* 91:1475-1479.
- Revankar, CM., Cimino, DF., Sklar, LA., Arterburn, JB. & Prossnitz, ER. (2005). A transmembrane intracellular estrogen receptor mediates rapid cell signaling. *Science*. 307:1625-1630.

- Ring, A. & Dowsett, M. (2004.) Mechanisms of tamoxifen resistance. *Endocr. Relat. Cancer.* 11:643-658.
- Robertson, JF. (1996). Oestrogen receptor: a stable phenotype in breast cancer. *Br. J Cancer.* 73: 5-12.
- Rocheftort, H. (1995). Oestrogen- and anti-oestrogen-regulated genes in human breast cancer. *Ciba. Found. Symp.* 191:254-265.
- Roger, P., Daures, JP., Maudelonde, T., Pignodel, C., Gleizes, M., Chapelle, J., Marty-Double, C., Baldet, P., Mares, P., Laffargue, F. & Rocheftort, H. (2000). Dissociated overexpression of cathepsin D and estrogen receptor alpha in preinvasive mammary tumors. *Hum. Pathol.* 31:593-600.
- Roger, P., Sahla, ME, Makela, S., Gustafsson, JA, Baldet, P. & Rocheftort, H. (2001). Decreased expression of estrogen receptor beta protein in proliferative preinvasive mammary tumors. *Cancer Res.* 61:2537-2541.
- Russell, KS., Haynes, MP., Sinha D., Clerisme, E. & Bender, JR. (2000). Human vascular endothelial cells contain membrane binding sites for estradiol, which mediate rapid intracellular signaling. *Proc Natl Acad Sci U S A.* 97:5930-5935.
- Safe, S. (2001). Transcriptional activation of genes by 17 beta-estradiol through estrogen receptor-Sp1 interactions. *Vitam. Horm.* 62:231-252.
- Saji, S, Sakaguchi, H., Andersson, S., Warner, M. & Gustafsson, J (2001). Quantitative analysis of estrogen receptor proteins in rat mammary gland. *Endocrinology.* 142:3177-3186.
- Santen, RJ. (2003). Inhibition of aromatase: insights from recent studies. *Steroids.* 68:559-567.
- Segars, JH. & Driggers, PH. (2002). Estrogen action and cytoplasmic signaling cascades. Part I: membrane-associated signaling complexes. *Trends Endocrinol. Metab.* 13:349-354.
- Shang, YF. & Brown, M. (2002). Molecular determinants for the tissue specificity of SERMs. *Science.* 295:2465-2468.
- Shang, YF., Hu, X., DiRenzo, J., Lazar, MA. & Brown, M. (2000). Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell.* 103:843-852.
- Sheikh, MS., Garcia, M., Pujol, P., Fontana, JA. & Rocheftort, H. (1994). Why are estrogen-receptor-negative breast cancers more aggressive than the estrogen-receptor-positive breast cancers? *Invasion Metastasis.* 14:329-336.
- Shi, .L, Dong, B., Li, Z., Lu, Y., Ouyang, T., Li, J., Wang, T., Fan, Z., Fan, T., Lin, B., Wang, ZY. & Xie, YT. (2009). Expression of ER- α 36, a novel variant of estrogen receptor α , and resistance to tamoxifen treatment in breast cancer. *J Clin. Oncol.* 27:3423-3429.
- Shoker, BS., Jarvis, C., Clarke, RB., Anderson, E., Hewlett, J., Davies, MP., Sibson, DR. & Sloane, JP (1999a). Estrogen receptor-positive proliferating cells in the normal and precancerous breast. *Am. J Pathol.* 155:1811-1815.
- Shoker, BS., Jarvis, C., Sibson, DR., Walker, C. & Sloane, JP. (1999b). Oestrogen receptor expression in the normal and pre-cancerous breast. *J Pathol.* 188:237-244.
- Shupnik, MA. (2004) Crosstalk between steroid receptors and the c-Src-receptor tyrosine kinase pathways: implications for cell proliferation. *Oncogene.* 23:7979-7989.
- Sibonga, JD., Dobnig, H., Harden, RM. & Turner, RT. (1998). Effect of the high-affinity estrogen receptor ligand ICI 182,780 on the rat tibia. *Endocrinology.* 139:3736-3742.
- Song, RX., McPherson, RA., Adam, L., Bao, Y., Shupnik, MA., Kumar, R. & Santen, RJ. (2002). Linkage of rapid estrogen action to MAPK activation by ER α -Shc association and Shc pathway activation. *Mol. Endocrinol.* 16:116-127.

