Steroids 75 (2010) 524–527

Contents lists available at ScienceDirect

Steroids

journal homepage: www.elsevier.com/locate/steroids

Cell proliferation regulated by estradiol receptor: Therapeutic implications

Gabriella Castoria, Antimo Migliaccio, Pia Giovannelli, Ferdinando Auricchio*

Dipartimento di Patologia Generale, II Università di Napoli, Via L. De Crecchio 7, 80138 Naples, Italy

ARTICLE INFO

Article history:
Available online 30 October 2009

Keywords:
Estradiol receptor
Non-genomic action
Nuclear export
Specific interfering peptides
Breast cancer therapy

ABSTRACT

Estrogen receptor (ER) is a ligand-regulated transcription factor that controls human breast cancer cell proliferation. About 60–70% of human breast cancers express ER. In spite of major progress in the therapy of human breast cancer, many patients become resistant to pharmacologic treatments and develop metastatic breast tumors. Several mechanisms have been proposed to explain tumor progression and resistance to the therapies. However, the causes of hormone-dependent breast tumor progression as well as therapy resistance are still debated. An increasing body of evidence from our and other laboratories shows that in breast cancer cells, in addition to its classical transcriptional action, ER stimulates proliferative and anti-apoptotic signaling pathways in response to either ligand binding or growth factors. This discovery has led to the synthesis of new compounds specifically interfering in the rapid responses mediated by ER. It also suggests that the modalities currently in use for breast cancer treatment need to be reconsidered.

1. Introduction

Breast cancer is very common in developed countries and the status of estradiol receptor (ER) is a prognostic marker in this tumor. Implication of ER in breast cancer progression is corroborated by the finding that about 60–70% of human breast cancers are ERalpha-positive. Most patients with ERalpha-positive breast cancer are treated with estradiol antagonists or aromatase inhibitors. Although these therapies improve the outcome of breast cancer, many patients become resistant to the treatment and develop metastatic breast tumors. Several mechanisms have been proposed to explain the causes of resistance to endocrine therapy [1]. These include expression of ER variants and ligand-independent activation of ER, as well as over-expression and activation of EGF-R and tyrosine kinases, including Src [2].

Estradiol controls proliferation and survival of breast cancer cells. This activity has until now been attributed to regulation of gene transcription [3]. In addition to well-known nuclear functions, ER (alpha or beta) participates in extra-nuclear and membrane-mediated signaling events. This non-genomic action has been linked to rapid responses elicited by estradiol and involves activation of Src, mitogen-activated protein kinase (MAPK), phosphatidylinositol-3-kinase (PI3-K), protein kinase C (PKC) and etherotrimeric G-proteins in cytoplasm or membrane of target cells [4]. In addition, cytoplasmic cross talk between EGF and extranuclear ER has been investigated in breast cancer cells [5]. Thus, important biological responses such as DNA synthesis, cell growth and processes related to ER cytoplasm re-localization occur upon activation of these signaling effectors [6–11].

In this review, we will highlight the role of the signaling network regulated by ER in breast cancer cells. Our findings are integrated with further data we obtained using peptides specifically interfering in this network.

2. Genomic and non-genomic models of estradiol action

In mammals, estradiol influences cell proliferation, inflammatory response, cardiovascular health, immunity, bone integrity, cognition and behaviour. Regulation of these effects may be mediated by a complex interface between signaling cascade activation and control of gene expression. ER (alpha or beta) in the cell nucleus regulates gene expression, whereas classical ER localized in the extra-nuclear compartment of target cells activates signal transduction pathways [4]. Genomic effects of estradiol usually occur via ligand-dependent binding of receptors to target gene promoters [3]. However, the resulting fluctuation in mRNAs and the proteins they encode takes place within hours or even days following hormonal exposure. In contrast, estradiol activation of signal transducing pathways occurs within seconds or minutes and is independent of RNA or protein synthesis. To date, all the members of the sex steroid hormone family exhibit non-genomic actions in reproductive as well as non-reproductive cells. These actions range

Abbreviations: CRM1, chromosome region maintenance 1; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ER, estradiol receptor; FKHR, forkhead in rhabdomyosarcoma; MAPK, mitogen-activated protein kinase; NES, nuclear export signal; PI3-K, phosphatidylinositol-3-kinase; PKC, protein kinase C.

