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Review

Structural and functional characterization of aromatase, estrogen receptor, and their genes in endocrine-responsive and –resistant breast cancer cells

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ABSTRACT

Aromatase and estrogen receptor α (ER) are two key proteins for the proliferation of endocrine-responsive and –resistant breast cancers. Aromatase is an enzyme involved in the conversion of androgen (such as testosterone) to estrogen (such as 17β-estradiol). It is also a very effective therapeutic target for the treatment of endocrine-responsive breast cancer. Comparing endocrine-responsive and -resistant breast cancer, aromatase protein levels do not change significantly. Aromatase activity; however, can be increased via PI3K/Akt/IGFR signaling pathways in endocrine resistant cells. The activity of aromatase has been reported to be modulated by phosphorylation. The ER is an important steroid nuclear receptor in the proliferation of both endocrine-responsive and -resistant cells. Although the mutation or amplification of ER can cause endocrine resistance, it is not commonly found. Some point mutations and translocation events have been characterized and shown to promote estrogen-independent growth. Phosphorylation by cross-talk with growth factor pathways is one of the main mechanisms for ligand-independent activation of ER. Taken together, both ER and aromatase are important in ER-dependent breast cancer and the development of endocrine resistance.

Contents

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1. Introduction

Breast cancers are mostly dependent on estrogens for proliferation and survival. Estrogens play an important role in hormone-receptor-positive breast cancer development, by binding to and activating the estrogen receptor (ER). Seventy-five percent of breast cancers are ER-positive and 65% are also progesterone (PgR) positive [1]. Receptor positive status is a stimulant for breast cancer development; thus, elimination of the production of estrogen from androgens and inhibition of the ER activation are important forms of hormonal therapy. Aromatase is an enzyme that converts androgens into estrogens, and aromatase inhibitors (AIs) can eliminate the production of estrogen. Furthermore, antiestrogens can bind to ER and antagonize its activity. Both of these strategies are successful in the clinic; however, some patients do not respond to the initial therapy, and a significant number of patients who respond will develop resistance to these therapies. A better understanding of the mechanisms of endocrine resistance will aid in the development of new therapeutic strategies to overcome the de novo and acquired resistance [2]. The structural and functional importance of ER and aromatase in endocrine-responsive and -resistant breast cancers will be discussed in more detail.

2. Estrogen receptor

2.1. ER α and β isoforms

The estrogen receptor exists in two isoforms: ERα and ERβ [3–5] with a 56% homology between the two isoforms [6]. Both ERs contain a DNA binding domain, a dimerization region, a ligand binding domain, and two transactivation domains-one located near the N-terminus (AF-1) and another near the C-terminus (AF-2). They share high sequence homology in the DNA binding region, but they are not redundant genes because they have different expression patterns and functions [7]. Recent data indicates that ERα is implicated in promoting growth and survival of breast epithelial cells, both cancerous and non-cancerous, while ERβ is involved in growth inhibitory properties [6,8,9]. The ERα is also able to form a heterodimer with ERβ, which has a similar binding affinity to DNA as the ERα homodimer, but a lower level of transcriptional activity [10]. Ligands such as estrogen (17β-estradiol/E2), tamoxifen and 4-hydroxytamoxifen (4-OHT), an activated derivative of tamoxifen, help to stabilize the ER binding to DNA; however, the antiestrogen ICI 182780 (referred to as ICI in this review and also known as fulvestrant) affects ERα and ERβ DNA binding differently. DNA binding capability of ERβ is less affected by ICI than that of ERα [11]. Another difference in the ERα and ERβ is in the ligand binding affinities, where estrogens bind to both isoforms with similar affinities [12]. The importance of ERα in breast cancer cell growth has been well studied and documented. On the other hand, the involvement of ERβ in estrogen signaling and breast cancer is not fully defined and remains controversial [13,14]; thus, will not be extensively discussed here. For simplicity, ERα will be referred to as ER.

2.2. Estrogen receptor structure and function

ER, a nuclear receptor, is mainly functional in the nucleus, where it activates transcription of ER-regulated genes, and its activity depends on binding of E2. ER is also found in the cytosol in an unliganded state, but enters the nucleus due to ligand-dependent and independent activation [6,15–17]. Within the cytosol, ER is bound to chaperone proteins such as HSP90 and HSP70. Chaperones are essential for stability of proto-oncogenes and hormone receptors such as ER and PR [18,19]. Upon E2 binding at the ligand binding domain (i.e., AF2) of ER, the receptor undergoes conformational changes. These changes include HSP dissociation from ER; ER dimerization; the receptor plus the bound hormone entering the nucleus; and the formation of a hydrophobic domain, exposing the two activating function (AF) sites to which co-activators (NCoAs) or co-repressors (NCoRs) bind [4–6,18].

ER function can be broadly classified as genomic or non-genomic. In the genomic pathway, ER forms a dimer upon binding of E2 (Fig. 1). The activated ER dimer then translocates into the nucleus and can bind the ERE in the promoter regions to initiate the "classical" transcriptional activation or repression. The ER can also interact with other transcription factors such as activator protein 1 (AP1) and specificity protein 1 (SP1) to bind DNA indirectly, and cause the activation or repression of target genes. This is also known as the "non-classical" or "ERE-independent" genomic action. A third genomic mechanism involves ligand-independent ER activation (at the AF1 domain) by phosphorylation via kinases in the growth factor receptor signaling pathways. With the aid of kinase signaling pathways, ER and its co-activators can be phosphorylated, independent of ligand, through the genomic or non-genomic mechanisms; thus, leading to endocrine resistance. These kinases include stress related kinases: p38 MAPK or JNK; p44/42 MAPK; PI3K/Akt; or p90rsk [20–22].

Testosterone (T) is converted into estrogen (E2) by the enzyme aromatase. Normal breast cells synthesize E2 which has autocrine and paracrine functions. Breast cancer cells express higher levels of aromatase; thus, their E2 concentration is higher than normal breast cell. Furthermore, ER-positive breast cells require E2 for growth and utilize certain genomic signaling pathways to transcribe ER-regulated genes. These pathways include: classical genomic (E2-ER complex binds to the ERE); ERE-independent genomic (E2-ER complex binds to transcription factor-TF-binding sites); and non-classical genomic (ER is phosphorylated in absence of E2 via kinase cascades).

2.3. ER phosphorylation

The C-terminus transactivation function 2 (AF2) of ER is activated by ligand binding of E2 [23] while the N-terminus transactivation domain (AF1) is activated by phosphorylation at several residues. Mostly post-translational modifications occur in the N-terminus, upon ligand binding, and upon ligand-independent growth factor signaling pathways [4,24,25]. Of the 14 serine residues in the N-terminus, several have been researched extensively due to their phosphorylation abilities. Serines 104, 106, 118, and 167 when mutated to alanine decrease ER transcription. Most notably, S167 is phosphorylated by PI3K/AKT [21] and S118 is phosphorylated by the Ras-MAPK signaling cascades [22], which are both important for the activation of ER and mediate ER binding to co-activators. MAPK activated S118 phosphorylation mediates SRC3 binding [26], and increases hypersensitivity to E2 [27]. S118