- Speir, E., Yu, ZX., Takeda, K., Ferrans, VJ. & Cannon, RO., 3rd. (2000). Competition for p300 regulates transcription by estrogen receptors and nuclear factor-kappaB in human coronary smooth muscle cells. *Circ. Res.* 87:1006-1011.
- Speirs, V., Parkes, AT., Kerin, MJ., Walton, DS., Carleton, PJ., Fox, JN. & Atkin, SL. (1999). Coexpression of estrogen receptor alpha and beta: poor prognostic factors in human breast cancer? *Cancer Res.* 59:525-528.
- Speirs, V., Skliris, GP., Burdall, SE. & Carder, PJ. (2002). Distinct expression patterns of ER alpha and ER beta in normal human mammary gland. *J Clin. Pathol.* 55:371-374.
- Stoner, M., Saville, B., Wormke, M., Dean, D., Burghardt, R. & Safe, S. (2002). Hypoxia induces proteasome-dependent degradation of estrogen receptor alpha in ZR-75 breast cancer cells. *Mol. Endocrinol.* 16:2231-2242.
- Sylvia, VL., Walton, J., Lopez, D., Dean, DD., Boyan, BD. & Schwartz, Z. (2001). 17 beta-estradiol-BSA conjugates and 17 beta-estradiol regulate growth plate chondrocytes by common membrane associated mechanisms involving PKC dependent and independent signal transduction. *J Cell Biochem.* 81:413-429.
- Tesarik, J. & Mendoza, C. (1997). Direct non-genomic effects of follicular steroids on maturing human oocytes: oestrogen versus androgen antagonism. *Hum. Reprod. Update.* 3:95-100.
- Tice, DA., Biscardi, JS., Nickles, AL. & Parsons, SJ. (1999). Mechanism of biological synergy between cellular Src and epidermal growth factor receptor. *Proc Natl Acad Sci U S A.* 96:1415-1420.
- Tong, JS., Zhang, QH., Wang, ZB., Li, S., Yang, CR., Fu, XQ., Hou, Y., Wang, ZY., Sheng, J. & Sun, QY. (2010). ER-alpha36, a novel variant of ER-alpha, mediates estrogen-stimulated proliferation of endometrial carcinoma cells via the PKCdelta/ERK pathway. *PLoS One.* 5:e15408.
- Toran-Allerand, CD., Guan, X., MacLusky, NJ., Horvath, TL., Diano, S., Singh, M., Connolly, ES., Jr., Nethrapalli, IS. & Tinnikov, A. (2002). ER-X: a novel, plasma membrane-associated, putative estrogen receptor that is regulated during development and after ischemic brain injury. *J Neurosci.* 22:8391-8401.
- Touitou, I., Mathieu, M. & Rochefort, H. (1990). Stable transfection of the estrogen receptor cDNA into Hela cells induces estrogen responsiveness of endogenous cathepsin D gene but not of cell growth. *Biochem. Biophys. Res. Commun.* 169:109-115.
- Troyer, KL. & Lee, DC. (2001.) Regulation of mouse mammary gland development and tumorigenesis by the ERBB signaling network. *J Mammary Gland Biol. Neoplasia.* 6:7-21.
- Tsai, EM., Wang, SC., Lee, JN. & Hung, MC. (2001). Akt activation by estrogen in estrogen receptor-negative breast cancer cells. *Cancer Res.* 61:8390-8392.
- Tzukerman, MT., Esty, A., Santiso-Mere, D., Danielian, P., Parker, MG., Stein, RB., Pike, JW. & McDonnell, DP. (1994). Human estrogen receptor transactivational capacity is determined by both cellular and promoter context and mediated by two functionally distinct intramolecular regions. *Mol. Endocrinol.* 8:21-30.
- Vranic, S., Gatalica, Z., Deng, H., Frkovic-Grazio, S., Lee, MJ., Olga Gurjeva, O. & Wang, Z-Y. (2011). ER- α 36, a novel isoform of ER- α 66, is commonly over-expressed in apocrine and adenoid cystic carcinomas of the breast". *J. of Clinical Pathology.* 64: 54-57.

