REVIEW PAPER

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Pathophysiological roles of ERα in the ER signaling mediated oncogenesis of breast cancer

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ABSTRACT

Introduction. Estrogen receptors (ER) are members of nuclear receptors that act in the ER signaling pathway regulating the pathophysiology of hormone-responsive target cells including breast tissue.

Aim. This detailled review literature was written on the pathophysiology of ER signaling as well as the effect altered ERα and associated pathway derangement in the oncogenesis of breast cancer.

Material and methods. This review was performed according to systematic literature search of three major bibliographic databases (Scopus, PubMed, and Cochran).

Analysis of the literature. In this pathway, estrogen receptor alpha (ERα) is a key estradiol-17β (E2) induced transcription factor that has been implicated in the initiation and development of the major fraction of breast cancers. Hence understanding the ERα-mediated ER signaling that results in alterations from normal phenotypic features of breast tissue to the oncogenic features of breast cancer is important. The oncogenic effect of ERα in ER signaling is driven by combinations of molecular assets within the cancer cells. Normally, the transcriptional activity of ERα is controlled by tight regulation of its protein level inside the cells. Altered stability and activity of ERα due to its phosphorylation, ubiquitination, glycosylation, sumoylation, and acetylation events can trigger oncogenic ER signaling.

Conclusion. The function and activity of ERα is also modulated by its interaction with coregulators as well as crosstalk with oncogenic factors from other oncogenic pathways. These all events increase the complexity of the progression of ER+ breast cancer and its response to endocrine therapy.

Keywords. breast, cancer, estrogen receptor alpha, oncogenesis

The list of abbreviations:

AF-1 ‑ activation function 1, **AF-2** ‑ activation function 2, **AIB1** ‑ amplified in breast cancer 1, AIs ‑ aromatase inhibitors, **AP-1** ‑ activator protein 1, **CYP19** ‑ cytochrome p450 family 19, **CYP2D6** ‑ cytochrome p450 family 2 subfamily D member 6, **DBD** ‑ DNA binding domain, **DCIS** ‑ ductal carcinoma in situ, **DNA** ‑ deoxyribose Nucleic Acid, **DR** ‑ drug resistance, **E2** ‑ estradiol-17β, **EGFR** ‑ epidermal growth factor receptor, **ER+** ‑ estrogen receptor positive, **ERE** ‑ Estrogen response element, **ERα** ‑ estrogen receptor alpha, **FISH** ‑ fluorescence in situ hybridization, **GREB1** ‑ growth regulation by es-

trogen in breast cancer 1, **GSK3** – glycogen synthase kinase 3, **HER2** – human epidermal growth factor receptor 2, **IDC** – invasive ductal carcinoma, **IHC** – immunohistochemistry, **ILC** – invasive lobular carcinoma, **LBD** – ligand binding domain, **LCIS** – lobular carcinoma in situ, **MRI** – magnetic resonance imaging, **NRF-1** – nuclear respiratory factor 1, **NRF2** – Notch, nuclear factor erythroid-derived 2, **PAM50** – prosigna of A 50-gene qPCR assay, **PCR** – polymerase chain reaction, **PEST** – proline (P), glutamic acid (E), serine (S), and threonine (T) region, **PI3K** – phosphatidylinositol 3-kinase, **PIN1** – peptidyl-prolyl cis-trans isomerase NIMA-interacting 1, **PR** – progesterone receptor, **RE** – response element, **RNA** – Ribonucleic acid, **SP1** – Specificity protein 1, **Src** – tyrosine-protein kinase, **TF** – transcription factor

Introduction

Cell signaling is a complex network of the communication process that cells normally use to respond to their microenvironment. Oncogenic signalling that drives oncogenesis happens when cellular signaling interactions and information processing is altered.[1,2] Cytogenetic aberrations in signaling pathways that control cell growth, apoptosis and cell-cycle progression are common hallmarks of cancer. The cancer genome atlas (TCGA) study on the oncogenic signaling pathways in 33 cancer types found alteration of cell cycle, Hippo, Myc, Notch signalling, nuclear factor erythroid-derived 2 (Nrf2), phosphoinositide 3-kinase (PI3K/Akt), receptor tyrosine kinase (RTK-RAS) signalling, tumor growth factor betta (TGFβ) signaling, p53 and Wnt/β-catenin signalling pathways.[3] On top of deranged expression of such potentially oncogenic factors in a given pathway, oncogenic signalling pathway cross-talk is also a common phenomenon as most solid tumors often undergo clonal evolution on top of primary cancer initiating oncogenic mutation.[4] The extent, mechanisms, and patters of co-occurrence of alterations in these pathways differ between individual tumors and tumor subtypes.

ER signalling pathway is important in tissue expressing ER for the normal development of breast tissue. However when this pathway is deranged, it has been implicated to trigger the oncogenesis of breast cancer.[5,6] Due to the central importance of ERα in the pathophysiology of E2 target tissues, understanding of E2-ER signaling events that results in alterations from normal phenotypic features to the oncogenic features of breast cancer is important.

So far there have been many studies on the mechanisms of E2/ERα-mediated breast cancer development where ERα plays critical roles among which epigenetic regulation of ERα expression, altered ERα stability and identification of many of ERα coregulators and their association with breast cancer.[7-21] Moreover, the oncogenic event of ER+ breast cancer has been found to more com-

plex especially when pathway crosstalk happens with other oncogenic signals.[22,23] This implicates that on top of the current ERα targeted endocrine therapy, there is a need of systematic analysis of oncogenic events and the resistance mechanisms of endocrine therapy for such major subtype of breast cancer. Hence, this detailed review literature was written on the pathophysiology of ER signaling as well as the effect altered ERα and associated pathway derangement in the oncogenesis of breast cancer.

Aim

This detailed review literature was written on the pathophysiology of ER signaling as well as the effect altered ERα and associated pathway derangement in the oncogenesis of breast cancer.

Breast cancer: Overview

Breast cancer is the most common malignancy as well as the leading cause of cancer death in women with increasing incidence rate all across the world.[24,25] According to global cancer incidence, mortality and prevalence (GLOBOCAN), breast cancer accounts for 25.1% of all cancers with a higher incidence rate in developed countries and relative greater mortality in less developed countries.[26]

Risk factors for breast cancer include being female, menarche at early ages and menopause in old ages, the use of preventive pregnancy hormones, opting not to have children, obesity after menopause, use hormones to prevent pregnancy, physical inactivity and alcohol consumption. In contrast, having children and breast-feeding are preventive factors.[27]

Formation of a lump in the breast tissue is the most common symptom of breast cancer, but symptoms vary in many cases. The other common symptoms of breast cancer include irregular enlargement of the breast, abnormal or bloody discharge from the nipple, dimpling and rash on the nipple. It has been reported that only one in 10 lumps is diagnosed as malignant.[28]

Primary screening for diagnosis of breast cancer commonly includes clinical examination followed by mammography which is specialized medical imaging system that uses a low-dose x-ray to detect breast cancer. Adjunctive screening for breast cancer uses breast ultrasonography and magnetic resonance imaging (MRI) which uses radio waves & magnetic fields to produce detailed images of breast tissue.[29] A biopsy of a small sample of breast tissue or fluid taken from the suspicious area is also analyzed to know the type of breast cancer cells, the grade of cancer as well as the hormone receptor status that can influence the patient treatment options.

Subtypes of breast cancer

The choice and improvement of best diagnosis, prognosis and treatment of breast cancer rely on the knowl-

edge of the clinical heterogeneity, genetic and intrinsic heterogeneity of breast cancer. In this regard breast cancer is classified into multiple subtypes based on the following three major subtyping features. These subtyping features are defined by histological analysis, molecular characterization and functional subtyping of the breast cancers.[30]

Histological subtyping classifies breast cancers based on their histological features and growth patterns. Breast cancer can be broadly categorized into pre-invasive (25%) or invasive carcinoma (75%).[28,29] Pre-invasive carcinoma can then be sub-divided into ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). The invasive carcinomas are more heterogeneous in that they can be categorized as invasive ductal carcinoma (IDC) which is the most common one, invasive lobular carcinoma (ILC), tubular carcinoma, infiltrating ductal carcinoma, mucinous, and medullary carcinoma.[29-31] The traditionally used histological staining like fluorescence in situ hybridization (FISH) and immunohistochemistry (IHC) allow us to classify the clinical specimen of breast cancer into ER+, progesterone receptor (PR+), and/or containing an amplification of the human epidermal growth factor receptor 2 (HER2).[32] But it does not segregate the luminal A and B subtype of ER+ cancer that may have a distinct clinical response to the given therapy.[33,34] The emerging needs for personalized therapy and prognostics also require more advanced breast tumor classification with greater diagnostic precision.