* Corresponding author. Tel.: +39 0815665676; fax: +39 081291327.
E-mail address: ferdinando.auricchio@unina2.it (F. Auricchio).

0039-128X/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.steroids.2009.10.007

from activation of Src, MAPKs, adenylyl cyclase and PI3-K to rises in intracellular calcium concentrations [12].

We previously focused on the analysis of interplay between estradiol-induced signaling activation and cell cycle progression in target cells. We identified and characterized ER/Src interaction that intersects PI3-K signaling in breast cancer cells [7,9,13–15]. This network controls G1/S progression in cultured MCF-7 cells [6–8], cell growth in mouse models of tumorigenesis [11] and even ER cytoplasm re-localization in MCF-7 cells [9,10]. This model of estradiol action points to the proliferative role of rapid hormonal action and the associated extra-nuclear localization of ER, thus linking signaling pathway activation by estradiol with sub-cellular ER localization.

3. The role of Src and PI3-K in non-genomic action of estradiol

Some years ago we observed that estradiol rapidly (within seconds) and transiently triggers Src activation as well as protein tyrosine phosphorylation in breast cancer-derived MCF-7 cells [16]. Evidence collected in our laboratory also pointed to the role of classical ER in mediating this response [13,16]. We subsequently showed that ERalpha directly interacts through its tyrosine 537 with the SH2 domain of Src. Activation of the Src-dependent pathway follows this association, with dramatic effects on nuclear events leading to cell proliferation [6,13,15]. A similar mechanism also takes place upon EGF stimulation of breast cancer MCF-7 cells [5]. EGF, like estradiol, induces ER/Src association, thereby activating the Src-dependent pathway. This activation triggers both DNA synthesis and motility of breast cancer MCF-7 cells [5]. Thus, ER/Src association represents a novel and promising target in therapy of breast cancer, since these tumors respond to both steroid hormones and growth factors.

Estradiol activation of Src occurs simultaneously with activation of PI3-K in MCF-7 cells, where it triggers the assembly of a multi-molecular complex made up of ER, Src, the regulatory subunit of PI3-K, p85 alpha, and other signaling effectors [7,8]. Estradiol-activated PI3-K triggers AKT and atypical PKC zeta activation. Once activated, AKT up-regulates cyclin D1 [7], while PKC zeta fosters p27 nuclear release by recruiting Ras to the ER/Src/PI3-K complex and inducing Erk-2 nuclear translocation [8]. This interplay between signaling effectors and cell cycle regulators controls cell cycle progression in MCF-7 cells.

Altogether, our findings suggested that interference in the ER/Src interaction inhibits estradiol-activated cell cycle progression in cultured cells and most likely impairs cell growth in vivo.

Data on estradiol activation of extra-nuclear signaling pathways point to the extra-nuclear localization of ER. In addition, it has been reported that ERalpha (or beta) exists as a monomer to caveolae rafts at cell surface, and that ligand addition induces receptor dimerization and recruitment of signaling effectors in different cell types [17]. Altogether, these findings show that ER extra-nuclear localization can orchestrate receptor-mediated signaling activation and biological responses in target cells.

It has been recently reported that estradiol activation of PI3-K/AKT pathway controls the nuclear export of ERalpha in MCF-7 cells. The nuclear export sequence has been mapped within the COOH-terminus of ER and it has been shown that an ERalpha mutant (NES-ERalpha mutant), unable to interact with CRM1, does not exit nuclei and impairs S-phase entry in MCF-7 cells [9]. Thus, estradiol activation of this signaling pathway has been linked with ER nuclear export and cell cycle progression in breast cancer cells.

