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Cross-talk between estradiol receptor and EGFR/IGF-IR signaling pathways in estrogen-responsive breast cancers: Focus on the role and impact of proteoglycans ☆

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ABSTRACT

In hormone-dependent breast cancer, estrogen receptors are the principal signaling molecules that regulate several cell functions either by the genomic pathway acting directly as transcription factors in the nucleus or by the non-genomic pathway interacting with other receptors and their adjacent pathways like EGFR/IGFR. It is well established in literature that EGFR and IGFR signaling pathways promote cell proliferation and differentiation. Moreover, recent data indicate the cross-talk between ERs and EGFR/IGFR signaling pathways causing a transformation of cell functions as well as deregulation on normal expression pattern of matrix molecules. Specifically, proteoglycans, a major category of extracellular matrix (ECM) and cell surface macromolecules, are modified during malignancy and cause alterations in cancer cell signaling, affecting eventually functional cell properties such as proliferation, adhesion and migration. The on-going strategies to block only one of the above signaling effectors result cancer cells to overcome such inactivation using alternative signaling pathways. In this article, we therefore review the underlying mechanisms in respect to the role of ERs and the involvement of cross-talk between ERs, IGFR and EGFR in breast cancer cell properties and expression of extracellular secreted and cell bound proteoglycans involved in cancer progression. Understanding such signaling pathways may help to establish new potential pharmacological targets in terms of using ECM molecules to design novel anticancer therapies.

1. Introduction

Breast cancer is a heterogeneous disease, with substantial genotypic and phenotypic diversity (Perou et al., 2000). Hormone receptors-bearing tumors are correlated with the mortality rate of the majority of breast cancer patients (Mook et al., 2011). Estrogen receptor (ER) and progesterone receptor (PgR) are indicated in hormone-dependent breast cancer. ER status is the most important discriminator of breast cancers and ER divides breast tumors into two major groups: ER-positive (luminal A and B) and ER-negative [normal-like, human epidermal growth factor receptor-2 (HER2) enriched, basal, and claudin-low] subtypes (Perou et al., 2000). ERs are ligand-regulated transcription factors that promote several cell functions, including cell proliferation and growth (Ascenzi et al., 2006). The family of ERs is distinguished in two classes: nuclear ERs and plasma membrane ERs or G protein-coupled estrogen receptors (GPERs). ERs are consisted from two different receptors, estrogen receptor-alpha (ERα) and estrogen receptor-beta (ERβ), that are encoded from ESR1 and ERS2 genes, respectively (McDonnell and Norris, 2002). The steroid sex hormone estradiol (E2) is the prime ligand of ERα and ERβ and in normal conditions it controls cell proliferation and differentiation.

ERα, PgR, and HER2 (also referred to as HER2/neu or erbB2) are the three mandatory prognostic and predictive factors in invasive breast cancer used in routine clinical practice today (Allred, 2010). Four main breast cancer subtypes drive treatment decisions: ER-positive and HER2-negative with a low or intermediate differentiation grade (luminal A); ER-positive and HER2-negative with a high differentiation

Abbreviations: ER, estrogen receptor; PgR, progesterone receptor; HER2/erbB2, human epidermal growth factor receptor-2; E2, estradiol; RTK, receptor tyrosine kinase; IGF-IR, insulin-like growth factor I receptor; EGFR, epidermal growth factor receptor; MAPK, mitogen activating protein kinase; HB-EGF, heparin-binding EGF; IRS-1, insulin receptor substrate-1; PI3K, phosphatidylinositol 3′-kinase; ECM, extracellular matrix; PGs, proteoglycans; GAGs, glycosaminoglycans; HS, heparan sulfate; SLRPs, small leucine-rich PGs; Met, hepatocyte growth factor receptor; HIF-1α, hypoxia inducible factor 1α; VEGFA, vascular endothelial growth factor A; SDC, syndecan; GPC, glypican; SDCBP, syndecan binding protein; MT1-MMP, membrane type 1-matrix metalloproteinase; CAFs, cancer associated fibroblasts.