Molecular subtyping is used as a compliment to primary screening and histological subtyping, as a prognostic indicator and to inform the choice of therapy. Molecular subtyping of breast tumors means categorizing tumors according to microarray analysis of their gene expression patterns. The two genomic tests are bluePrint/mammaPrint and the prosigna breast cancer prognostic gene signature assay (PAM50 assay). Based on the expression analysis of ER, PR, HER2, Claudin, epidermal growth factor receptor (EGFR), Keratin 5/14, E-cadherin, Vimentin and its major combinations, breast cancers are grouped into four major molecular subtypes. These are Luminal A (ER/RP+ HER2- and low Ki-67 expression), Luminal B (ER/RP+ and HER2+ or HER2- but high Ki-67 expression), HER2-enriched and Basal subtype (triple negative). In addition to these four subtypes, a normal-like, Basal-like and Claudin-low subtype have also been identified.[33-35]

The functional subtyping of breast cancer is an extension of molecular subtyping. It is based on the newly emerging hypothetical concept that the functional outcome of the tumor may depend on the perigenetic alterations present in the tumor-initiating mammary stem cell or progenitor cell being transformed by various oncogenes.[36,37] Molecular features associated with a biological function or clinical outcome of particular subtypes within a given tumor may lead to the heterogeneity of breast carcinoma.[36,38] Hence functional genetic screening is needed to identify genetic signatures that play a critical role for growth and drug response of specific subtype of breast cancer. For example, the patterns of sensitivities to the targetable oncogenes such as PTEN mutation or functional genetic screening of a given kinase inhibitors helps to define the functional viability profiles of breast cancer.[39]

Treatment strategies and endocrine resistance

The treatment plan for breast cancer depends on the biology and behavior of cancer and the status of the patient. As a result of this, treatment recommendations and options are very personalized and many factors are often taken in to account including, the tumor's subtype, stage of the tumor, the presence of an inherited mutation and genomic markers, the patient's general health status, age, menopausal status and patient preferences.

The common breast cancer treatment includes surgery, hormonal therapy, radiation therapy, chemotherapy and targeted therapy. ER and PR are standard biomarkers used in clinical practice to characterize breast cancers. ERα+ breast cancers can be effectively targeted endocrine therapy which includes selective ER modulators such as Tamoxifen, selective ER down-regulators such as Fulvestrant and Aromatase inhibitors (AIs). About 30% of cases of ERα+ breast cancers treated with Tamoxifen develop resistance.[40] In some patients, this *de novo* tamoxifen resistance could be culminated by treatment with Fulvestrant and AIs indicating that hormonal-based therapy reduces the recurrence risks and confers survival benefit for ER+ breast cancer.[41-43] However, the risk of disease recurrence albeit 5 years after adjuvant-based tamoxifen treatment is still substantial.[44,45] Activation of an alternative signalling pathway during endocrine therapy remains a challenge as it results in the growth of treatment resistance clones, recurrence of cancer and treatment failure.

So far several acquired endocrine resistance mechanisms have been proposed. Upregulation of the ERα co-regulator Amplified in breast cancer 1 (AIB1) potentiates tamoxifen agonistic effects, especially in the presence of HER2 expression.[46] Phosphatidylinositol 3-kinase (PI3K) and mitogen-activated protein kinases (MAPK) pathways activation by aberrant growth factor signaling have also been implicated in resistance to tamoxifen, as well as AIs.[47-50] Aberrant expression of genes such as c-Myc, BCL2 associated agonist of cell death (BAD) and apoptosis regulator B-cell lymphoma 2 (BCL2) and breast cancer anti-estrogen resistance 3 (BCAR3) have been reported to allow cancer survival and proliferation under endocrine therapy.[51,52] Efficacy of tamoxifen and AIs is also influenced by functional

polymorphisms, with cytochrome p450 family 2 subfamily D member 6 (CYP2D6) and cytochrome p450 family 19 (CYP19), being the most widely studied.[53,54]

Taking the central role of ERα in the tumorigenesis and drug response of breast cancer, understanding the regulation of cellular status of ERα is by far important. Altered ERα expression or mutations which give rise to an active ligand-independent form or epigenetic silencing also contribute to endocrine resistance.[8-12] One of the identified modifications which have been reported to induce resistance to tamoxifen is serine-305 phosphorylation at the hinge region of ERα.[55] ER+ tumours generally respond less well to chemotherapy.[56,57] It was observed that if ERα is reconstituted back to ER-negative cells it became less responsive to chemotherapeutic agents, raising the possibility that ERα modulates chemo-response.[58,59] Since the underlying mechanisms are barely known and likely complex, tamoxifen co-administration with chemotherapy has not proven effective choice to treat cases of primary breast cancer.

Estrogen Receptor Signaling in Normal Human Physiology

Estrogen receptors are members of a large superfamily of nuclear receptors that act as transcription factors. The activity of estrogen receptors is modulated by steroid hormones; hydrophobic hormones generally synthesized from cholesterol in the gonads and adrenal glands. The best-characterized estrogen receptors are those responsible for membrane-initiated estradiol signaling. These include the classical ERα and Estrogen Receptor beta (ERβ) isoforms. ERα and ERβ are encoded by separate genes and each has different and specific roles in mammals.[60-63]

Estrogens are one class of steroid hormones that include estriol, estradiol and estrone. E2, the most potent circulating hormone involved in the detailed array of important physiology. E2 regulates development of reproductive organs, regulation of musculoskeletal, cardiovascular as well as immune system, and homeostasis of the central nervous system.[5,6] ER signaling in normal breast tissue is activated by E2 and it modulates the normal development of the mammary gland. The activity and expression of ERα are tightly controlled at transcriptional and post-translational levels.[64]

E2 can enter cells and interacts with the ER in two ways. Being lipophilic, E2 passes through the plasma membrane of any cell freely and then interact with cytoplasmic ER. E2 can also enter the cell through ER-mediated membrane signaling. Starting from such slightly distinct intracellular localization paths, E2 triggers three major ER signaling events that can follow five interconnected signalling pathways (Figure 1). The three signaling events are ER-mediated membrane signaling event, ER-mediated mitochondrial events and ER-mediated nuclear signaling events.

The plasma membrane-initiated signaling is a rapid and transcriptional-independent E2 signaling event. Following binding of E2 to ER, the membrane-integrated and palmitoylated ER monomer dimerizes as shown on the second (II) signalling pathway in Figure 1 below. Once dimerized, it detaches from the membrane and localizes to the nucleus where it induces nuclear ER signaling. The E2 induced plasma membrane-associated ER dimerization may also allow the recruitment of G protein that can collaterally activate kinases allowing the activation of the tyrosine-protein kinase (Src) and RTK oncogenic signaling pathway.[65-68] ERα palmitoylation was found to be important in this signaling cascade, as mutation of Cystine-451 residue to Alanine within the ligand binding domain (LBD) prevents the trafficking of the receptor to the membrane thereby abrogating nuclear ER-mediated transcriptional events. Introducing this mutation into mice resulted in infertility, abnormal regulation of the pituitary hormone, abnormal ovaries, arrested the development of mammary gland and altered vasculatures.[69,70]

The mitochondria are an important target of ER-mediated E2 action in which both ERα and ERβ can localize to inhibit early stage of apoptosis induced by several stimuli.[71-74] The E2-ER complex alters mitochondrial function by directly acting on mitochondrial deoxyribose nucleic acid (DNA) to induce mitochondrial gene expression; including expression of mitochondrial adenosine triphosphate (ATP) synthase subunits and manganese superoxide dismutase.[75,76] Here the superoxide generated by E2 induced mitochondrial biogenesis is counterbalanced by manganese superoxide dismutase encoded from mitochondrial DNA to prevent apoptosis (Figure 1: signaling pathway V). The E2-induced nuclear receptor signalling can also indirectly act on mitochondria by activating the expression of Nuclear respiratory factor 1 (NRF-1) that can enter into the mitochondrial scaffold to regulate mitochondrial function and maintain cellular integrity.[71,77,78]

Nuclear ER-mediated signaling is the major effector arm of the ER signaling pathway regulating the pathophysiology of hormone-responsive target cells. ER has a nuclear localization signal that interacts with importin, a nuclear membrane component, thus maintaining ER's nuclear localization.[79] Once E2-ER complex moves to the nucleus, it follows two well-known separate modes of action. These are the E2 response element (ERE)-dependent (sometimes called the genomic) signaling pathway and ERE-independent or non-genomic signaling pathway.

