Steroids 81 (2014) 64–69

Contents lists available at ScienceDirect

Steroids

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

Recent insights into non-nuclear actions of estrogen receptor alpha

Subhashis Banerjee, Ken L. Chambliss, Chieko Mineo, Philip W. Shaul *

Division of Pulmonary and Vascular Biology, Department of Pediatrics, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd., Dallas, TX 75390-9063, USA

ARTICLE INFO

Article history:
Available online 16 November 2013

Keywords:
Bone mineralization
Caveolae
Endothelium
Estrogen receptor
Nitric oxide synthase
Vascular smooth muscle

ABSTRACT

Estrogen receptors (ER) classically function as transcription factors regulating gene expression. More recently, evidence has continued to accumulate that ER additionally serve numerous important functions remote from the nucleus in a variety of cell types, particularly in non-reproductive tissues. The identification of post-translational modifications of ERα and protein–protein interactions with the receptor that are critical to its non-nuclear functions has afforded opportunities to gain greater insights into these novel non-genomic roles of the receptor. The development of a stable ligand that selectively activates non-nuclear ER has also been invaluable. In this review focused on ERα, recent new understanding of the processes underlying non-nuclear ER action and their in vivo consequences will be discussed. Further research into the non-nuclear capacities by which ER modulate cellular behavior is essential to ultimately harnessing these processes for therapeutic gain in numerous disease contexts.

1. Introduction

Via direct or indirect binding to regulatory elements on target genes, estrogen receptors alpha and beta (ERα, ERβ) classically function as steroid hormone nuclear receptors regulating gene transcription [1,2]. Over the past 15–20 years it has also become apparent that ER serve functions outside the cell nucleus that entail unique post-translational modifications and protein–protein interactions of the receptor with adaptor molecules, G proteins and kinases. Upon ligand binding to non-nuclear ER, a variety of signaling events are prompted that can themselves modify cellular behavior, or that influence cellular responses to nuclear ER activation through complex non-nuclear to nuclear cross-talk events [3–5]. In this review we will focus on recent insights gained regarding non-nuclear ER actions and their physiologic and pathologic consequences. Using endothelial cells as an example of a model system, a summary of non-nuclear ER signaling mechanisms will first be provided. Related works in non-endothelial cells that have yielded complementary insights will also be discussed. This will be followed by an overview of adaptor proteins that have been implicated in non-genomic ER function. These topics set the stage for a discussion of genetic approaches that have more recently been taken to better understand the cell biology of non-nuclear ER actions. This will be followed by a summarization of recent attempts to query the biology of non-nuclear ER in vivo in a finite number of tissue contexts, and also a review of the recent discovery of a novel partnership of another nuclear receptor with ER in plasma membrane caveolae/lipid rafts. Finally, we will point out the potential future directions for research in this field by highlighting the questions that remain to be answered and the current challenges.

2. Non-nuclear ERα signaling mechanisms

The signaling events initiated by the non-nuclear subpopulation of ER have been elucidated in a variety of cell types. These include breast cancer cells, oocytes, osteoblasts, osteoclasts, vascular smooth muscle cells, endothelial cells and other specific cell types [4–7]. Because of the immediate consequences on a variety of processes in the vascular wall that greatly impact vascular health and disease, the mechanisms underlying the coupling of non-nuclear ERα to endothelial NO synthase (eNOS) in particular have received considerable attention [8]. These efforts followed the initial discovery by two laboratories that estradiol (E2) activates eNOS within minutes of hormone application to cultured endothelial cells [9,10]. To illustrate the approaches that have been used to interrogate non-nuclear ER mechanisms of action and the types of signaling events that are often operative, studies performed in the context of E2 activation of eNOS in endothelial cells will be summarized. Where applicable, related studies in other cell types will be mentioned to provide complementary information unavailable

Abbreviations: 27HC, 27-hydroxycholesterol; E2, estradiol; E2-BSA, bovine serum albumin-conjugated E2; EDC, estrogen dendrimer conjugate; eNOS, endothelial NO synthase; ER, estrogen receptor; ERE, estrogen response element; HPIP, hematopoietic PBX-interacting protein; LXR, liver X receptor; MNAR, modulator of nongenomic activity of ER; PAMAM, poly(amido)amine; PP2Ac, protein phosphatase 2Ac; PTX, pertussis toxin; SERM, selective estrogen receptor modulator; VSM, vascular smooth muscle.