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grade (luminal B), aggressive type of HER2-positive and triple-negative breast cancer (ER-, PgR- and HER2-negative) with aggressive behavior and poor prognosis, or with better prognosis very similar to hormone receptor positive breast cancers. ERα regulates growth and differentiation of normal breast epithelial cells and is assessed to predict response to hormonal therapies (Jensen and Jordan, 2003). ERα regulates the expression of PgR, whose presence indicates that the estrogen–ERα pathway is intact and functional. HER2 is a proto-oncogene encoding a receptor tyrosine kinase (RTK), but the relationship between HER2 status and clinical outcome is complex and varies with the setting. Notably, there is a weak but significant association between poor outcome and positive HER2, i.e., amplified and/or overexpressed (Allred, 2010).

Under normal conditions, the levels of the two ERs are maintaining low (Helguero et al., 2005). In pathological situations, and especially in breast cancer, the ratio between ERα and ERβ is increased vertically, due to lower expression of ERβ. The majority of data in the literature is focused on the role of ERα in comparison to ERβ in cancer. The precise biologic role of ERβ remains unclear, in part due to the presence of several isoforms. In same cases, it is documented that ERα and ERβ exhibit an antagonistic action, regulating differentially the cell behavior in breast cancer initiation and progression. For instance, in the presence of ERα estrogens trigger breast cancer cells proliferation, whereas the presence of ERβ down-regulates this effect (Lazennec et al., 2001; Marotti et al., 2010).

Two thirds of breast cancers are ERα positive. ERα plays an important role in the development, progression and treatment of breast cancer and is of special interest because its protein level is elevated in premalignant and malignant breast lesions, but not in normal tissue (Allred and Mohsin, 2000). Immunohistochemical studies have shown that ERα expression only occurs in 6–10% of normal mammary epithelial cells, whereas 60% of primary breast cancers are ERα-positive (Dickson and Lippman, 1988; Jacquemier et al., 1990). As a result, ERα is a valuable predictive and prognostic factor in the clinical management of breast cancer (Fisher et al., 1988). However, the majority of ERα-positive tumors, even if initially respond to treatment with anti-estrogenic reagents, such as tamoxifen, will eventually develop resistance to this treatment, generally without any alteration in their ER profile (Hanstein et al., 2004) and likely at last lead to endometrial carcinoma (O'Regan et al., 1998).

2. The interplay between ERs and EGFR/IGF-IR as potential regulator of breast cancer cell signaling and properties

When activated by E2, ERα plays an important role in the stimulation of cancer cell proliferation and prevention of apoptosis (Ali and Coombes, 2000). The biological actions of E2 are mediated both by genomic transcriptional effects in the nucleus and by non-genomic actions via ERα acting outside of the nuclear compartment. In the canonical genomic ER-mediated transcription mechanism, binding of E2 to ERα results in its dissociation from heat-shock protein 90 (Hsp90) and its conformation is altered. Active ER dimerizes and then the dimer binds directly or indirectly to genes containing estrogen response elements (ERE). It may also recruit various co-activators or act as co-activator of transcription factors, regulating transcription and subsequent protein biosynthesis (Nilsson et al., 2001; Heldring et al., 2007). The indirect activation of ERE is conducted by AP-1 and Sp-1 transcription factors (Heldring et al., 2007). The ER-mediated transactivation actions are mainly regulated by the phosphorylation of ER and introduce a complementary role to the action of E2-ER complex. Depending on the cell type and context, the non-genomic effects of E2 can lead to the rapid activation of many signaling molecules, such as insulin-like growth factor I receptor (IGF-IR) and epidermal growth factor receptor (EGFR), $p21^{ras}$ and Raf-1, mitogen activating protein kinase (MAPK) and Akt, protein kinase C, release of nitric oxide and stimulation of prolactin secretion, and alteration of calcium and Maxi-K channels (Yee and Lee, 2000; Cheskis, 2004). Both genomic and non-genomic actions of E2 play pivotal roles in E2-induced cancer cell proliferation and survival (Alexaki et al., 2004).