In the ERE-dependent pathway, the E2-ER complex directly binds to the consensus motif sequence (5'-GGTCAnnnTGACC-3') often found on the promoter of ER target genes to regulate its expression (Figure 1: signaling pathway I and II). The E2 liganded ERα

binds to ERE through the sequence specificity P-box residue (Glutamine-203, Glycine-204 and Alanine-207) and conserved Arginine-211 residue recruiting co-regulators, chromatin modifiers as well as the transcription initiation complex.[80-83] Briefly, the E2-ER complex first recruits the p160 co-activator family members (Sirtuin 1-3, AIB1, Nuclear receptor coactivator 2) which have histone acetyltransferase activity to open-up the chromatin. This phenomenon allows the recruitment of the p300 co-integrator that further relaxes the chromatin. p300 activates the subsequent binding of RNA polymerase II on to the transcription initiation sites to start transcription of the target genes.[84-86]
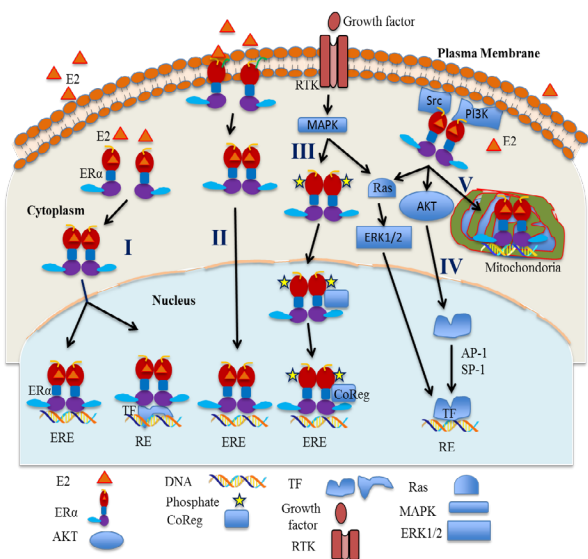

Fig. 1. Estrogen Receptor Signalling Pathways: I) In ER-dependent nuclear signalling, E2 can freely inter the cell, bind and dimerize ERα. Once ERα dimer localizes to nucleus it may bind directly to an ERE or binds to another response element (RE) through transcription factors (TF). II) ER-mediated membrane signaling pathway. Here receptor associated ER monomer binds to E2 and dimerizes. III) E3-independent signalling pathway/growth factor-mediated ER signalling. Here MAPK/Ras-mediates the cascade or MAPK phosphorylates ERα. Phosphorylated ERα dimerize and localizes to the nucleus where it recruits co-activators and bind to ERE. IV) ER-mediated Src, PI3K/ AKT signalling crosstalk which involves other TF (AP-1 and SP-1). V) ER-mediated mitochondrial signalling events also happen when ER localizes and binds to mitochondrial DNA (modified)[87,88]

The ERE-independent pathway involves the mechanism whereby E2 liganded and/or antagonist/agonist liganded ER dimer interacts with co-regulatory proteins or transcription factors such as specificity protein 1(SP-1) and activator protein 1 (AP-1) that have their own cognate response elements to modulate the expression of genes involved in cell proliferation, differentiation or

cell death.[89-96] The critical involvement of co-regulators accordingly makes a good platform to diversify the E2 responsive genes (Figure 1: signalling pathway IV). The involvement of co-regulators in this pathway diversifies the chromatin binding coverage for the genes responsive to ER signalling pathway.

To further understand the role of the ERE-independent pathway, the P-box residues (Glycine-204, Glutamine-203 and Alanine-207) found on the DNA binding domain of ERα were mutated in human cell lines and in mice thereby ERE independent E2 mediated signalling pathway model were developed [97]. Later-on to reduce the effect of such mutation on the co-regulator binding, Arginine-211 was mutated to Glutamine-211 along with ER203/204 thereby created ERE-binding null ER. These mutations were sufficient to abrogate the cardinal ERE response while still allowing activation of subsets of ERE-independent E2 responsive genes. The *in vivo* mouse knock-in model of these mutants showed the phenotype of hypoplastic uteri, hemorrhagic ovary, reduced mammary gland development which are phenotypic features of ERα-knockout mouse.[98,99] Furthermore, ERE oligonucleotide used as decoy DNA transfected into ER+ breast cancer cells were shown to halt E2 induced growth.[100] These examples indicate that the ERE-dependent signalling pathway accounts for the majority of physiologically relevant E2-ER signalling.

Structure and function of ER

Estrogen receptors (ERα and ERβ) have some basic structural features that underlie the similarity of their function. Linking the ER structural topology to its function, there are five segments of ER functional domains encoded by 8 exons. ERα is encoded on chromosome 6 and is 595 amino acid residues in length or 66-kD when translated. In comparison, ERβ is smaller in size, encoded from chromosome 14 and it is 530 amino acid in length, or 60-kD when translated. The homology of the two ER domains varies across the domain with the highest similarity (97% homology) in C segment of DNA binding domain (DBD) (Figure 2).

The amino-terminus A/B domain of ER is highly disordered AF-1 region but co-operatively assemble to keep the structural integrity of activation function 2 (AF-2) region of LBD in accordance with the signaling milieu.[103-107] The A/B domain of ER can display a variety of dynamic conformations that change with modifications such as phosphorylation or upon interaction with another protein partner.[108] Compared to ERα, ER-β has a truncated AF-1 region and thus it's interaction with other protein partner is impaired.[109-112]

In ERE-dependent ER signaling, ER bind to chromatin through the C domain called DBD. This domain is highly conserved between the two estrogen receptors and it dimerizes and assembles itself on the DNA

double helix forming a zinc finger module, the P-box and D-box determining half-site spacing and DNA-sequence binding specificity.[80-82] It has been also reported that this domain is linked to the hinge region (D domain) containing nuclear localization signal and allow the binding of associated transcription factor during modulation of ERE-independent signaling pathway activation [113]. The D segment of ER also contains multiple ubiquitination residues that undergo posttranslational modification to determine the half-life of ERα.[114]
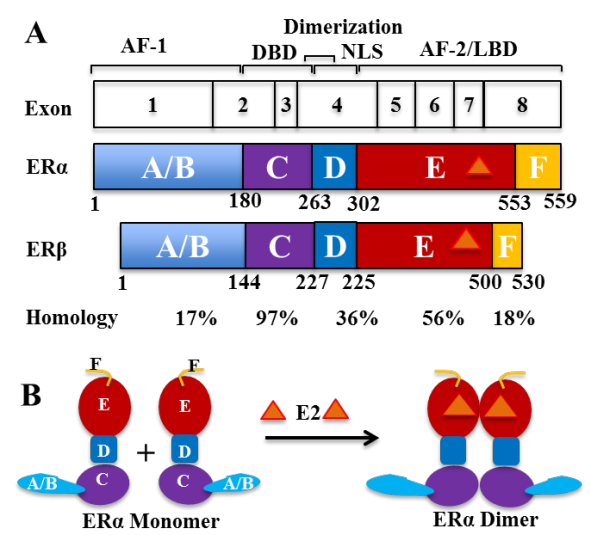

Fig. 2. Human ERα and ERβ Domain Structure. A) Schematic presentation of ERα and ERβ domains with corresponding percent homology. Both ERα and ERβ genes are expressed from 8 exons and have five interconnected segments of functional domains. The percent homology between the two ER for each segment is indicated. B) ERα domain arrangement and conformation change during E2 induced dimerization (adapted)[101,102]

The LBD of ERα is predominantly involved in the dimerization of ERα following the formation of ligand binding scaffold; a very important segment for ligand-dependent activation of ER signalling.[115] E2 binds to this AF-2 domain thereby recruit co-activators.[116-118] The conformational status of the dynamically mobile H12 helicase motif in the LBD determines the choice of agonist or antagonists as well as the co-activator binding events. Thus LBD has been chosen as the best pocket-docking site for drugs.[117,119]

Roles of ERα in ER Signaling in Breast Cancer

The oncogenic effects of ER signalling are driven by combinations of molecular assets within the cancer cells. E2 induced ER signaling has been also implicated in the initiation and development of breast cancer.[5,6] Functional study in this pathway also showed that impairing estrogen signalling by removing the ERα causes

the defects on the reproductive system and brain in both female and male mice, whereas prolonged exposure of exogenous E2 by using contraceptives or other hormone therapy has been shown to promote the incidence and progression of many hormone-dependent; breast, ovary, and prostate cancers.[120-124]

Normally, the transcriptional activity of ERα is controlled by tightly regulating its protein level inside the cells. In the oncogenesis of breast cancer, E2 influences uncontrolled cell proliferation or promote ER independent signalling to sidestep the physiologically controlled ER signalling.[125,126] Few reports also showed that breast cancers express a protein that stabilizes ERα to promote proliferation.[127,128] Thus understanding the cellular and molecular events in breast cancer that regulate ERα stability and function is very important.

Altered ERα stability triggers oncogenic ER signaling

Given that the majority of breast cancers are ERα+ and the cellular level ERα is a critical determinant both as a diagnostic marker as well as a targetable molecule, understanding the mechanisms that underlie the tight regulation of ERα will greatly impact breast cancer therapy. It has been reports that ERα undergoes an intricate interplay of post-translational modifications such as phosphorylation, ubiquitination, glycosylation, sumoylation, and acetylation events that can modulate un-liganded or liganded ERα stability as well as function thereby triggers oncogenic ER signaling in breast cancer.[13-19]

The post-translational modification of ER that regulate ER stability in cancer is often mediated by several proteins that interact and protect ER from degradation by the ubiquitin-proteasome system.[129] Through diverse mechanisms, these proteins prevent polyubiquitination and degradation of ER, leading to an increase in ER protein levels; consequently, estrogen signaling and its physiologic effects are enhanced in breast cancer cells. Thus, increased protein stability seems to be one of the main reasons that ER is upregulated in breast cancer. For instance there are coactivators that stabilize the level ER protein, the kinases like GSK3, LMTK3, and ABL interact with and stabilize ER, non-nuclear mechanisms for maintaining ER stability and involvement of other proteins like MUC1, PIN1, GSK3, LMTK3, RNF31, RB, and ABL have been reported to be involved in ER stability in breast cancer.[127-138] More recently TRIM56 has been identified to be a novel regulatory factor prolongs ERα protein stability in breast cancer, through targeting ER alpha K63-linked ubiquitination.[139] Some of the depicted mechanism how such post-translatioal modification affect ER stability is diacussed below.