* Corresponding author. Tel.: +1 214 648 2015; fax: +1 214 648 2096.
E-mail address: Philip.shaul@utsouthwestern.edu (P.W. Shaul).

0039-128X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.steroids.2013.11.002

from work performed so far in endothelial cells. It is important to note that although the initial signaling events prompted by non-nuclear ER activation in different cell types are often similar, it should not be assumed that the processes initiated in one cell type occur in another cell type. In addition, although initial signaling events may be shared between different cell types, the ultimate impact of the signaling on cellular behavior cannot be extrapolated from one cell type to another. An example is that although the selective activation of non-nuclear ERα in MCF-7 breast cancer cells and endothelial cells causes Erk1/2 activation in both cell types, selective non-nuclear ERα stimulation invokes cell proliferation in endothelial cells but not in MCF-7 cells [11,12].

In early studies of the processes underlying ER coupling to eNOS, roles for tyrosine kinases and MAP kinases were initially implicated by the findings that genistein and the MAP kinase kinase inhibitor PD98059 prevent E2 activation of eNOS [13]. Further work using either assessments of changes in protein phosphorylation or pharmacologic approaches in studies of E2 treatment of isolated arteries or cultured primary endothelial cells revealed participation of PI3 kinase, Akt kinase and Erk1/2, and also increases in the phosphorylation of eNOS Ser1177 [14–17], which is frequently involved in the activation of the enzyme by extracellular stimuli whose actions on the enzyme are mediated by plasma membrane-associated receptors [18]. However, in a unique manner ERα interacts directly with the p85α regulatory subunit of PI3 kinase in a ligand-dependent fashion [17]. E2 activation of PI3 kinase and its downstream target Akt require c-Src kinase, whose SH2 domain interacts with phosphorylated ERa Tyr537. Further studies have revealed that this interaction between c-Src and the receptor likely also promotes the plasma membrane recruitment of ERα [19–21]. Along with recognition that ERα and eNOS are both associated with endothelial cell plasma membranes, it has been further demonstrated that they are co-residents of plasma membrane caveolae/lipid rafts, which are domains enriched in cholesterol and sphingomyelin that serve to compartmentalize signaling molecules on the cell surface [22]. In studies performed primarily in MCF-7 breast cancer cells, it has been shown that the methylation of ERα Arg260, which resides within the DNA binding domain of the receptor, by the arginine methyltransferase PRMT1 promotes the interactions of the receptor with both the PI3 kinase p85 subunit and c-Src. Additional work revealed that this mechanism participates in non-nuclear signaling downstream of the receptor [23]. Thus, kinases, many of which form a complex with ERα, and posttranslational modifications including methylation are operative in the signaling invoked by the receptor outside the nucleus.