Blockade of E2 synthesis with aromatase inhibitors or antagonism of its action with anti-estrogens represents first-line treatments for patients with ER-positive breast cancer. However, primary or secondary resistance to hormonal therapy commonly occurs and may reflect enhanced activation of the growth factor receptor functions of IGF-IR and EGFR/HER2 (Nicholson et al., 2001; Baserga et al., 2003). Accumulating evidence suggests that ERα is involved in the development of hormone resistance, in which extranuclear actions of this receptor are operative (Santen et al., 2004). Some studies suggest a mechanistic link between growth factor pathways and extranuclear ERα in breast cancer cells whereby ERα binds to the IGF-IR and activates its downstream signaling pathways (Song et al., 2004; Knowlden et al., 2005). IGF-IR is important in cellular biological processes, including cell differentiation and proliferation, the establishment and maintenance of transformation, and protection against apoptosis (Fig. 1D) (Baserga et al., 2003). It is a hetero-tetrameric transmembrane glycoprotein comprising two α- and two β-subunits. The β-subunits possess intrinsic tyrosine kinase activity upon ligand binding to the α-subunits. The EGFR is a type I RTK that mediates many biological processes, including cell migration, proliferation, and protection from apoptosis in response to ligands, such as EGF, transforming growth factor α (TGFα), and/or heparin-binding EGF (HB-EGF) (Hart et al., 2005). Interestingly, both IGF-IR and EGFR initiate some common downstream signaling pathways, such as activation of MAPK and Akt cascades (Adams et al., 2004). Ligand binding on IGF-IR or EGFR initiates their autophosphorylation at tyrosine residues and their activation. A variety of docking proteins – for example the adapter protein Shc, insulin receptor substrate-1 (IRS-1), and the p85α-subunit of phosphatidylinositol 3′-kinase (PI3K) that contain Src homology-2- and phosphotyrosine-binding domains – bind to the phosphorylated tyrosine residues on the receptors, leading to activation of the downstream signaling cascades of MAPK and Akt.

E2 is known to rapidly activate many signaling molecules, including IGF-IR, EGFR, and MAPK in breast cancer cells (Kahlert et al., 2000; Pietras, 2003; Song et al., 2004). Furthermore, the cross-talk between growth factors' receptors, intracellular proteins and steroids in both the cytoplasm and nucleus regulates cancer cell growth (Fox et al., 2009). Mounting evidence suggests that the cross-talk between non-genomic ERα action and growth factor receptor pathways is one mechanism in the development of endocrine-resistance (Nicholson and Johnston, 2005). Treatment of breast cancer cells with estrogens triggers IGF-I signaling through up-regulation of several components of this pathway such as IGF-IR, IRS-1 and IGF-I (Mauro et al., 2001). These upregulated proteins act locally in autocrine loops to mediate the biologic effects of estrogen. A close relationship between ERα and IGF-IR has been confirmed by the fact that uterine cells do not respond to estrogen upon blocking of the IGF-IR pathway and that IGF-I also loses its effect on gene transactivation and cell proliferation in ERα knockout cells (Klotz et al., 2002). A direct interaction between the two pathways also occurs at the receptor level whereby estrogen can induce IGF-IR phosphorylation in uterine epithelial cells, ERα-transfected COS-7 cells, and MCF-7 breast cancer cells (Richards et al., 1996; Kahlert et al., 2000). These data indicate that the IGF-IR-mediated signaling pathway is important in estrogen action. Interestingly, the expression of EGFR correlates inversely with the expression of ERα. However, a variety of evidence shows that both receptors are functional in the regulation of breast cancer growth regardless of which one is dominantly expressed (Klijn et al., 1992; van Agthoven et al., 1994). For example, EGFR is functionally involved in estrogen-induced MAPK activation in MCF-7 cells, a cell line with high expression of IGF-IR and ERα but low EGFR. ERα seems to interact with EGFR after E2 activation and through MAPK pathway induce the levels of ERα and IGF-IR (Fig. 1B). Notably, both EGF and E2 also cross-talk at other levels; for example, administration of the anti-estrogen ICI 182780 reduces the response to EGF in the mammary gland (Ankrapp et al., 1998). Studies in ERα-knockout mice

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