Steroids 114 (2016) 2–6

Contents lists available at ScienceDirect

Steroids

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

Review

Interplay between steroid hormone activation of the unfolded protein response and nuclear receptor action

Xiaobin Zheng [a], Neal Andruska [a,b], Liqun Yu [a], Chengjian Mao [a], Ji Eun Kim [a], Mara Livezey [a], William G. Helferich [d], David J. Shapiro [a,b,c,*]

[a] Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[b] College of Medicine, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[c] Food Science and Human Nutrition, University of Illinois at Urbana-Champaign, Urbana, IL, USA
[d] University of Illinois Cancer Center, University of Illinois at Urbana-Champaign, Urbana, IL, USA

ARTICLE INFO

Article history:
Received 25 January 2016
Received in revised form 17 March 2016
Accepted 22 March 2016
Available online 23 March 2016

Keywords:
Steroid receptor
Breast cancer
Unfolded protein response
Cell death
Hormone action
Cancer

ABSTRACT

To identify new pathways of estrogen action and novel estrogen receptor α (ERα) biomodulators, we performed high throughput screening and used follow on assays and bioinformatics to identify small molecule ERα inhibitors with a novel mode of action. These studies led to identification of rapid extranuclear activation of the endoplasmic reticulum stress sensor, the unfolded protein response (UPR), as a new pathway of estrogen-ERα action. Moreover, increasing evidence indicates that the mechanism underlying anticipatory activation of the UPR is shared among steroid and peptide hormones and is conserved from insects to humans. It is likely that this newly unveiled extranuclear pathway is used by diverse mitogenic hormones to prepare cells for the increased protein folding load that will occur during subsequent cell proliferation. Demonstrating biological relevance, elevated expression of a UPR gene signature in ERα positive breast cancer is a powerful new prognostic marker tightly correlated with subsequent resistance to tamoxifen, tumor recurrence and poor survival. In addition, overexpression of epidermal growth factor receptor and HER2/neu is positively correlated with increased UPR activation in breast cancer. This review describes recent research that demonstrates the importance of anticipatory UPR activation in therapy resistant tumors and discusses a promising small molecule biomodulator that inhibits tumor growth by tuning this UPR signaling pathway.

Contents

1. Introduction

The endoplasmic reticulum (EnR) plays a key role in synthesis, folding and transport of nascent peptides. Protein maturation in the EnR is a critical step in normal cell function and in cell survival. Modest changes in the cellular environment, such as changes in the intracellular Ca^{2+} level in the lumen of the EnR, nutrient availability, redox state, or in the rate of protein synthesis, can cause accumulation of misfolded or unfolded proteins. The resulting EnR stress [1,2] leads to activation of the EnR stress response pathway, the unfolded

Abbreviations: AR, androgen receptor; CAMKIII/eEF2K, eukaryotic elongation factor 2 kinase; Ec, ecdysone; EcR, ecdysone receptor; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; eIF2α, eukaryotic initiation factor 2α; EnR, endoplasmic reticulum; ERα, estrogen receptor α; IP_3, inositol triphosphate; IP_3R, inositol triphosphate receptor; PERK, protein kinase RNA-like endoplasmic reticulum kinase; PLCγ, phospholipase C gamma; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; UPR, unfolded protein response; VEGF, vascular endothelial growth factor.

* Corresponding author at: Department of Biochemistry, 419 Roger Adams Lab, University of Illinois, 600 S. Mathews Avenue, Urbana, IL 61801, USA.
E-mail address: djshapir@illinois.edu (D.J. Shapiro).

http://dx.doi.org/10.1016/j.steroids.2016.03.014
0039-128X/

protein response (UPR). The UPR consists of three main branches that together balance the synthesis of new proteins with the availability of chaperones and other proteins to help fold and transport proteins within cells. EnR stress activates autophosphorylation of the transmembrane kinase PERK (protein kinase RNA-like endoplasmic reticulum kinase) [2,3]. Activated p-PERK phosphorylates eukaryotic initiation factor 2α (eIF2α), resulting in transient inhibition of most protein synthesis and increased translation of $p58^{IPK}$ and GADD34. If the stress is moderate, the $p58^{IPK}$ binds PERK, inhibiting PERK activation, and the GADD34 dephosphorylates eIF2α. This ultimately reverses PERK activation and protein synthesis is restored [4,5]. The other arms of the UPR initiate with activation of the transcription factor ATF6α, leading to increased protein folding capacity and activation of the splicing factor IRE1α, which alternatively splices the transcription factor XBP1, leading to production of active spliced XBP1 (sp-XBP1), increased protein folding capacity and altered mRNA decay and translation (Fig. 1) [1–3].

Diverse mitogenic hormones, acting via their respective receptors, stimulate cell proliferation and tumor growth [6–11]. Enhanced cell proliferation requires increased protein production, potentially leading to insufficient protein folding capacity and EnR stress. Although UPR activation has been described in multiple cancers [2,12–15], until recently, it has not been a major research focus in hormone-dependent cancers. This review focuses on the pathophysiological importance of anticipatory UPR activation in hormone signaling as an early component of the cellular proliferation program and discusses the preclinical promises of targeting the UPR.