In addition to the novel direct interactions demonstrated between ERα and c-Src and PI3 kinase, E2-ER modulation of eNOS enzymatic activity involves unique G protein coupling of the receptor. The potential participation of G proteins was initially revealed in studies showing that E2 activation of eNOS is fully prevented by pertussis toxin (PTX), that ERα and Gαi can be coimmunoprecipitated from the plasma membranes of endothelial cells treated with E2, and that the coimmunoprecipitation is prevented by PTX [24]. Since these findings suggested possible direct protein–protein interaction between the receptor and Gαi, pull-down experiments were performed and they demonstrated that ERα binds directly not only to Gαi, but also to Gβγ. Deletion mutagenesis experiments and strategies employing blocking peptide then showed that the interaction between ERα and Gαi and between ERα and Gβγ occur via amino acids 251–260 and 271–595 of human ERα, respectively. Studies employing complexes comprised of purified recombinant proteins then showed that E2 causes the release of both Gαi and Gβγ from their interaction with ERα, without stimulating guanine nucleotide exchange. To test the functional significance of the G protein interactions with the receptor and the G protein release upon ligand binding, further work was done in COS-7 cells. The disruption of ERα–Gαi interaction by deletion mutagenesis of the receptor or by expression of a blocking peptide comprised of the Gαi binding domain (amino acids 251–260), as well as transfection with cDNA encoding the β-adrenergic receptor kinase C terminus to sequester Gβγ, blunted the activation of both Src kinase and Erk1/2 by E2. As such, liberated Gβγ mediates the activation of Src and downstream signaling events by the ERα–G protein complex. In endothelial cells the disruption of the ERα–Gαi interaction leads to the prevention of eNOS activation, it attenuates the ability of E2 to blunt monocyte adhesion to endothelial cells, and it prevents the stimulation of endothelial cell migration by E2 [25]. Thus, direct ERα–G protein interaction is required for functional coupling of ERα to the kinase cascades and resulting downstream cellular responses to E2 that occur in endothelial cells.

Along with activating kinase signaling cascades in endothelial cells, there is evidence that non-nuclear ER stimulation modulates intracellular calcium homeostasis. In studies of human endothelial cells, E2 caused a rapid increase in intracellular calcium at physiologically-relevant hormone concentrations (10^{-9} M), this involved intracellular calcium store release, and simultaneous NO release was apparent. Importantly, these observations were made not only in cultured human endothelial cells but also in human internal thoracic artery endothelium in situ. The ER antagonist tamoxifen antagonized these responses, and E2 and bovine serum albumin-conjugated E2 (E2-BSA), which has been used to evaluate cell surface receptor function, yielded similar findings [26]. It should be noted that caution is warranted in the interpretation of findings with E2-BSA because certain E2-BSA preparations are of very high molecular weight, suggesting extreme protein crosslinking, free immunoassayable E2 can often be detected in freshly-prepared solutions of the reagent, and the E2 in E2-BSA is linked to the albumin via groups on E2 that are important for its binding to ER [27,28]. E2 and E2-BSA also caused comparable calcium transients in cultured rat endothelial cells, and these responses were blunted by the ER antagonist ICI 182,780 [29]. In contrast, in other work a rapid rise in intracellular calcium was not demonstrable in endothelial cells in response to E2 [10,30], despite the fact that the non-nuclear activation of eNOS by E2 is calcium-dependent [9,31]. Since E2 stimulation of eNOS in isolated endothelial cell plasma membranes and caveolae occurs in the absence of added calcium yet it is completely prevented by calcium chelation [22], these disparities may be explained by the participation of highly-localized, caveolae-associated calcium pools and caveolae-associated proteins that regulate calcium homeostasis [32]. Thus, mirroring observations in certain other cell types, intracellular calcium homeostasis in endothelial cells is modulated by non-nuclear ER.

3. Non-nuclear ER adaptor proteins

Since a subpopulation of ERα is localized to endothelial cell caveolae, and the receptor has both physical and functional interaction with other signaling molecules in that specialized microdomain, adaptor proteins are likely to be important to non-nuclear actions of ERα in endothelial cells. One such adaptor molecule is the ERα binding protein striatin, which is a member of the WD-repeat protein family. In studies performed in the endothelial cell line Eahy.926, striatin and ERα were coimmunoprecipitated, and overexpression of striatin increased plasma membrane abundance of ERα. In addition, treatment of cells with E2 promoted the formation of a complex containing ERα and striatin and Gαi. Pull-down experiments with purified proteins demonstrated direct protein-protein interaction between ERα and striatin that requires amino acids 183–253 of the receptor, and a peptide containing amino acids 176–253 of ERα prevented E2 activation of Akt,