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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-

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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 complex 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 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.

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.

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 breastfeeding 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 downregulators 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]

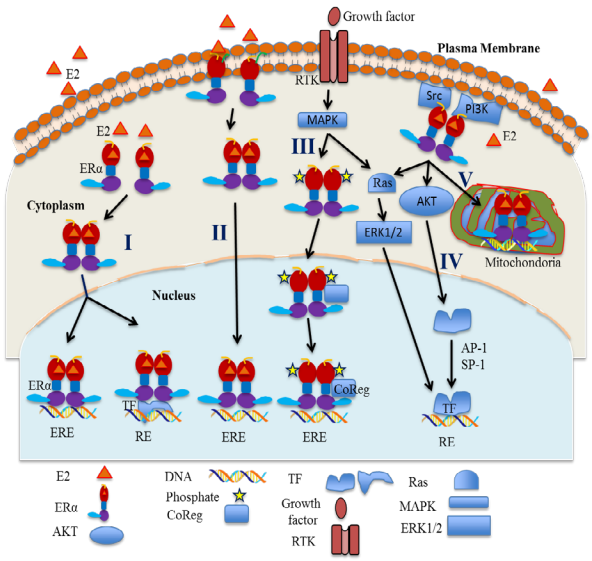

Fig. 1. Estrogen Receptor Signalling Pathways: I) In ER-dependent nuclear signalling, E2 can freely inter the cell, bind and dimerize ERα. Once ERα dimer localizes to nucleus it may bind directly to an ERE or binds to another response element (RE) through transcription factors (TF). II) ER-mediated membrane 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]

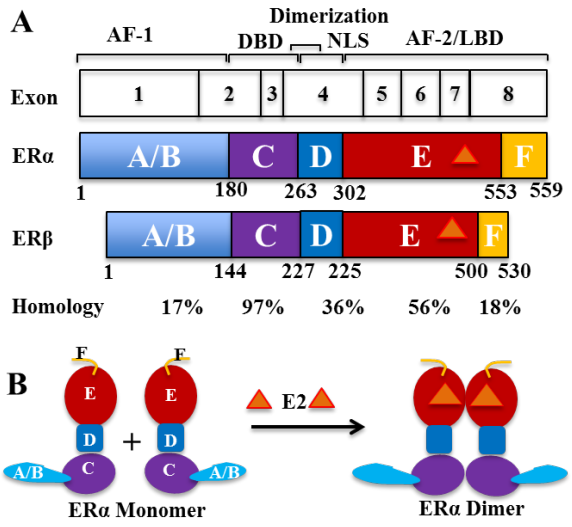

Fig. 2. Human ERα and ERβ Domain Structure. A) Schematic presentation of ERα and ERβ domains with corresponding percent homology. Both ERα and ERβ genes are expressed from 8 exons and have five interconnected segments of functional domains. The percent homology between the two ER for each segment is 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.

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