2. Steroid/peptide hormone activation of the UPR

Steroid and peptide hormones execute their biological functions through direct interaction with hormone-specific receptors [8,9]. These include binding of mitogenic steroid hormones, 17β-estradiol (E_2; estrogen), dihydrotestosterone (DHT; androgen) and ecdysone (Ec) to their respective nuclear receptors (ERα, AR and EcR) and of the peptide hormones epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF) to their receptors (EGFR and VEGFR). Steroid hormones exert their molecular functions by regulating gene expression in the nucleus and cross-talking with diverse extranuclear signal transduction pathways. In the classical genomic action of steroid hormones, here illustrated using estrogen, estrogens bind to ERα; this results in receptor dimerization. Estrogen-ERα binds directly to genomic response elements and interacts with DNA indirectly through tethering to other proteins. This results in activation of a genomic program that alters the expression of thousands of genes and plays an important role in promoting the proliferation of ERα positive cancer cells [16–18].

While the genomic actions of steroid hormones are initiated rapidly, they play out over many hours. A disparate set of rapid extranuclear actions of steroid receptors, often initiated at or near the plasma membrane, influence diverse cell functions and also play a pivotal role in modulating the receptors genomic program [19–21]. While much attention focused on rapid effects of steroid hormones on established signal transduction pathways, rapid effects of estrogen and other steroid hormones on activation of the UPR were largely unexplored. We recently showed that, within 1 min, estrogens, acting via ERα, activate phospholipase C gamma (PLCγ), producing inositol triphosphate (IP_3). The IP_3 binds to and opens the EnR inositol triphosphate receptor (IP_3R) calcium channels allowing rapid efflux of calcium from the lumen of the EnR into the cytosol (Fig. 1). This rapid calcium efflux activates the UPR, inducing chaperones (Fig. 1). Notably, inhibition or knockdown of pathway components strongly inhibits estrogen stimulated cell proliferation and nearly abolishes subsequent estrogen-ERα induction and repression of gene expression (Fig. 1) [22]. Moreover, analysis of data from approximately 1000 ERα positive breast cancers shows that elevated expression of a UPR gene signature at diagnosis is a powerful new prognostic marker tightly correlated with subsequent resistance to tamoxifen, tumor recurrence and poor survival [22].

The well-studied oncogenic mitogen EGF, acting through EGF receptors, rapidly activates the ERK and AKT signaling pathways and alters gene expression. EGF-EGFR activation of these pathways promotes tumor growth and invasion, and is antiapoptotic [23–25]. Although EGF is a peptide hormone and EGFR is a plasma

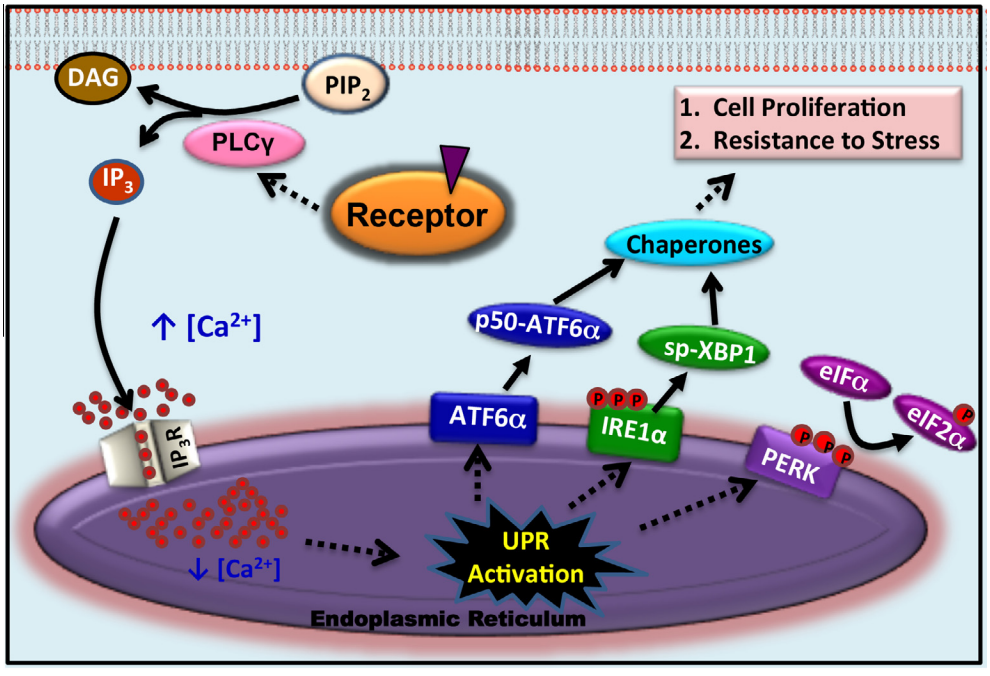

Fig. 1. Model for activation of the UPR by steroid or peptide hormones.