ERα Ubiquitination

The cellular level of ERα is primarily regulated by ubiquitination, especially when ERα is transcriptionally ac-

tive. Reports have shown some proteins dynamically interact with and prevent degradation of ERα by the ubiquitin-proteasome system.[114,127,128,134] The oncogenesis of breast cancer could emanate evolving the molecular mechanisms whereby cancer cell bypasses this polyubiquitination and degradation of ERα, thus increases its stability.

An oncogenic glycoprotein Mucin-1(MUC1) has been reported to binds directly to the ERα DBD and stabilize ERα by blocking its ubiquitination and degradation.[137] Another protein called Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1) interacts with ERα to prevent the binding of E3 ligase, E6AP, to ERα thereby prevents the ubiquitination and degradation of ERα.[138] Recently, a Ring-between-Ring E3 ligase family protein; Ring Finger Protein 31 has been found to associate with ERα and catalyzes ERα monoubiquitination but blocking its polyubiquitination thereby increasing ER protein stability.[127] Retinoblastoma transcriptional corepressor (RB) has also been reported to interact with and stabilize ERα, protecting it from degradation by the ubiquitin-proteasome system (UPS) in breast cancer.[139,140]

ERα Phosphorylation

Phosphorylation of ERα also regulates its stability and function. ERα is phosphorylated following the activation of various kinases. It has been reported that MAPK, AKT, PKA, RSK and Src kinase-associated pathway phosphorylates various residues of ERα.[141-143] The kinases like glycogen synthase kinase 3 (GSK3), lemur tyrosine kinase 3, abelson tyrosine-protein kinase and casein kinase 2 also phosphorylate and stabilize ERα.[130-135] Phosphorylation impacts ERα in various ways including altering its ubiquitination, chromatin interactions, recruitment of coregulators and the expression target genes that trigger the growth of breast tumor and patient response to endocrine therapy.[40,144-147] Concomitant to phosphorylation, there are also other mechanisms and proteins associated with ERα stability. For example, phosphatidylethanolamine binding protein 4 that competes with ERα for components of the UPS and other posttranslational modifications like acetylation, palmitoylation, can also affect ERα stability by affecting its phosphorylation.[148-150] On top of this, c-Abl regulates ERα transcription activity through its stabilization by phosphorylation.[132]

ERα Glycosylation

So far, there is no experimentally validated glycosylation of human ERα. However, mouse ERβ has been reported to undergo an alternative O-glycosylation/O-phosphorylation. This posttranslational modification occurs on Serine-16 near to the transactivation domain and Threonine-575 as part of a PEST region (a peptide sequence that is rich in proline, glutamic acid, serine, and threonine which acts as a signal peptide for prompt protein degradation) on mouse ERβ suggesting that glycosylation may regulate transactivation and turnover of ERβ indicating that such saccharide modification may also play a role in modulating the dimerization, stability, or transactivation functions of Estrogen receptors.[151-153] More recently ERα Glycosylation by N-Acetylgalactosaminyltransferase 6 (GALNT6) found to affect its nuclear localization in breast cancer cells.[154] Moer recently GREB1 a well known top E2 responsive ER target that regulate ER signaling in breast cancer was computationaly identified as a putative glycosyltransferase enzyme.[155,156] Another study also showed that the amino-terminal of GREB1 interracts with ERα thereby trigger progression of breast cancer.[157] Nevertheless whether the conserved c-terminal GREB1 domain predicted to have glycosyltransferase activity affects the transcriptional co-activator function of ERα or albeit affect ERα stability remains to be biochemically investigated.

Mutation of ERα

ERα gene (*ESR1*) somatic mutations have been linked to the acquired resistance to endocrine therapies in breast cancer.[12,158-161] The most prevalent point mutations of ERα are Y537S and D538G.[158] These mutations which are found on the LBD of ERα have been reported to affect the ERα co-activator binding conformation as well as chaperone-mediated regulation of ER stability.[158] In breast tumour cells, ERα mutations at the sites linked to ERα degradation were also reported to regulate its stability.[114,134]

Altered ERα expression

The deregulation of E2–ER signaling plays a critical role in the initiation and progression of target tissue malignancies as well as in ER-driven neoplastic processes and also the development of endocrine resistance in the treatment of estrogen target tissue malignancies, exemplified by breast cancers.[19,162-164]

The regulation of ERα signaling could be altered due to alteration of ER expression via epigenetic events that leads to the initiation and/or progression of numerous types of cancer including breast cancer. Epigenetic dysregulation of the GC-rich promoter of ER due to methylation-mediated ERα gene silencing during tumor progression happens in one-third of breast cancers that initially express ERα.[165]

Altered reregulation of ERα function and activity

The E2 induced ER signaling could underlay the carcinogenesis of breast cancer if the ERα function and activity is altered. For instance the loss of control over cell cycle progression following overexpression of Cyclin D1 could be due to heightened E_2 induced ER sig-

naling that recruits various transcription factors that involve ATF-2 and c-Jun without ERE requirement on the cyclin D1 promoter.[166,167] The extranuclear actions of ER also affect breast cancer cell proliferation, migration, drug resistance, and apoptotic inhibition by stimulating various pathway cross-talks.[168,169] Rapid E_2 action leads to the activation of IGF-1R and EGFR as well as stimulation of the Src kinase, MAPK, PI3K, and protein kinase C (PKC) pathways in the cytosol for breast cancer cells. [22,23]

On top of this, there several studies that show ERα coregulators in ER signaling also play role in the development breast cancer. Many well-characterized coregulators with the potential to influence the ERα-mediated breast cancers by interacting with ERα thereby regulate chromatin remodeling and directly or indirectly regulate target gene expression.[20,21] For instance, the AIB1 coactivator activates ERα-dependent transcription by recruiting HAT such as p300 and P/CAF to ERα target gene chromatin.[170] AIB1 interacts with ERα in a ligand-dependent fashion and it leads to ERα stabilization in the presence of E2, thereby regulating ERα activity, as well as ERα protein degradation mediated by the ubiquitin proteasome pathway.[171]

Recently, GREB1 was reported to function as a transcription co-activator of ERα. Loss or dysregulation of GREB1 substantially decreased ERα-mediated gene transcription and reduced tumor growth.[155,172-174] One study found GREB1-ERα interactions in 50% of ER+ cancers and showed GREB1 expression was correlated with a good clinical outcome.[151] Nevertheless, little is known about the exact role of GREB1 in the cascade of hormone action, though it appears to be a key E2-induced gene having a role in ER signaling. MUC1 is also a potent coactivator of ERα. It regulates ERα activity by directly binding to the DNA binding domain of ERα and stabilizes ERα by blocking its ubiquitination and degradation in breast cancer cells.[175]

In contrast to coactivators, corepressors recruit histone deacetylases (HDACs) to ERα target gene chromatin, which leads to the chromatin condensation and the inhibition of ERα target gene expression in breast cancer cells.[7] The corepressors counterbalance the actions of coactivators to orchestrate the magnitude of E2 responses, which leads to the inhibition of ERα target gene expression. Therefore, the loss of ERα corepressors promotes breast cancer.[176] For instance, MTA1 containing nucleosome remodeling and histone deacetylation complex (NuRD) suppresses ERα-mediated gene expression, resulting in invasive breast cancer phenotype.[177] While Nuclear receptor corepressor 1 (NCOR1) is another well-defined corepressor of ERα that inhibits ERα transcriptional activity by binding to the ligand-binding domain. Low expression of NCOR1 is associated with shorter relapse-free survival in breast

cancer patients, which shows that loss of NCOR1 enhances breast cancer development.[178] As ERα-mediated physiological response results from the coordination between ERα, coactivators, and corepressors, targeting expression-profiles for all coregulators may help patient diagnosis and treatment of the breast cancer subtypes.

Conclusions

ER+ breast cancer constitutes a major fraction of breast cancers. Thus, tremendous efforts have been made to explore ERα function and its relevance to breast cancer. As a result, many novel mechanisms of E2/ERα-mediated breast cancer development were discovered including epigenetic regulation of ERα expression, altered stability and identification of hundreds of ERα coregulators and their association with breast cancer development. The extensive posttranslational modification of estrogen receptor regulating ERα stability shows the complexity of ER signals, especially when pathway crosstalk happens with other oncogenic signals. Hence, a better understanding of oncogenic events that drives expression, activity, stability of ERα may play a critical role in the development of diagnostic and prognostic biomarker as well as to overcome endocrine therapy resistance.

Acknowledgments

References

1. Vlahopoulos SA, Cen O, Hengen N, et al. Dynamic aberrant NF-kappaB spurs tumorigenesis: a new model encompassing the microenvironment. *Cytokine Growth Factor Rev.* 2015;26(4):389-403.

2. Wang K, Grivennikov SI, Karin M. Implications of anti--cytokine therapy in colorectal cancer and autoimmune diseases. *Ann Rheum Dis.* 2013;72, 2:ii100-103.

3. Sanchez-Vega F, Mina M, Armenia J, et al. Oncogenic Signaling Pathways in The Cancer Genome Atlas. *Cell.* 2018;173(2):321-337 e310.

4. Sawyers C. Targeted cancer therapy. *Nature.* 2004;432(7015):294-297.

5. Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. Production and actions of estrogens. *N Engl J Med.* 2002;346(5):340-352.

6. Nelson LR, Bulun SE. Estrogen production and action. *J Am Acad Dermatol.* 2001;45(3):116-124.

7. Feng Q, Zhang Y. The NuRD complex: linking histone modification to nucleosome remodeling. *Curr Top Microbiol Immunol.* 2003;274:269-290.

8. Sharma D, Blum J, Yang X, Beaulieu N, Macleod AR, Davidson NE. Release of methyl CpG binding proteins and histone deacetylase 1 from the Estrogen receptor alpha (ER) promoter upon reactivation in ER-negative human

breast cancer cells. *Mol Endocrinol.* 2005;19(7):1740-1751.

9. Garcia-Becerra R, Santos N, Diaz L, Camacho J. Mechanisms of resistance to endocrine therapy in breast cancer: focus on signaling pathways, miRNAs and genetically based resistance. *Int J Mol Sci.* 2012;14(1):108-145.

10. Barone I, Cui Y, Herynk MH, et al. Expression of the K303R estrogen receptor-alpha breast cancer mutation induces resistance to an aromatase inhibitor via addiction to the PI3K/Akt kinase pathway. *Cancer Res.* 2009;69(11):4724-4732.

11. Li Y, Meeran SM, Patel SN, Chen H, Hardy TM, Tollefsbol TO. Epigenetic reactivation of estrogen receptor-alpha (ERalpha) by genistein enhances hormonal therapy sensitivity in ERalpha-negative breast cancer. *Mol Cancer.* 2013;12:9.

12. Robinson DR, Wu YM, Vats P, et al. Activating ESR1 mutations in hormone-resistant metastatic breast cancer. *Nat Genet.* 2013;45(12):1446-1451.

13. Giuliano M, Trivedi MV, Schiff R. Bidirectional Crosstalk between the Estrogen Receptor and Human Epidermal Growth Factor Receptor 2 Signaling Pathways in Breast Cancer: Molecular Basis and Clinical Implications. *Breast Care (Basel).* 2013;8(4):256-262.

14. Levin ER. Bidirectional signaling between the estrogen receptor and the epidermal growth factor receptor. *Molecular endocrinology.* 2003;17(3):309-317.

15. Trevino LS, Weigel NL. Phosphorylation: a fundamental regulator of steroid receptor action. *Trends Endocrinol Metab.* 2013;24(10):515-524.

16. Anbalagan M, Huderson B, Murphy L, Rowan BG. Post-translational modifications of nuclear receptors and human disease. *Nucl Recept Signal.* 2012;10:e001.

17. Le Romancer M, Poulard C, Cohen P, Sentis S, Renoir JM, Corbo L. Cracking the estrogen receptor's posttranslational code in breast tumors. *Endocr Rev.* 2011;32(5):597-622.

18. Zhou W, Slingerland JM. Links between oestrogen receptor activation and proteolysis: relevance to hormone-regulated cancer therapy. *Nat Rev Cancer.* 2014;14(1):26-38.

19. Leung YK, Lee MT, Lam HM, Tarapore P, Ho SM. Estrogen receptor-beta and breast cancer: translating biology into clinical practice. *Steroids.* 2012;77(7):727-737.

20. Green KA, Carroll JS. Oestrogen-receptor-mediated transcription and the influence of co-factors and chromatin state. *Nat Rev Cancer.* 2007;7(9):713-722.

21. Lonard DM, O'Malley BW. The expanding cosmos of nuclear receptor coactivators. *Cell.* 2006;125(3):411-414.

22. Yamnik RL, Digilova A, Davis DC, Brodt ZN, Murphy CJ, Holz MK. S6 kinase 1 regulates estrogen receptor alpha in control of breast cancer cell proliferation. *J Biol Chem.* 2009;284(10):6361-6369.

23. Mann M, Krishnan S, Vadlamudi RK. Emerging significance of estrogen cancer coregulator signaling in breast cancer. *Minerva Ginecol.* 2012;64(1):75-88.

24. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin.* 2011;61(2):69-90.

25. Clegg LX, Reichman ME, Miller BA, et al. Impact of socioeconomic status on cancer incidence and stage at diagnosis: selected findings from the surveillance, epidemiology, and end results: National Longitudinal Mortality Study. *Cancer Causes Control.* 2009;20(4):417-435.

26. Ghonche M, Pournamdar Z, Salehiniya H. Incidence and Mortality and Epidemiology of Breast Cancer in the World. *Asian Pac J Cancer Prev.* 2016;17(3):43-46.

27. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. *CA Cancer J Clin.* 2015;65(2):87-108.

28. Pruthi S. Detection and evaluation of a palpable breast mass. *Mayo Clin Proc.* 2001;76(6):641-647;647-648.

29. Bevers TB, Anderson BO, Bonaccio E, et al. NCCN clinical practice guidelines in oncology: breast cancer screening and diagnosis. *J Natl Compr Canc Netw.* 2009;7(10):1060-1096.

30. Malhotra GK, Zhao X, Band H, Band V. Histological, molecular and functional subtypes of breast cancers. *Cancer Biol Ther.* 2010;10(10):955-960.

31. Recommendations for the reporting of breast carcinoma. Association of Directors of Anatomic and Surgical Pathology. *Am J Clin Pathol.* 1995;104(6):614-619.

32. Hon JD, Singh B, Sahin A, et al. Breast cancer molecular subtypes: from TNBC to QNBC. *Am J Cancer Res.* 2016;6(9):1864-1872.

33. Sorlie T, Perou CM, Tibshirani R, et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proceedings of the National Academy of Sciences of the United States of America.* 2001;98(19):10869-10874.

34. Sorlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proceedings of the National Academy of Sciences of the United States of America.* 2003;100(14):8418-8423.

35. Perou CM, Sorlie T, Eisen MB, et al. Molecular portraits of human breast tumours. *Nature.* 2000;406(6797):747-752.

36. Pfefferle AD, Spike BT, Wahl GM, Perou CM. Luminal progenitor and fetal mammary stem cell expression features predict breast tumor response to neoadjuvant chemotherapy. *Breast Cancer Res Treat.* 2015;149(2):425-437.

37. Visvader JE. Keeping abreast of the mammary epithelial hierarchy and breast tumorigenesis. *Genes Dev.* 2009;23(22):2563-2577.

38. Spike BT, Engle DD, Lin JC, Cheung SK, La J, Wahl GM. A mammary stem cell population identified and characterized in late embryogenesis reveals similarities to human breast cancer. *Cell Stem Cell.* 2012;10(2):183-197.

39. Brough R, Frankum JR, Sims D, et al. Functional viability profiles of breast cancer. *Cancer Discov.* 2011;1(3):260-273.

40. Riggins RB, Schrecengost RS, Guerrero MS, Bouton AH. Pathways to tamoxifen resistance. *Cancer Lett.* 2007;256(1):1-24.

41. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet.* 2005;365(9472):1687-1717.

42. Forbes JF, Cuzick J, Buzdar A, Howell A, Tobias JS, Baum M. Effect of anastrozole and tamoxifen as adjuvant treatment for early-stage breast cancer: 100-month analysis of the ATAC trial. *Lancet Oncol.* 2008;9(1):45-53.

43. Thurlimann B, Keshaviah A, Coates AS, et al. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. *N Engl J Med.* 2005;353(26):2747-2757.

44. Early Breast Cancer Trialists' Collaborative G. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet.* 2005;365(9472):1687-1717.

45. Pagani O, Price KN, Gelber RD, et al. Patterns of recurrence of early breast cancer according to estrogen receptor status: a therapeutic target for a quarter of a century. *Breast Cancer Res Treat.* 2009;117(2):319-324.

46. Osborne CK, Bardou V, Hopp TA, et al. Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. *J Natl Cancer Inst.* 2003;95(5):353-361.

47. Zhang Y, Moerkens M, Ramaiahgari S, et al. Elevated insulin-like growth factor 1 receptor signaling induces antiestrogen resistance through the MAPK/ERK and PI3K/Akt signaling routes. *Breast Cancer Res.* 2011;13(3):R52.

48. Turner N, Pearson A, Sharpe R, et al. FGFR1 amplification drives endocrine therapy resistance and is a therapeutic target in breast cancer. *Cancer Res.* 2010;70(5):2085-2094.

49. Gilani RA, Kazi AA, Shah P, et al. The importance of HER2 signaling in the tumor-initiating cell population in aromatase inhibitor-resistant breast cancer. *Breast Cancer Res Treat.* 2012;135(3):681-692.

50. Burris HA, 3rd. Overcoming acquired resistance to anticancer therapy: focus on the PI3K/AKT/mTOR pathway. *Cancer Chemother Pharmacol.* 2013;71(4):829-842.

51. Planas-Silva MD, Bruggeman RD, Grenko RT, Smith JS. Overexpression of c-Myc and Bcl-2 during progression and distant metastasis of hormone-treated breast cancer. *Exp Mol Pathol.* 2007;82(1):85-90.

52. van Agthoven T, van Agthoven TL, Dekker A, van der Spek PJ, Vreede L, Dorssers LC. Identification of BCAR3 by a random search for genes involved in antiestrogen resistance of human breast cancer cells. *Embo J.* 1998;17(10):2799-2808.

53. Schroth W, Goetz MP, Hamann U, et al. Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. *JAMA.* 2009;302(13):1429-1436.

54. Liu L, Bai YX, Zhou JH, et al. A polymorphism at the 3'-UTR region of the aromatase gene is associated with the efficacy of the aromatase inhibitor, anastrozole, in metastatic breast carcinoma. *Int J Mol Sci.* 2013;14(9):18973-18988.

55. Michalides R, Griekspoor A, Balkenende A, et al. Tamoxifen resistance by a conformational arrest of the estrogen receptor alpha after PKA activation in breast cancer. *Cancer Cell.* 2004;5(6):597-605.

56. Berry DA, Cirrincione C, Henderson IC, et al. Estrogen-receptor status and outcomes of modern chemotherapy for patients with node-positive breast cancer. *JAMA.* 2006;295(14):1658-1667.

57. Petit T, Wilt M, Velten M, et al. Semi-quantitative evaluation of estrogen receptor expression is a strong predictive factor of pathological complete response after anthracycline-based neo-adjuvant chemotherapy in hormonal-sensitive breast cancer. *Breast Cancer Res Treat.* 2010;124(2):387-391.

58. Sui M, Huang Y, Park BH, Davidson NE, Fan W. Estrogen receptor alpha mediates breast cancer cell resistance to paclitaxel through inhibition of apoptotic cell death. *Cancer Res.* 2007;67(11):5337-5344.

59. Tabuchi Y, Matsuoka J, Gunduz M, et al. Resistance to paclitaxel therapy is related with Bcl-2 expression through an estrogen receptor mediated pathway in breast cancer. *Int J Oncol.* 2009;34(2):313-319.

60. Green S, Walter P, Kumar V, et al. Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. *Nature.* 1986;320(6058):134-139.

61. Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. *FEBS Lett.* 1996;392(1):49-53.

62. Evans RM. The steroid and thyroid hormone receptor superfamily. *Science.* 1988;240(4854):889-895.

63. Ribeiro RC, Kushner PJ, Baxter JD. The nuclear hormone receptor gene superfamily. *Annu Rev Med.* 1995;46:443-453.

64. Zhou Z, Qiao JX, Shetty A, et al. Regulation of estrogen receptor signaling in breast carcinogenesis and breast cancer therapy. *Cell Mol Life Sci.* 2014;71(8):1549.

65. Marino M, Ascenzi P, Acconcia F. S-palmitoylation modulates estrogen receptor alpha localization and functions. *Steroids.* 2006;71(4):298-303.

66. Galluzzo P, Ascenzi P, Bulzomi P, Marino M. The nutritional flavanone naringenin triggers antiestrogenic effects by regulating estrogen receptor alpha-palmitoylation. *Endocrinology.* 2008;149(5):2567-2575.

67. Razandi M, Pedram A, Park ST, Levin ER. Proximal events in signaling by plasma membrane estrogen receptors. *The Journal of biological chemistry.* 2003;278(4):2701-2712.

68. Sanchez AM, Flamini MI, Baldacci C, Goglia L, Genazzani AR, Simoncini T. Estrogen receptor-alpha promotes breast cancer cell motility and invasion via focal

adhesion kinase and N-WASP. *Molecular endocrinology.* 2010;24(11):2114-2125.

69. Adlanmerini M, Solinhac R, Abot A, et al. Mutation of the palmitoylation site of estrogen receptor alpha in vivo reveals tissue-specific roles for membrane versus nuclear actions. *Proceedings of the National Academy of Sciences of the United States of America.* 2014;111(2):E283-290.

70. Pedram A, Razandi M, Lewis M, Hammes S, Levin ER. Membrane-localized estrogen receptor alpha is required for normal organ development and function. *Dev Cell.* 2014;29(4):482-490.

71. Chen JQ, Cammarata PR, Baines CP, Yager JD. Regulation of mitochondrial respiratory chain biogenesis by estrogens/estrogen receptors and physiological, pathological and pharmacological implications. *Biochim Biophys Acta.* 2009;1793(10):1540-1570.

72. Klinge CM. Estrogenic control of mitochondrial function and biogenesis. *J Cell Biochem.* 2008;105(6):1342-1351.

73. Monje P, Boland R. Subcellular distribution of native estrogen receptor alpha and beta isoforms in rabbit uterus and ovary. *J Cell Biochem.* 2001;82(3):467-479.

74. Simpkins JW, Yang SH, Sarkar SN, Pearce V. Estrogen actions on mitochondria--physiological and pathological implications. *Mol Cell Endocrinol.* 2008;290(1-2):51-59.

75. Chen JQ, Yager JD, Russo J. Regulation of mitochondrial respiratory chain structure and function by estrogens/estrogen receptors and potential physiological/pathophysiological implications. *Biochim Biophys Acta.* 2005;1746(1):1-17.

76. Pedram A, Razandi M, Wallace DC, Levin ER. Functional estrogen receptors in the mitochondria of breast cancer cells. *Mol Biol Cell.* 2006;17(5):2125-2137.

77. Papa L, Germain D. Estrogen receptor mediates a distinct mitochondrial unfolded protein response. *J Cell Sci.* 2011;124(Pt 9):1396-1402.

78. Germain D. Estrogen carcinogenesis in breast cancer. *Endocrinol Metab Clin North Am.* 2011;40(3):473-484, vii.

79. Echeverria PC, Picard D. Molecular chaperones, essential partners of steroid hormone receptors for activity and mobility. *Biochim Biophys Acta.* 2010;1803(6):641-649.

80. Green S, Kumar V, Theulaz I, Wahli W, Chambon P. The N-terminal DNA-binding 'zinc finger' of the oestrogen and glucocorticoid receptors determines target gene specificity. *Embo J.* 1988;7(10):3037-3044.

81. Mader S, Kumar V, de Verneuil H, Chambon P. Three amino acids of the oestrogen receptor are essential to its ability to distinguish an oestrogen from a glucocorticoid-responsive element. *Nature.* 1989;338(6212):271-274.

82. Kumar V, Green S, Stack G, Berry M, Jin JR, Chambon P. Functional domains of the human estrogen receptor. *Cell.* 1987;51(6):941-951.

83. Schwabe JW, Chapman L, Rhodes D. The oestrogen receptor recognizes an imperfectly palindromic response element through an alternative side-chain conformation. *Structure.* 1995;3(2):201-213.

84. Hall JM, McDonnell DP, Korach KS. Allosteric regulation of estrogen receptor structure, function, and coactivator recruitment by different estrogen response elements. *Molecular endocrinology.* 2002;16(3):469-486.

85. Freedman LP, Luisi BF. On the mechanism of DNA binding by nuclear hormone receptors: a structural and functional perspective. *J Cell Biochem.* 1993;51(2):140-150.

86. Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell.* 2000;103(6):843-852.

87. Yasar P, Ayaz G, User SD, Gupur G, Muyan M. Molecular mechanism of estrogen-estrogen receptor signaling. *Reprod Med Biol.* 2017;16(1):4-20.

88. Cui J, Shen Y, Li R. Estrogen synthesis and signaling pathways during aging: from periphery to brain. *Trends Mol Med.* 2013;19(3):197-209.

89. Huang J, Li X, Hilf R, Bambara RA, Muyan M. Molecular basis of therapeutic strategies for breast cancer. *Curr Drug Targets Immune Endocr Metabol Disord.* 2005;5(4):379-396.

90. Hall JM, Couse JF, Korach KS. The multifaceted mechanisms of estradiol and estrogen receptor signaling. *The Journal of biological chemistry.* 2001;276(40):36869-36872.

91. Kushner PJ, Agard DA, Greene GL, et al. Estrogen receptor pathways to AP-1. *J Steroid Biochem Mol Biol.* 2000;74(5):311-317.

92. Safe S. Transcriptional activation of genes by 17 beta-estradiol through estrogen receptor-Sp1 interactions. *Vitam Horm.* 2001;62:231-252.

93. Bjornstrom L, Sjoberg M. Mutations in the estrogen receptor DNA-binding domain discriminate between the classical mechanism of action and cross-talk with Stat5b and activating protein 1 (AP-1). *The Journal of biological chemistry.* 2002;277(50):48479-48483.

94. Cheung E, Acevedo ML, Cole PA, Kraus WL. Altered pharmacology and distinct coactivator usage for estrogen receptor-dependent transcription through activating protein-1. *Proceedings of the National Academy of Sciences of the United States of America.* 2005;102(3):559-564.

95. Karin M, Liu Z, Zandi E. AP-1 function and regulation. *Curr Opin Cell Biol.* 1997;9(2):240-246.

96. Safe S, Abdelrahim M. Sp transcription factor family and its role in cancer. *Eur J Cancer.* 2005;41(16):2438-2448.

97. Nott SL, Huang Y, Li X, et al. Genomic responses from the estrogen-responsive element-dependent signaling pathway mediated by estrogen receptor alpha are required to elicit cellular alterations. *The Journal of biological chemistry.* 2009;284(22):15277-15288.

98. Ahlbory-Dieker DL, Stride BD, Leder G, et al. DNA binding by estrogen receptor-alpha is essential for the transcriptional response to estrogen in the liver and the uterus. *Molecular endocrinology.* 2009;23(10):1544-1555.

99. Walker VR, Korach KS. Estrogen receptor knockout mice as a model for endocrine research. *Ilar J.* 2004;45(4):455-461.

100. Wang LH, Yang XY, Zhang X, Mihalic K, Xiao W, Farrar WL. The cis decoy against the estrogen response element suppresses breast cancer cells via target disrupting c-fos not mitogen-activated protein kinase activity. *Cancer research.* 2003;63(9):2046-2051.

101. Gibson DA, Saunders PT. Estrogen dependent signaling in reproductive tissues - a role for estrogen receptors and estrogen related receptors. *Mol Cell Endocrinol.* 2012;348(2):361-372.

102. Muyan M, Gupur G, Yasar P, et al. Modulation of Estrogen Response Element-Driven Gene Expressions and Cellular Proliferation with Polar Directions by Designer Transcription Regulators. *PLoS One.* 2015;10(8):e0136423.

103. Kumar R, Thompson EB. Transactivation functions of the N-terminal domains of nuclear hormone receptors: protein folding and coactivator interactions. *Molecular endocrinology.* 2003;17(1):1-10.

104. Tora L, White J, Brou C, et al. The human estrogen receptor has two independent nonacidic transcriptional activation functions. *Cell.* 1989;59(3):477-487.

105. Metzger D, Ali S, Bornert JM, Chambon P. Characterization of the amino-terminal transcriptional activation function of the human estrogen receptor in animal and yeast cells. *The Journal of biological chemistry.* 1995;270(16):9535-9542.

106. Bocquel MT, Kumar V, Stricker C, Chambon P, Gronemeyer H. The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific. *Nucleic acids research.* 1989;17(7):2581-2595.

107. Tasset D, Tora L, Fromental C, Scheer E, Chambon P. Distinct classes of transcriptional activating domains function by different mechanisms. *Cell.* 1990;62(6):1177-1187.

108. Rajbhandari P, Finn G, Solodin NM, et al. Regulation of estrogen receptor alpha N-terminus conformation and function by peptidyl prolyl isomerase Pin1. *Mol Cell Biol.* 2012;32(2):445-457.

109. Yi P, Bhagat S, Hilf R, Bambara RA, Muyan M. Differences in the abilities of estrogen receptors to integrate activation functions are critical for subtype-specific transcriptional responses. *Molecular endocrinology.* 2002;16(8):1810-1827.

110. Cowley SM, Parker MG. A comparison of transcriptional activation by ER alpha and ER beta. *J Steroid Biochem Mol Biol.* 1999;69(1-6):165-175.

111. Delaunay F, Pettersson K, Tujague M, Gustafsson JA. Functional differences between the amino-terminal domains of estrogen receptors alpha and beta. *Mol Pharmacol.* 2000;58(3):584-590.

112. Huang J, Li X, Maguire CA, Hilf R, Bambara RA, Muyan M. Binding of estrogen receptor beta to estrogen response element in situ is independent of estradiol and impaired by its amino terminus. *Molecular endocrinology.* 2005;19(11):2696-2712.

113. Teyssier C, Belguise K, Galtier F, Chalbos D. Characterization of the physical interaction between estrogen receptor alpha and JUN proteins. *The Journal of biological chemistry.* 2001;276(39):36361-36369.

114. Berry NB, Fan M, Nephew KP. Estrogen receptor-alpha hinge-region lysines 302 and 303 regulate receptor degradation by the proteasome. *Molecular endocrinology.* 2008;22(7):1535-1551.

115. Tamrazi A, Carlson KE, Daniels JR, Hurth KM, Katzenellenbogen JA. Estrogen receptor dimerization: ligand binding regulates dimer affinity and dimer dissociation rate. *Molecular endocrinology.* 2002;16(12):2706-2719.

116. Vajdos FF, Hoth LR, Geoghegan KF, et al. The 2.0 A crystal structure of the ERalpha ligand-binding domain complexed with lasofoxifene. *Protein Sci.* 2007;16(5):897-905.

117. Brzozowski AM, Pike AC, Dauter Z, et al. Molecular basis of agonism and antagonism in the oestrogen receptor. *Nature.* 1997;389(6652):753-758.

118. Pike AC, Brzozowski AM, Hubbard RE. A structural biologist's view of the oestrogen receptor. *J Steroid Biochem Mol Biol.* 2000;74(5):261-268.

119. Shiau AK, Barstad D, Loria PM, et al. The structural basis of estrogen receptor/coactivator recognition and the antagonism of this interaction by tamoxifen. *Cell.* 1998;95(7):927-937.

120. Emmen JM, Korach KS. Estrogen receptor knockout mice: phenotypes in the female reproductive tract. *Gynecol Endocrinol.* 2003;17(2):169-176.

121. Hwang CJ, Yun HM, Park KR, et al. Memory Impairment in Estrogen Receptor alpha Knockout Mice Through Accumulation of Amyloid-beta Peptides. *Mol Neurobiol.* 2015;52(1):176-186.

122. Cheng CK, Chow BK, Leung PC. An activator protein-1-like motif mediates 17beta-estradiol repression of gonadotropin-releasing hormone receptor promoter via an estrogen receptor alpha-dependent mechanism in ovarian and breast cancer cells. *Mol Endocrinol.* 2003;17(12):2613-2629.

123. Jones LP, Tilli MT, Assefnia S, et al. Activation of estrogen signaling pathways collaborates with loss of Brca1 to promote development of ERalpha-negative and ERalpha-positive mammary preneoplasia and cancer. *Oncogene.* 2008;27(6):794-802.

124. Yang M, Wang J, Wang L, et al. Estrogen induces androgen-repressed SOX4 expression to promote progression of prostate cancer cells. *Prostate.* 2015;75(13):1363-1375.

125. Yager JD, Davidson NE. Estrogen carcinogenesis in breast cancer. *N Engl J Med.* 2006;354(3):270-282.

126. Yue W, Wang JP, Li Y, et al. Effects of estrogen on breast cancer development: Role of estrogen receptor independent mechanisms. *Int J Cancer.* 2010;127(8):1748-1757.

127. Zhu J, Zhao C, Kharman-Biz A, et al. The atypical ubiquitin ligase RNF31 stabilizes estrogen receptor alpha and modulates estrogen-stimulated breast cancer cell proliferation. *Oncogene.* 2014;33(34):4340-4351.

128. Zhuang T, Yu S, Zhang L, et al. SHARPIN stabilizes estrogen receptor alpha and promotes breast cancer cell proliferation. *Oncotarget.* 2017;8(44):77137-77151.

129. Tecalco-Cruz AC, Ramirez-Jarquin JO. Mechanisms that Increase Stability of Estrogen Receptor Alpha in Breast Cancer. *Clin Breast Cancer.* 2017;17(1):1-10.

130. Williams CC, Basu A, El-Gharbawy A, Carrier LM, Smith CL, Rowan BG. Identification of four novel phosphorylation sites in estrogen receptor alpha: impact on receptor-dependent gene expression and phosphorylation by protein kinase CK2. *BMC Biochem.* 2009;10:36.

131. Murphy LC, Seekallu SV, Watson PH. Clinical significance of estrogen receptor phosphorylation. *Endocr Relat Cancer.* 2011;18(1):R1-14.

132. He X, Zheng Z, Song T, et al. c-Abl regulates estrogen receptor alpha transcription activity through its stabilization by phosphorylation. *Oncogene.* 2010;29(15):2238-2251.

133. Giamas G, Filipovic A, Jacob J, et al. Kinome screening for regulators of the estrogen receptor identifies LMTK3 as a new therapeutic target in breast cancer. *Nat Med.* 2011;17(6):715-719.

134. Tecalco-Cruz AC, Ramirez-Jarquin JO. Mechanisms that Increase Stability of Estrogen Receptor Alpha in Breast Cancer. *Clin Breast Cancer.* 2017;17(1):1-10.

135. Grisouard J, Medunjanin S, Hermani A, Shukla A, Mayer D. Glycogen synthase kinase-3 protects estrogen receptor alpha from proteasomal degradation and is required for full transcriptional activity of the receptor. *Molecular endocrinology.* 2007;21(10):2427-2439.

136. Zhao G, Guo J, Li D, et al. MicroRNA-34a suppresses cell proliferation by targeting LMTK3 in human breast cancer mcf-7 cell line. *DNA Cell Biol.* 2013;32(12):699-707.

137. Wei X, Xu H, Kufe D. MUC1 oncoprotein stabilizes and activates estrogen receptor alpha. *Mol Cell.* 2006;21(2):295-305.

138. Rajbhandari P, Schalper KA, Solodin NM, et al. Pin1 modulates ERalpha levels in breast cancer through inhibition of phosphorylation-dependent ubiquitination and degradation. *Oncogene.* 2014;33(11):1438-1447.

139. Xue M, Zhang K, Mu K, et al. Regulation of estrogen signaling and breast cancer proliferation by an ubiquitin ligase TRIM56. *Oncogenesis.* 2019;8(5):30.

140. Caligiuri I, Toffoli G, Giordano A, Rizzolio F. pRb controls estrogen receptor alpha protein stability and activity. *Oncotarget.* 2013;4(6):875-883.

141. Lannigan DA. Estrogen receptor phosphorylation. *Steroids.* 2003;68(1):1-9.

142. Likhite VS, Stossi F, Kim K, Katzenellenbogen BS, Katzenellenbogen JA. Kinase-specific phosphorylation of the estrogen receptor changes receptor interactions with ligand, deoxyribonucleic acid, and coregulators associated with alterations in estrogen and tamoxifen activity. *Mol Endocrinol.* 2006;20(12):3120-3132.

143. de Leeuw R, Neefjes J, Michalides R. A role for estrogen receptor phosphorylation in the resistance to tamoxifen. *Int J Breast Cancer.* 2011;2011:232435.

144. Chen D, Washbrook E, Sarwar N, et al. Phosphorylation of human estrogen receptor alpha at serine 118 by two distinct signal transduction pathways revealed by phosphorylation-specific antisera. *Oncogene.* 2002;21(32):4921-4931.

145. Sarwar N, Kim JS, Jiang J, et al. Phosphorylation of ERalpha at serine 118 in primary breast cancer and in tamoxifen-resistant tumours is indicative of a complex role for ERalpha phosphorylation in breast cancer progression. *Endocr Relat Cancer.* 2006;13(3):851-861.

146. Yamashita H, Nishio M, Toyama T, et al. Low phosphorylation of estrogen receptor alpha (ERalpha) serine 118 and high phosphorylation of ERalpha serine 167 improve survival in ER-positive breast cancer. *Endocr Relat Cancer.* 2008;15(3):755-763.

147. Gee JM, Robertson JF, Ellis IO, Nicholson RI. Phosphorylation of ERK1/2 mitogen-activated protein kinase is associated with poor response to anti-hormonal therapy and decreased patient survival in clinical breast cancer. *Int J Cancer.* 2001;95(4):247-254.

148. Liu H, Qiu J, Li N, Chen T, Cao X. Human phosphatidylethanolamine-binding protein 4 promotes transactivation of estrogen receptor alpha (ERalpha) in human cancer cells by inhibiting proteasome-dependent ERalpha degradation via association with Src. *J Biol Chem.* 2010;285(29):21934-21942.

149. Cui Y, Zhang M, Pestell R, Curran EM, Welshons WV, Fuqua SA. Phosphorylation of estrogen receptor alpha blocks its acetylation and regulates estrogen sensitivity. *Cancer Res.* 2004;64(24):9199-9208.

150. La Rosa P, Pesiri V, Leclercq G, Marino M, Acconcia F. Palmitoylation regulates 17beta-estradiol-induced estrogen receptor-alpha degradation and transcriptional activity. *Mol Endocrinol.* 2012;26(5):762-774.

151. Cheng X, Cole RN, Zaia J, Hart GW. Alternative O-glycosylation/O-phosphorylation of the murine estrogen receptor beta. *Biochemistry.* 2000;39(38):11609-11620.

152. Cheng X, Hart GW. Glycosylation of the murine estrogen receptor-alpha. *J Steroid Biochem Mol Biol.* 2000;75(2-3):147-158.

153. Jiang MS, Hart GW. A subpopulation of estrogen receptors are modified by O-linked N-acetylglucosamine. *J Biol Chem.* 1997;272(4):2421-2428.

154. Deng B, Tarhan YE, Ueda K, et al. Critical Role of Estrogen Receptor Alpha O-Glycosylation by N-Acetylgalactosaminyltransferase 6 (GALNT6) in Its Nuclear Localization in Breast Cancer Cells. *Neoplasia.* 2018;20(10):1038-1044.

155. Mohammed H, D'Santos C, Serandour AA, et al. Endogenous purification reveals GREB1 as a key estro-

gen receptor regulatory factor. *Cell Rep.* 2013;3(2): 342-349.

156. Iyer LM, Zhang D, Burroughs AM, Aravind L. Computational identification of novel biochemical systems involved in oxidation, glycosylation and other complex modifications of bases in DNA. *Nucleic Acids Res.* 2013;41(16):7635-7655.

157. 157. Haines CN, Braunreiter KM, Mo XM, Burd CJ. GREB1 isoforms regulate proliferation independent of ERalpha co-regulator activities in breast cancer. *Endocr Relat Cancer.* 2018;25(7):735-746.

158. Toy W, Shen Y, Won H, et al. ESR1 ligand-binding domain mutations in hormone-resistant breast cancer. *Nat Genet.* 2013;45(12):1439-1445.

159. Merenbakh-Lamin K, Ben-Baruch N, Yeheskel A, et al. D538G mutation in estrogen receptor-alpha: A novel mechanism for acquired endocrine resistance in breast cancer. *Cancer research.* 2013;73(23):6856-6864.

160. Li S, Shen D, Shao J, et al. Endocrine-therapy-resistant ESR1 variants revealed by genomic characterization of breast-cancer-derived xenografts. *Cell reports.* 2013;4(6):1116-1130.

161. Jeselsohn R, Yelensky R, Buchwalter G, et al. Emergence of constitutively active estrogen receptor-alpha mutations in pretreated advanced estrogen receptor-positive breast cancer. *Clin Cancer Res.* 2014;20(7):1757-1767.

162. Giuliano M, Trivedi MV, Schiff R. Bidirectional Crosstalk between the Estrogen Receptor and Human Epidermal Growth Factor Receptor 2 Signaling Pathways in Breast Cancer: Molecular Basis and Clinical Implications. *Breast Care (Basel).* 2013;8(4):256-262.

163. Trevino LS, Weigel NL. Phosphorylation: a fundamental regulator of steroid receptor action. *Trends Endocrinol Metab.* 2013;24(10):515-524.

164. Zhou W, Slingerland JM. Links between oestrogen receptor activation and proteolysis: relevance to hormone-regulated cancer therapy. *Nat Rev Cancer.* 2014;14(1):26-38.

165. Yang X, Yan L, Davidson NE. DNA methylation in breast cancer. *Endocr Relat Cancer.* 2001;8(2):115-127.

166. Sabbah M, Courilleau D, Mester J, Redeuilh G. Estrogen induction of the cyclin D1 promoter: involvement of a cAMP response-like element. *Proc Natl Acad Sci U S A.* 1999;96(20):11217-11222.

167. Herber B, Truss M, Beato M, Muller R. Inducible regulatory elements in the human cyclin D1 promoter. *Oncogene.* 1994;9(4):1295-1304.

168. Conzen SD. Minireview: nuclear receptors and breast cancer. *Mol Endocrinol.* 2008;22(10):2215-2228.

169. Kim R, Kaneko M, Arihiro K, et al. Extranuclear expression of hormone receptors in primary breast cancer. *Ann Oncol.* 2006;17(8):1213-1220.

170. Chen H, Lin RJ, Schiltz RL, et al. Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell.* 1997;90(3):569-580.

171. Shao W, Keeton EK, McDonnell DP, Brown M. Coactivator AIB1 links estrogen receptor transcriptional activity and stability. *Proc Natl Acad Sci U S A.* 2004;101(32):11599-11604.

172. Rae JM, Johnson MD, Scheys JO, Cordero KE, Larios JM, Lippman ME. GREB 1 is a critical regulator of hormone dependent breast cancer growth. *Breast Cancer Res Treat.* 2005;92(2):141-149.

173. Rae JM, Johnson MD, Cordero KE, et al. GREB1 is a novel androgen-regulated gene required for prostate cancer growth. *Prostate.* 2006;66(8):886-894.

174. Laviolette LA, Hodgkinson KM, Minhas N, Perez-Iratxeta C, Vanderhyden BC. 17beta-estradiol upregulates GREB1 and accelerates ovarian tumor progression in vivo. *International journal of cancer.* 2014;135(5):1072-1084.

175. Wei X, Xu H, Kufe D. MUC1 oncoprotein stabilizes and activates estrogen receptor alpha. *Mol Cell.* 2006;21(2):295-305.

176. Dobrzycka KM, Townson SM, Jiang S, Oesterreich S. Estrogen receptor corepressors -- a role in human breast cancer? *Endocr Relat Cancer.* 2003;10(4):517-536.

177. Mazumdar A, Wang RA, Mishra SK, et al. Transcriptional repression of oestrogen receptor by metastasis-associated protein 1 corepressor. *Nat Cell Biol.* 2001;3(1):30-37.

178. Girault I, Lerebours F, Amarir S, et al. Expression analysis of estrogen receptor alpha coregulators in breast carcinoma: evidence that NCOR1 expression is predictive of the response to tamoxifen. *Clin Cancer Res.* 2003;9(4):1259-1266.