FKHR is a member of the FOXO transcriptional factor family which targets different factors, including cyclin D1 [18]. Since FKHR nuclear export depends on its phosphorylation by AKT [19] and estradiol activation of the PI3-K/AKT pathway positively controls S-phase entry in MCF-7 cells [7], we speculated that FKHR might play a role in this network. Thus, we observed that the estradiol-activated PI3-K/AKT pathway leads to phosphorylation of FKHR at Ser 256 in MCF-7 cells. In addition, over-expression of the FKHR triple mutant (FKHR-AAA), which cannot be phosphorylated by AKT, inhibits estradiol-induced S-phase entry in MCF-7 cells [9]. This FKHR mutant is permanently localized in nuclei, thereby inducing G1 arrest in cells [20]. We observed that over-expression of this mutant sequesters ERalpha in the nuclear compartment of estradiol-treated MCF-7 cells and that, in turn, over-expression of NES-ERalpha mutant retains wild type FKHR in the nuclei. Thus, estradiol simultaneously regulates ER and FKHR nuclear export. From this and other experimental approaches, we concluded that FKHR phosphorylation induced by estradiol in MCF-7 breast cancer cells is required for its association with receptor as well as the export of the FKHR/ER complex with the consequent release of FKHR-mediated cell cycle inhibition. Thus, in ER-positive breast cancer cells, FKHR moves from nucleus to cytoplasm via a receptor-dependent mechanism [9].

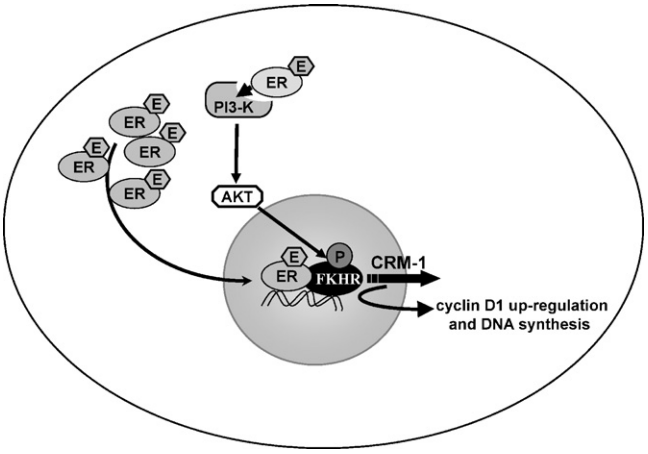

Fig. 1. Model of ERalpha nuclear export. Estradiol stimulates the PI3-K/AKT pathway, which triggers FKHR phosphorylation and the associated export of FKHR/ER. This export results in cyclin D1 up-regulation and DNA synthesis stimulation.

FKHR nuclear export is regulated by PI3-K/AKT pathway, which through FKHR export induces cell cycle progression up-regulating cyclin D1 and down-regulating p27. In addition, FOXO factors down-regulate cyclin D1 and up-regulate p27 expression through a transcriptional mechanism [21–23]. In both cases, cell cycle arrest follows. Recent findings from our laboratory suggest that estradiol-induced FKHR phosphorylation controls cyclin D1 expression in MCF-7 cells (unpublished data). This, together with previous results on induction of cyclin D1 transcription by estradiol-activated PI3-K/AKT pathway in MCF-7 cells [7], led us to propose the model presented in Fig. 1. According to this model, estradiol-stimulated PI3-K/AKT pathway induces FKHR phosphorylation and positively controls the nuclear export of FKHR/ERalpha complex. In this way, the inhibitory action of FKHR on cyclin D1 transcription is removed and breast cancer cells enter S-phase. These findings also imply that interference in ER nuclear export blocks cell cycle progression by inhibiting cytoplasmic release of FKHR in breast cancer cells.

4. An ER-derived peptide specifically interferes in ER/Src association

The observation that ERalpha phosphotyrosine 537 is required for the association of estradiol receptor alpha with the Src-SH2 domain [15] led us to design and synthesize a small peptide derived from ERalpha sequence surrounding Tyr537 [11]. This six-amino