- Wang, ZY., Zhang, XT., Shen, P., Loggie, BW., Chang, Y. & Deuel, TF. (2005). Identification, cloning, and expression of human estrogen receptor-alpha36, a novel variant of human estrogen receptor-alpha66. *Biochem. Biophys. Res. Commun.* 336:1023-1027.
- Wang, ZY., Zhang, XT., Shen, P., Loggie, BW., Chang, Y. & Deuel, TF. (2006). A variant of estrogen receptor- α , hER- α 36: transduction of estrogen- and antiestrogen-dependent membrane-initiated mitogenic signaling. *Proc Natl Acad Sci U S A.* 103:9063-9068.
- Warner, M., Saji, S. & Gustafsson, JA. (2000.) The normal and malignant mammary gland: a fresh look with ER-beta onboard. *J Mammary Gland Biol. Neoplasia.* 5:289-294.
- Watson, C., Norfleet, A., Pappas, T. & Gametchu, B. (1999). Rapid actions of estrogens in GH3/B6 pituitary tumor cells via a plasma membrane version of estrogen receptor- α . *Steroids.* 64:5-13
- Watters, JJ., Campbell, JS., Cunningham, MJ., Krebs, EG. & Dorsa, DM. (1997). Rapid membrane effects of steroids in neuroblastoma cells: effects of estrogen on mitogen activated protein kinase signaling cascade and c-fos immediate early gene transcription. *Endocrinology.* 138:4030-4033.
- Xie, H., Sun, M., Liao, XB., Yuan, LQ., Sheng, Z.F, Meng, JC., Wang, D., Yu, ZY., Zhang, LY, Zhou, HD., Luo, XH., Li, H., Wu, XP., Wei, QY., Tang, SY., Wang, ZY. & Liao, EY. (2011). Estrogen receptor alpha36 mediates a bone-sparing effect of 17beta-estrodial in postmenopausal women. *J Bone Miner. Res.* 26:156-168.
- Yamashita, H., Iwase, H., Toyama, T. & Fujii, Y. (2003). Naturally occurring dominant-negative Stat5 suppresses transcriptional activity of estrogen receptors and induces apoptosis in T47D breast cancer cells. *Oncogene.* 22:1638-1652.
- Zeps, N., Bentel, JM., Papadimitriou, JM., Dawkins, HJ. (1999). Murine progesterone receptor expression in proliferating mammary epithelial cells during normal pubertal development and adult estrous cycle. Association with eralpha and erbeta status. *J Histochem. Cytochem.* 47:1323-1330.
- Zhang, XT., Kang, LG., Ding, L., Vranic, S., Gatalica, Z. & Wang, ZY. (2011). A positive feedback loop of ER-alpha36/EGFR promotes malignant growth of ER-negative breast cancer cells. *Oncogene.* 30:770-780.
- Zhao, L., O'Neill, K. & Brinton, RD. (2006). Estrogenic agonist activity of ICI 182,780 (Faslodex) in hippocampal neurons: implications for basic science understanding of estrogen signaling and development of estrogen modulators with a dual therapeutic profile. *J Pharmacol. Exp. Ther.* 319:1124-1132.
- Zhao, L., O'Neill, K., Diaz Brinton, R. (2005,) Selective estrogen receptor modulators (SERMs) for the brain: current status and remaining challenges for developing Neuro-SERMs. *Brain Res. Rev.* 49:472-493.
- Zheng, WH., Andersson, S., Cheng, G., Simpson, ER., Warner, M. & Gustafsson, JA. (2003). Update on estrogen signaling. *FEBS Lett.* 546:17-24.
- Zou, Y., Ding, L., Coleman, M. & Wang, ZY. (2009). Estrogen receptor-alpha (ER-alpha) suppresses expression of its variant ER-alpha 36. *FEBS Lett.* 583:1368-1374.



Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways

Edited by Prof. Mehmet Gunduz

ISBN 978-953-307-714-7

Hard cover, 732 pages

Publisher InTech

Published online 30, November, 2011

Published in print edition November, 2011

Cancer is the leading cause of death in most countries and its consequences result in huge economic, social and psychological burden. Breast cancer is the most frequently diagnosed cancer type and the leading cause of cancer death among females. In this book, we discussed various aspects of breast cancer carcinogenesis from clinics to its hormone-based as well as genetic-based etiologies for this deadly cancer. We hope that this book will contribute to the development of novel diagnostic as well as therapeutic approaches.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

ZhaoYi Wang, XianTian Zhang and LianGuo Kang (2011). ER-Alpha36 Mediates Non-Genomic Estrogen and Anti-Estrogen Signaling in Breast Cancer Cells, Breast Cancer - Carcinogenesis, Cell Growth and Signalling Pathways, Prof. Mehmet Gunduz (Ed.), ISBN: 978-953-307-714-7, InTech, Available from: <http://www.intechopen.com/books/breast-cancer-carcinogenesis-cell-growth-and-signalling-pathways/er-alpha36-mediates-non-genomic-estrogen-and-anti-estrogen-signaling-in-breast-cancer-cells>

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen