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REVIEW ARTICLE

Estrogen Receptor and the Unfolded Protein Response: Double-Edged Swords in Therapy for Estrogen Receptor-Positive Breast Cancer

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Abstract

Estrogen receptor α (ERα) is a target for the treatment of ER-positive breast cancer patients. Paradoxically, it is also the initial site for estrogen (E_2) to induce apoptosis in endocrine-resistant breast cancer. How ERα exhibits distinct functions, in different contexts, is the focus of numerous investigations. Compelling evidence demonstrated that unfolded protein response (UPR) is closely correlated with ER-positive breast cancer. Treatment with antiestrogens initially induces mild UPR through ERα with activation of three sensors of UPR—PRK-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1α (IRE1α), and activating transcription factor 6 (ATF6)—in the endoplasmic reticulum. Subsequently, these sensors interact with stress-associated transcription factors such as c-MYC, nuclear factor-κB (NF-κB), and hypoxia-inducible factor 1α (HIF1α), leading to acquired endocrine resistance. Paradoxically, E_2 further activates sustained secondary UPR via ERα to induce apoptosis in endocrine-resistant breast cancer. Specifically, PERK plays a key role in inducing apoptosis, whereas IRE1α and ATF6 are involved in endoplasmic reticulum stress-associated degradation after E_2 treatment. Furthermore, persistent activation of PERK deteriorates stress responses in mitochondria and triggers of NF-κB/tumor necrosis factor α (TNFα) axis, ultimately determining cell fate to apoptosis. The discovery of E_2-induced apoptosis has clinical relevance for treatment of endocrine-resistant breast cancer. All of these findings demonstrate that ERα and associated UPR are double-edged swords in therapy for ER-positive breast cancer, depending on the duration and intensity of UPR stress. Herein, we address the mechanistic progress on how UPR leads to endocrine resistance and commits E_2 to inducing apoptosis in endocrine-resistant breast cancer.

Key Points

Estrogen receptor α (ERα) is a target for the treatment of ER-positive breast cancer patients. Paradoxically, it is also the initial site for estrogen (E_2) to induce apoptosis in endocrine-resistant breast cancer.

The ERα-associated unfolded protein response (UPR) confers antiestrogen resistance, whereas it commits E_2 to inducing apoptosis in endocrine-resistant breast cancer, depending on the duration and intensity of stress.

The UPR-associated transcription factors such as c-MYC and nuclear factor-κB (NF-κB) display features of double-edged swords in determining cell fate under different cellular contexts.

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1 Introduction

Tamoxifen is the first targeted therapy to treat all stages of estrogen receptor (ER)-positive breast cancer, and the first medicine for the reduction of breast cancer incidence in high-risk pre- and post-menopausal women [1]. Currently, endocrine therapies targeting ER with selective ER modulators (SERMs) and selective ER degraders (SERDs) or preventing synthesis of estrogen (E_2) via aromatase inhibitors (AIs) are the standards of care for ER-positive breast cancer patients [2, 3]. However, acquired resistance to endocrine therapies is a major challenge for the treatment in these patients [4]. Although it remains unclear how acquired resistance occurs, many mechanisms underlie acquired resistance to endocrine therapies in breast cancer. One of the widely accepted mechanisms is activation of tyrosine kinase receptors, including HER2, epithelial growth factor receptor, and insulin-like growth factor-1 receptor β [5–7]. Additionally, *PIK3CA* mutation over activates downstream signaling pathways such as mammalian target of rapamycin (mTOR) and cyclin-dependent kinase 4/6 (CDK4/6), which results in antiestrogen resistance [8–10]. Thus, therapies targeting growth factor receptors, *PIK3CA* mutation, mTOR, and CDK4/6 are administered for advanced breast cancer [8–10]. All of these resistance mechanisms including ERα mutations have been recently reviewed [4, 11–13].

Our findings have demonstrated that E_2 deprivation and SERMs produce the same selective evolutionary pressure on ER-positive breast cancer cells to create configured cellular populations with activated unfolded protein response (UPR), inflammatory stress, and metabolic disorders during adaptation to hypoxia, nutritional deprivation, or therapy-induced stress [14, 15] (Fig. 1). The expression of UPR-associated proteins closely correlates with ERα in breast cancer [16, 17]. The ERα regulates the functions of three sensors of UPR (PRK-like endoplasmic reticulum kinase [PERK], inositol-requiring enzyme 1α [IRE1α], and activating transcription factor 6 [ATF6]) and the chaperone protein glucose-regulated protein 78 (GRP78) [18, 19]. Under endoplasmic reticulum stress conditions, GRP78 dissociates from these three sensors and binds to unfolded proteins in the lumen of the endoplasmic reticulum, facilitating the activation of PERK, IRE1α, and ATF6. Subsequently, the three sensors activate their downstream signals with the initial purpose of maintaining homeostasis in the endoplasmic reticulum [18, 19]. The final cell fate is determined on the duration and intensity of stress [18–20]. In hormone-sensitive

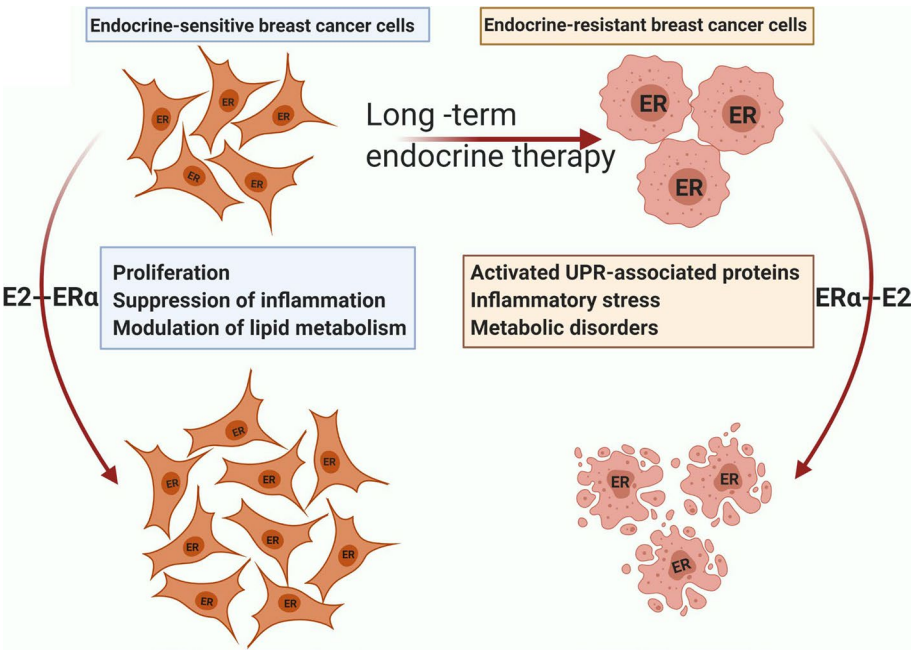

Fig. 1. Evolution of cell population after long-term endocrine therapy with activated UPR. ERα is the therapeutic target for endocrine therapy in hormone-sensitive breast cancer. In these breast cancer cells, ERα is a proliferative factor that suppresses inflammatory responses and regulates lipid metabolism. After long-term endocrine therapy, cell populations recapitulate activated UPR, inflammatory stress, and metabolic disorders, along with new morphologies. Simultaneously, UPR-associated transcription factors, such as c-MYC and NF-κB, are activated. All of these molecular alterations lead to the acquired endocrine resistance. Alternatively, they create a special microenvironment for E_2 to induce apoptosis in endocrine-resistant breast cancer. *E_2* estrogen, *ERα* estrogen receptor α, *NF-κB* nuclear factor-κB, *UPR* unfolded protein response

breast cancer cells, E_2 and antiestrogens activate mild UPR, which promotes cell growth and facilitates antiestrogen resistance [18, 21]. Recently, a UPR-associated gene expression signature has been identified as a powerful prognostic marker that predicts tamoxifen resistance of ERα-positive breast cancer [18]. In support of this finding, elevated expression of UPR-associated proteins have been observed in ERα-positive breast cancer cells after long-term E_2 deprivation and antiestrogen therapies [15, 22, 23]. Furthermore, the three sensors interact with transcription factors such as c-MYC, nuclear factor-κB (NF-κB), and hypoxia-inducible factor 1α (HIF1α), ultimately resulting in antiestrogen resistance [15, 22, 23]. These alterations create a special interaction for the distinct response to E_2 when resistance occurs (Fig. 1).

To study mechanisms in the laboratory, we serendipitously discovered E_2-induced apoptosis in laboratory models of human breast cancer [24]. A study of long-term tamoxifen therapy in breast cancer in vivo demonstrates that treatment for 5 years alters the tumor response to low-dose E_2 therapy. Instead of growing, E_2 regresses tamoxifen-stimulated tumors [25, 26]. Following that, two independent research groups demonstrated E_2-induced apoptosis in endocrine-resistant breast cancer in vitro [27, 28]. These complementary laboratory data focus on the mechanisms for the modulation of E_2-induced apoptosis in endocrine-resistant breast cancer cells [27, 28]. Recent findings demonstrate that UPR is persistently activated by E_2 as a major mechanism to induce apoptosis [19, 29–32]. This discovery has clinical relevance, as clinicians subsequently successfully used E_2 to treat AI-resistant breast cancer in clinical trials [33] and to interpret the results of the Women's Health Initiative (WHI) study demonstrating a decrease in breast cancer incidence in women taking conjugated equine estrogen (CEE) alone as hormone replacement therapy (HRT) [34]. By contrast, medroxyprogesterone acetate (MPA), a synthetic progestin used in HRT to prevent the E_2-induced development of endometrial cancer, also possesses glucocorticoid activity [35]. As a result, MPA reverses the anticancer effect of E_2 and increases breast cancer incidence in postmenopausal women [34, 35]. Mechanistically, glucocorticoids activate glucocorticoid receptor (GR) that suppresses the DNA-binding activity of NF-κB, thereby blocking E_2-induced apoptosis in endocrine-resistant breast cancer cells [36]. This topic has been covered in detail in a recent review [37].

In this review, we address the progress on how ERα integrally regulates UPR and associated transcription factors to confer antiestrogen resistance, whereas it commits E_2 to inducing apoptosis in endocrine-resistant breast cancer. All of these findings will provide an insight into novel strategies for the treatment of advanced ER-positive breast cancer.

2 The UPR and cell fates

2.1 Mild ERα-Driven UPR Confers Antiestrogen Resistance to ER-Positive Breast Cancer

Long-term antiestrogen therapies are chronic stress for ERα-positive breast cancer cells [14], eliciting mild UPR in an attempt to restore homeostasis in cells [15]. Consistently, elevated UPR gene expression is correlated with tamoxifen resistance in ERα-positive breast tumors [18]. ERα regulates three sensors of UPR and the chaperone protein GRP78 in response to hypoxia, nutrient deprivation, and therapy-induced stress in ERα-positive breast cancer cells [15, 18, 19, 21]. Among UPR-regulated proteins, GRP78 and IRE1α/X-box binding protein 1 (XBP1) are implicated in antiestrogen resistance [15, 23, 38, 39]. The expression of these UPR-associated proteins correlates with ERα in luminal tumors [16, 17]. Notably, GRP78 and XBP1 are overexpressed in 60–70% and 80–90% of breast tumors, respectively [16, 17], which may account for decreased sensitivity of ER-positive breast cancer to endocrine therapies [15, 23, 38, 39]. All of these observations demonstrate that both E_2 deprivation and antiestrogens (tamoxifen and fulvestrant) activate UPR with protective effects (also called anticipatory UPR), which contribute to the development of antiestrogen resistance [15, 38, 39].

2.1.1 Regulation of GRP78 by ERα

Overexpression of GRP78 after E_2 deprivation or tamoxifen-based treatment is an important biomarker of resistance to antiestrogen therapies [38, 39]. In addition to being a chaperone protein, GRP78 is a multifunctional protein that is highly associated with ERα in the lumen of the endoplasmic reticulum [40]. Also, it regulates fatty acid metabolism through sterol regulatory element-binding transcription factor 1 (SREBP1), resulting in fatty acid accumulation and consequent cytotoxicity after downregulation of GRP78 in antiestrogen-resistant breast cancer cells [41]. Importantly, GRP78 promotes proliferation and metastasis after translocation to the cell membrane, activating the PI3K/Akt pathway and enhancing angiogenesis in the tumor microenvironment [42, 43]. Additionally, GRP78 is involved in induction of autophagy, which integrally regulates the balance between UPR and apoptosis in antiestrogen-resistant breast cancer cells [38]. As a result, depletion of GRP78 converts antiestrogen-resistant cells into antiestrogen-sensitive ones [38].

2.1.2 Regulation of IRE1α and ATF6 by ERα

The UPR branch IRE1α is the most conserved sensor with kinase and endonuclease activities (Fig. 2). Upon activation, IRE1α splices XBP1 mRNA and generates a transcription factor called spliced XBP1 (XBP1s) that regulates the

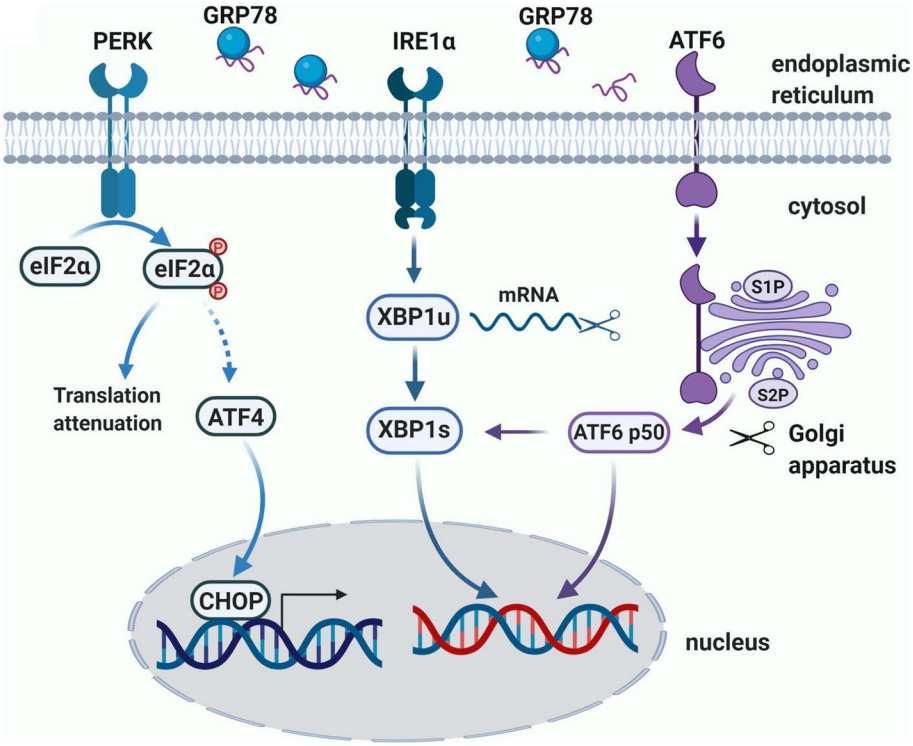

Fig. 2. Activation of the three sensors of UPR. Under stress conditions, the chaperone protein GRP78 releases from the three sensors and binds to unfolded proteins in the lumen of the endoplasmic reticulum. Next, the three sensors are activated, with different functions. PERK phosphorylates eIF2α to attenuate protein translation. Given that PERK activation is mild and transient, ATF4 is selectively activated for autophagy, but the downstream proapoptotic protein CHOP is not induced. IRE1α is the most ancient and conserved sensor of UPR. Upon activation, IRE1α splices XBP1 mRNA to XBP1s, which regulates the expression of genes to promote protein folding and induce ERAD. ATF6 is cleaved by S1P and S2P after translocation to the Golgi apparatus upon stress occurrence. Active ATF6 p50 is then released to increase the transcriptional activity of XBP1 and regulate the expression of UPR-associated target genes. *ATF* activating transcription factor, *CHOP* C/EBP homologous protein, *eIF2α* eukaryotic initiation factor 2α, *ERAD* endoplasmic reticulum stress-associated degradation, *GRP78* glucose-regulated protein 78, *IRE1α* inositol-requiring enzyme 1α, *PERK* PRK-like endoplasmic reticulum kinase, *S1P* Site-1 protease, *S2P* Site-2 protease, *UPR* unfolded protein response, *XBP1* X-box binding protein 1, *XBP1u* unspliced XBP1, *XBP1s* spliced XBP1

expression of genes to promote protein folding and induce endoplasmic reticulum stress-associated degradation [28, 44]. Accumulated data demonstrate that ERα directly regulates IRE1α and XBP1 expression in breast cancer cells [19, 29, 45]. *XBP1* is an E_2-responsive gene that is regulated by ERα on its promoter [45, 46]. In another way, XBP1 can increase ERα expression and transcriptional activities in an E_2-independent manner [46]. Thus, high expression of XBP1 in ER-positive tumors [16, 17] promotes E_2-independent growth and induces resistance to antiestrogens. In line with this result, both IRE1α and XBP1 are upregulated in resistant breast cancer cells after E_2 deprivation or antiestrogen therapy [15, 23]. Additionally, IRE1α and XBP1 are involved in the regulation of fatty acid metabolism [47] and glucose metabolism through interaction with the transcription factor Forkhead box O1 (FOXO1) [48] and regulatory subunits of PI3K [49]. The importance of IRE1α and XBP1 in mediation of endocrine resistance is confirmed by the finding that knockdown of IRE1α and XBP1 restores sensitivity to antiestrogen therapy [23]. As for ATF6, its function always overlaps that of IRE1α and XBP1 [29, 44], which is activated by Site-1 protease (S1P) and Site-2 protease (S2P) proteolysis after translocation to the Golgi apparatus upon endoplasmic reticulum stress (Fig. 2). Active ATF6 increases the transcriptional activity of XBP1 and contributes to cell survival during chronic stress [29, 44, 50]. In addition, ATF6 upregulates the chaperone proteins GRP78 and GRP94, which are indicators of ATF6 activation [51]. However, ATF6 activation and its association with antiestrogen resistance have received much less attention than IRE1α [50].

2.1.3 Regulation of PERK by ERα

The PERK is a major UPR sensor that attenuates protein translation through phosphorylation of eukaryotic initiation factor 2α (eIF2α) [52], but it selectively increases translation of activating transcription factor 4 (ATF4) [53]. If

stress is severe or prolonged, ATF4 can further activate the transcription factor C/EBP homologous protein (CHOP) to initiate apoptosis (Fig. 2) [54]. Substantial findings demonstrate that PERK/eIF2α/ATF4 signaling is activated by ERα in breast cancer cells, including hormone-sensitive and hormone-insensitive cells [18, 19, 29–32, 55]. The final cell fate depends on the intensity of stress activating the PERK/eIF2α/ATF4 axis [18, 19, 29–32, 55]. At modest levels of stress, such as hypoxia, the transcription factor HIF1α is directly upregulated by ERα because *HIF1α* gene bears an estrogen responsive element (ERE) [56]. Clinical data also suggest that expression of HIF1α is associated with poor outcome of tamoxifen therapy for ER-positive breast cancer [56, 57]. Investigators found that hypoxia is a potent activator of the PERK/eIF2α signaling pathway, which promotes nodal metastasis [58]. The PERK pathway contributes to the adaptive response to hypoxia through integration of its two substrates, ATF4 and nuclear factor erythroid 2-related factor 2 (NRF-2), which transcriptionally regulate the activity of antioxidants to reduce reactive oxygen species (ROS) generation, thereby maintaining cellular redox homeostasis [59, 60]. Additionally, ATF4 and NRF-2 participate in the induction of autophagy, which protects cancer cells from hypoxia-induced apoptosis [61]. Another important feature of tamoxifen-resistant breast cancer cells is epithelial-to-mesenchymal transition (EMT), which enhances invasion and facilitates metastasis [62]. Recent findings demonstrate that EMT activates PERK, which sensitizes cells in EMT to invade and metastasize [63]. Furthermore, the key EMT-related transcription factor is cAMP responsive element-binding protein 3-like 1 (CREB3L1); this protein is driven by PERK, which promotes invasion and metastasis of ER-positive breast cancer [64]. Consistently, inhibition of the PERK pathway remarkably mitigates the metastatic phenotype of breast cancer cells undergoing EMT [63, 64]. These findings underscore the key role of PERK in the promotion of endocrine resistance of ER-positive breast cancer.

2.2 The UPR-Associated Transcription Factors are Activated After Endocrine Therapy

In addition to being a proliferative factor, ERα modulates biological processes such as metabolism and inflammation in breast cancer cells [65, 66]. As a result, antiestrogen therapies, as well as menopause, cause metabolic disorders and inflammatory stress [15, 65, 67]. Similarly, ERα-regulated UPR enhances various signaling pathways related to inflammatory stress and metabolic dysfunction, contributing to poor clinical outcome of antiestrogen therapies [15, 68, 69]. Furthermore, many transcription factors, such as c-MYC, NF-κB, and HIF1α [22, 23, 32, 58, 70, 71], are activated by UPR and interact with ERα to integrally regulate metabolic alterations and inflammatory response [22, 23, 32, 58, 70, 71]. In particular, activation of c-MYC and NF-κB by UPR has been linked with antiestrogen resistance of breast cancer [22, 23, 32, 71, 72].

2.2.1 c-MYC and UPR in Endocrine Resistance

The oncogene *c-MYC* is a classical E_2-responsive gene [15, 72] that is overexpressed in endocrine-resistant breast cancer cells and implicated as a driver to promote the malignancy of breast cancer [22, 71, 72]. Notably, c-MYC functions as a key regulator of metabolism, leading to the current novel opportunities for treatment of hormone-responsive cancers [22, 73–77]. The metabolic reprogramming controlled by c-MYC includes that of glutamine, glucose, and lipid metabolism [22, 73–75]. Thus, the function of c-MYC is closely linked with UPR, resulting in multiple levels of interaction between c-MYC and UPR in cancer cells. Additionally, c-MYC–dependent proliferation demands synthesis of more proteins and activates UPR. Meanwhile, UPR-induced autophagy promotes MYC-dependent transformation and tumor growth [78]. The IRE1α/XBP1 axis has attracted attention regarding its association with c-MYC in the treatment of cancer [22, 77, 79, 80]. Specifically, IRE1α/XBP1s signaling is required for the sustained growth and survival of c-MYC–overexpressing cells [79, 80], and XBP1s acts as an enhancer of c-MYC for its overexpression and activation [79]. In addition, c-MYC transcriptionally activates the IRE1α/XBP1 pathway by binding directly to the promoter and enhancer of IRE1α [77]. Therefore, blockade of the RNase activity of IRE1α by the RNase-specific inhibitor MKC8866 and knockdown of XBP1 can effectively inhibit c-MYC–driven tumor growth [77, 79, 80] and reverse the acquired endocrine resistance of breast cancer [22, 71, 72].

2.2.2 NF-κB and UPR in Endocrine Resistance

The transcription factor NF-κB serves as a fundamental modulator of inflammation [81] that mainly regulates lipid metabolism through interaction with lipogenic transcription factors such as CCAAT/enhancer binding protein β (C/EBPβ) and peroxisome proliferator-activated receptor γ (PPARγ) [32, 82]. Accordingly, a variety of adipose inflammatory factors, including tumor necrosis factor α (TNFα) and interleukin-6 (IL-6), and redox homeostasis are regulated by NF-κB [32, 82]. Unlike c-MYC, NF-κB has an inverse relationship with ERα in breast cancer cells [83, 84]. ERα potently suppresses the activation of NF-κB [32, 83]. Thus, enhanced NF-κB activity is widely found in ER-negative breast cancer cell lines and tumors [85, 86]. Consistent with these observations, antiestrogen therapies lead to constitutive activation of NF-κB and its target genes, which increases cell proliferation and inflammatory response [23, 32, 87, 88]. The latter desensitizes breast cancer cells

to antiestrogen treatment and facilitates the association between NF-κB and UPR [23, 32, 89–92]. More evidence has demonstrated that both PERK and IRE1α can activate NF-κB via different mechanisms in antiestrogen-resistant breast cancer cells [23, 32, 90]. The ultimate cell fate (proliferation or apoptosis) relies on the functional dominance of distinct sensors and the cellular context [23, 32, 90]. The IRE1α/XBP1 axis is found to be closely linked with NF-κB for its contribution to endocrine resistance. The mutual activation between IRE1α/XBP1 and NF-κB escalates the process of antiestrogen resistance of breast cancer [23]. The two isoforms of XBP1 [XBP1s or unspliced XBP1 (XBP1u)] can activate NF-κB. The difference between them is that XBP1u needs ERα expression to mediate this activation, whereas XBP1s activates NF-κB independently of ERα [23]. In addition, NF-κB has the potential to further activate IRE1α and XBP1. Inhibition of NF-κB activity and knockdown of IRE1α and XBP1 make resistant cells sensitive to endocrine therapy [23, 93]. Taken together, these results suggest that IRE1α/XBP1 is a central branch of UPR that interacts with transcription factors and results in endocrine resistance. Therefore, targeting of IRE1α and XBP1 has been considered a novel treatment strategy for c-MYC– and NF-κB–driven cancers, including antiestrogen-resistant breast cancer [45, 77].

2.3 Sustained ERα-Driven UPR Leads to Apoptosis of Endocrine-Resistant Breast Cancer Cells

The UPR activated by antiestrogen treatment to ensure cell survival leads to acquired resistance of breast cancer [15, 23, 38, 39]. Unexpectedly, apoptosis of endocrine-resistant cells occurs upon administration of E_2 [25–28]. This discovery provides rationale for clinical trials to treat endocrine-resistant breast cancer [33]. It is also used to interpret the results of the WHI study demonstrating that administration of CEE alone as HRT reduces breast cancer incidence in postmenopausal women [34, 37]. Breast cancer incidence increases in postmenopausal women (no hysterectomy) taking CEE plus MPA [34] because MPA has glucocorticoid activity that blocks E_2-induced apoptosis [35–37]. Our decades of laboratory research have demonstrated that SERMs, E_2 deprivation, and menopause produce the same selective pressure on ERα-positive breast cancer cells and induce regrowth of cell populations susceptible to E_2-induced apoptosis [14, 94, 95]. The cell population selection is the fundamental mechanism for E_2 to induce apoptosis in endocrine-resistant breast cancer cells [14, 94, 95]. Based on this theory, a period of at least 5 years of E_2 deprivation after menopause, also called the gap time [95, 96], is required for the selection of vulnerable breast cancer cells to apoptosis [95–97]. The clinical outcome in the Million Women Study Collaborators confirms the necessity of this duration of cell selection in postmenopausal women [96].

Our laboratory findings have defined molecular mechanisms of E_2-induced apoptosis that occur via accumulation of stress responses, including endoplasmic reticulum, oxidative, and inflammatory stress [15, 19, 20]. Among these stress responses, UPR is the first one initiated by E_2 in the endoplasmic reticulum after hours of treatment [19, 20, 32]. The three sensors of UPR described above are all activated by E_2, with different functions in endocrine-resistant breast cancer cells: PERK activates eIF2α to attenuate protein translation, while IRE1α and ATF6 mainly mediate endoplasmic reticulum stress-associated degradation of the PI3K/Akt/mTOR pathway [20, 29]. Specifically, sustained activation of PERK, but not IRE1α or ATF6, plays a key role in the mediation of E_2-induced apoptosis [19, 20, 29]. In addition to activation of ATF4/CHOP, this apoptotic effect of PERK is not solely dependent on the phosphorylation of eIF2α [20, 32, 36] (Fig. 3). The PERK kinase is closely linked with the function of mitochondria to regulate the oxidative stress that leads to the highest ROS production and BH3-only proteins expression at the point of apoptosis [19, 20, 32] (Fig. 3). Furthermore, PERK participates in the regulation of inflammatory response. Particularly, induction of TNFα expression by E_2 takes place in a delayed pattern that relies on PERK for the activation of NF-κB [19, 20, 32, 36]. All of these features make PERK crucial for the regulation of intrinsic and extrinsic apoptotic pathways in endocrine-resistant breast cancer after treatment with E_2 (Fig. 3) [19, 20, 32, 36]. Interestingly, the G-protein-coupled estrogen receptor (GPER) activated by G1 induces UPR through Ca^{2+} depletion from the endoplasmic reticulum and results in cell death in MCF-7 breast cancer cells [98]. Consistently, Andruska et al. reported that treatment with an ERα biomodulator, BHPI, induces necrosis in several drug-resistant breast cancer models due to persistent activation of PERK [21]. Of note, both E_2 and BHPI induce excessive ERα-dependent PERK activation, causing cell death [19, 21]. However, E_2 activates classical ERα transcription pathways with increasing ERE activity [19], whereas BHPI suppresses ERα-regulated transcription [21]. These results support our findings that classical ERα-dependent ERE pathways are not necessary for the initiation of UPR to induce apoptosis of ERα-positive endocrine-resistant breast cancer cells [19]. In another way, nuclear ERα activates tethering pathways such as AP-1, which very possibly activates UPR due to the accumulations of short half-life protein c-Fos in the endoplasmic reticulum [20] (Fig. 4). Additionally, BHPI suppresses IRE1α and XBP1 expression due to functional inhibition of ERα, suggesting that activation of PERK is sufficient to induce apoptosis/necrosis [21]. In line with this view, Lin and coworkers reported divergent functions of PERK and IRE1 in determining cell fate [99, 100]. They

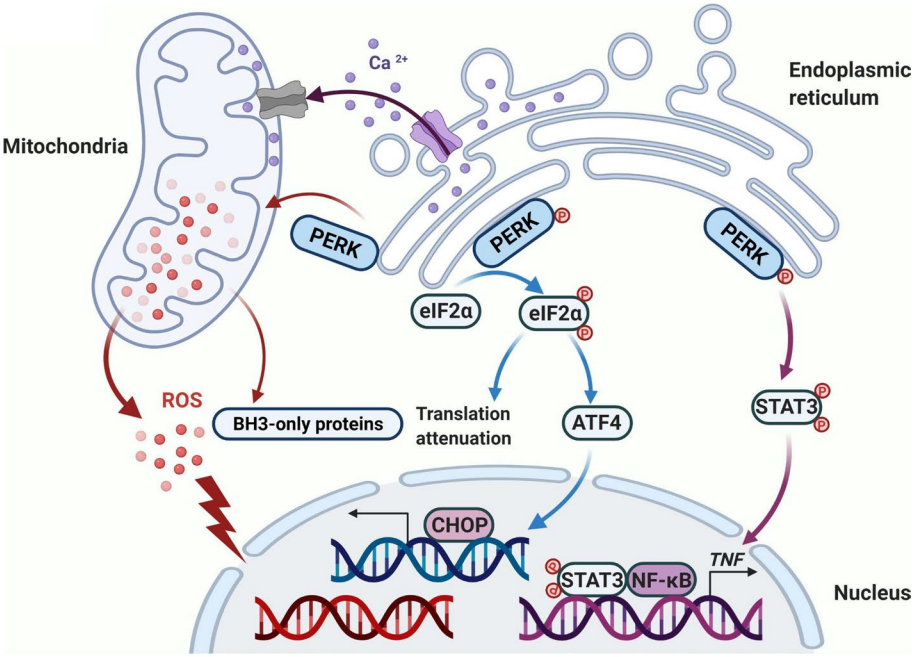

Fig. 3. Three functional ways of PERK activation to induce apoptosis of endocrine-resistant breast cancer cells. The PERK is persistently activated by E_2/ERα in resistant cells. Next, apoptosis is induced by PERK in three main ways [20]. First, in addition to routine attenuation of protein translation through phosphorylated eIF2α, sustained PERK activity induces activation of ATF4 and the downstream proapoptotic protein CHOP. Second, PERK regulates the function of mitochondria and results in release of ROS and overexpression of the BH3-only proteins. Third, the extrinsic apoptotic pathway is activated by PERK, which is mediated by STAT3 to increase DNA-binding activity of NF-κB and subsequent induction of TNFα expression. *ATF* activating transcription factor, *CHOP* C/EBP homologous protein, E_2 estrogen, *eIF2α* eukaryotic initiation factor 2α, *ERα* estrogen receptor α, *NF-κB* nuclear factor-κB, *PERK* PRK-like endoplasmic reticulum kinase, *ROS* reactive oxygen species, *STAT3* signal transducer and activator of transcription 3, *TNF* tumor necrosis factor

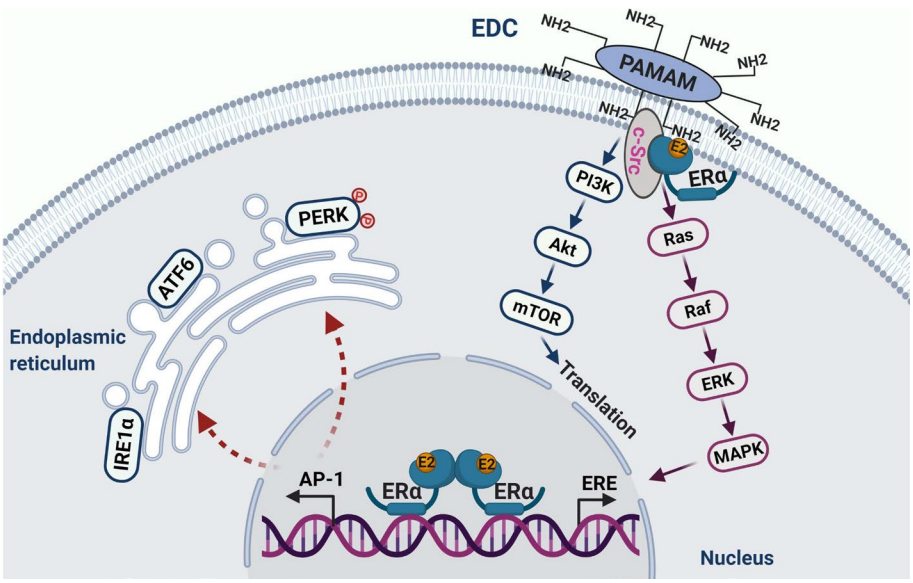

Fig. 4. E_2 initiates apoptosis through over activation of nuclear ERα. The macromolecule EDC specifically activates non-genomic pathways of ERα mediated by c-Src, which stimulates the proliferation of endocrine-resistant breast cancer cells. In the nucleus, E_2 activates classical transcription pathway ERE, which is involved in cellular proliferation. Simultaneously, E_2 consistently activates the tethering pathway of ERα, particularly AP-1 family members. This leads to stress responses in the endoplasmic reticulum. *AP-1* activator protein 1, *ATF* activating transcription factor, E_2 estrogen, *EDC* estrogen-dendrimer conjugate, *ERK* extracellular signal-regulated kinase, *ERα* estrogen receptor α, *ERE* estrogen responsive element, *IRE1α* inositol-requiring enzyme 1α, *MAPK* mitogen-activated protein kinase, *mTOR* mammalian target of rapamycin, *PAMAM* polyamidoamine, *PERK* PRK-like endoplasmic reticulum kinase

employed chemical-genetic strategies to activate these two sensors individually in HEK293 cells. Their data demonstrate that sustained PERK signaling promotes apoptosis, whereas equivalent durations of IRE1 signaling enhance cell proliferation [99, 100]. All of these findings emphasize the importance of PERK in the mediation of apoptosis of endocrine-resistant breast cancer cells [19–21, 32, 36, 99].

2.4 The UPR-Associated Transcription Factors Create a Microenvironment for E_2 to Induce Apoptosis of Endocrine-Resistant Breast Cancer Cells

Our data demonstrate that nuclear ERα is the initial site of apoptosis induction by E_2 [19, 20]. Andruska et al. [21] confirmed that BHPI persistently activates PERK in an ERα-dependent manner in endocrine-resistant breast cancer. The result that inhibition of c-Src tyrosine kinase blocks E_2-induced apoptosis seems to demonstrate that the nongenomic pathway of ERα plays an important role in apoptosis induction [19, 101]. To confirm the function of the ERα nongenomic pathway in E_2-induced apoptosis, we used a synthetic macrocompound, estrogen-dendrimer conjugate (EDC), to specifically activate the extranuclear area of ERα (Fig. 4) [20]. As expected, EDC rapidly activates the nongenomic pathway of ERα but does not induce apoptosis in endocrine-resistant breast cancer cells [19, 20]. Instead, EDC increases cellular proliferation [19, 20]. Further investigations revealed that the oncogene c-Src participates in the stress responses induced by ERα, thereby blocking E_2-induced apoptosis after inhibition of its tyrosine kinase activity [19, 101].

Although expression of ERα remains in the majority of cases of breast cancer after acquired endocrine resistance [4], the function of it in endocrine-resistant breast cancer is different from that in endocrine-sensitive breast cancer [102]. This functional difference in ERα leads to abnormal lipid metabolism and active inflammatory status in endocrine-resistant breast cancer patients, as well as menopausal women [15, 65, 66, 103, 104]. Remarkably, the action of ERα is suppressed by antiestrogens, whereas NF-κB, PPARγ, and c-MYC are activated in endocrine-resistant breast tumors [22, 32, 82]. All of these activated transcription factors ultimately alter metabolism and create an inflammatory microenvironment for E_2 to induce apoptosis in endocrine-resistant breast cancer cells [15, 19, 20, 29, 32, 36, 82]. We observed constitutive activation of NF-κB after E_2 deprivation [32]. Upon treatment with E_2, the DNA-binding activity of NF-κB is further increased by PERK kinase, resulting in induction of TNFα expression and causing apoptosis [32]. The stress-associated transcription factor, signal transducer and activator of transcription

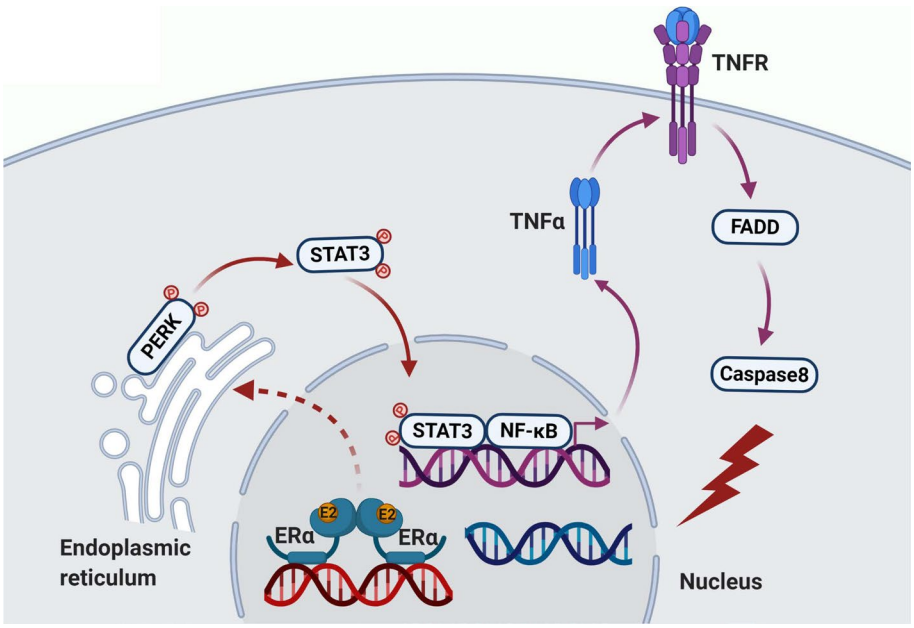

Fig. 5. The PERK/NF-κB/TNFα axis is activated by E_2 in endocrine-resistant breast cancer cells. E_2 activates nuclear ERα and results in the accumulation of unfolded proteins in the endoplasmic reticulum, activating PERK in response to the presence of misfolded proteins. The stress kinase PERK phosphorylates STAT3 and increases its DNA-binding activity. Subsequently, activated STAT3 promotes NF-κB DNA binding and induction of TNFα expression. Ultimately, TNFα binds to its receptor TNFR on plasma membrane and sequentially activates FADD/caspase-8 signal cascades to induce apoptosis. *E_2* estrogen, *ERα* estrogen receptor α, *FADD* Fas-associated death domain protein, *NF-κB* nuclear factor-κB, *PERK* PRK-like endoplasmic reticulum kinase, *STAT3* signal transducer and activator of transcription 3, *TNF* tumor necrosis factor

3 (STAT3), mediates this process to increase the nuclear activity of NF-κB (Fig. 5). Accordingly, PERK conveys a stress signal to the nucleus through STAT3 and NF-κB. We identified that the PERK/NF-κB/TNFα axis is critical for induction of apoptosis by E_2 [32] (Fig. 5). This finding provides further opportunities to modulate E_2-induced apoptosis through regulation of NF-κB activity. PPARγ is one of the transcription factors that suppress the activity of NF-κB and subsequent induction of TNFα expression, thereby blocking E_2-induced apoptosis of endocrine-resistant breast cancer cells [82]. In contrast, the PPARγ antagonist increases the activity of NF-κB and improves the therapeutic effects of E_2-induced apoptosis [82]. Similarly, many medications administered for other therapeutic purposes, such as anti-inflammatory glucocorticoids and MPA used in HRT [35, 36], block E_2-induced apoptosis through activation of GR to repress the DNA-binding activity of NF-κB in endocrine-resistant breast cancer [35, 36]. This is the basic mechanism by which the classical HRT taking CEE plus MPA in postmenopausal women increases breast cancer incidence for decades of the WHI study [34–37]. All of these results suggest that multiple transcription factors can alter the nuclear activity of NF-κB, thereby modulating E_2-induced apoptosis (Fig. 6). Additionally, deprivation of glutamine selectively induces apoptosis of MYC-overexpressing cancer cells [22, 105]. Emerging evidence demonstrates that MYC alters mitochondrial metabolism, making cancer cells rely on exogenous glutamine for survival. Depletion of glutamine activates ATF4-dependent, BH3-only proteins and induces apoptosis [106, 107]. Overall, long-term antiestrogen therapies alter the cellular metabolism and interaction of ERα with other stress-associated transcription factors, leading to the creation of a vulnerable microenvironment for apoptosis. These findings provide an important rationale for enhancing the therapeutic effects of novel synthetic estrogens for the treatment of endocrine-resistant breast cancer.

2.5 Therapeutic Potential of UPR and Future Challenges

The initial purpose of antiestrogen therapies is to block the proliferative potential of ERα in breast tumors [1–3]. Despite the fact that ERα does not remarkably increase cell growth of endocrine-resistant breast cancer, it is a potent modulator of UPR and inflammatory adaptation through a variety of interactions with other transcription factors and promotes the aggressiveness of breast cancer. Therefore, ERα remains a therapeutic target for antiestrogen resistant breast cancer [94, 106]. The functions of all UPR-related proteins are regulated by ERα. As a result, UPR and its association with transcription factors have attracted wide attention for therapy of advanced ER-positive breast cancer [22, 23, 32, 36, 77, 82]. Antiestrogen therapies over activate MYC, which reprograms the metabolism of cells and increases their dependency on glutamine for survival. MYC transcriptionally activates IRE1 and XBP1 to promote cell proliferation. Thus, inhibitors of glutamine metabolism and the selective IRE1 RNase inhibitor MC8866 are effective at preventing

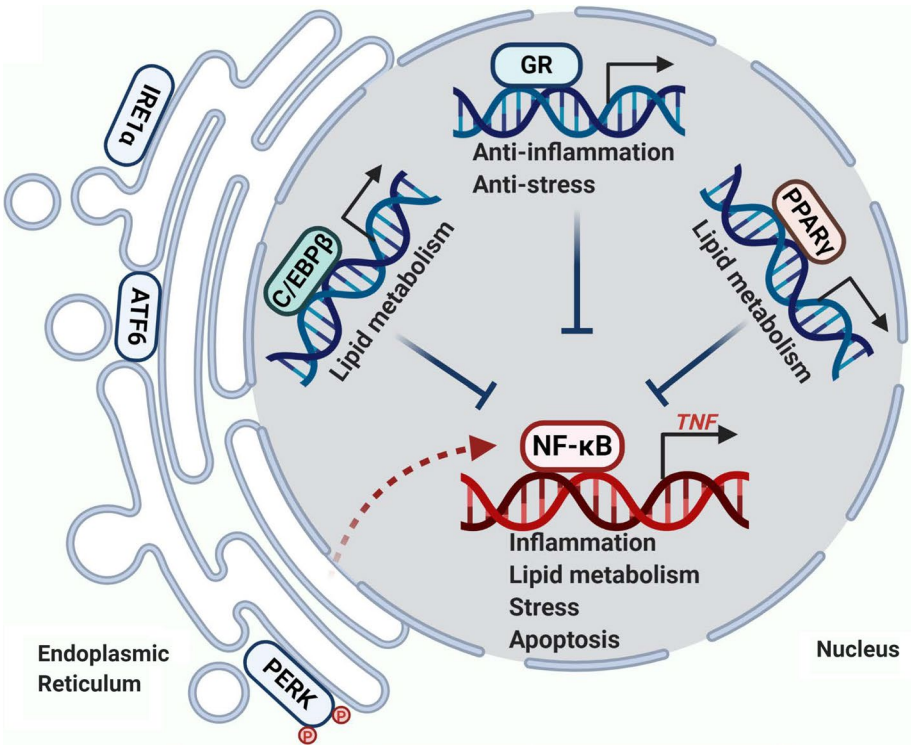

Fig. 6. Regulation of NF-κB DNA-binding activity by other transcription factors in endocrine-resistant breast cancer cells. E_2/ERα persistently activates PERK and subsequently increases NF-κB DNA-binding activity. However, the lipid metabolism-associated transcription factors C/EBPβ and PPARγ and inflammation modulator GR all suppress the DNA-binding activity of NF-κB, thereby inhibiting E_2-induced apoptosis in endocrine-resistant breast cancer cells. *ATF* activating transcription factor, *C/EBPβ* CCAAT/enhancer binding protein β, E_2 estrogen, *ERα* estrogen receptor α, *GR* glucocorticoid receptor, *IRE1α* inositol-requiring enzyme 1α, *NF-κB* nuclear factor-κB, *PERK* PRK-like endoplasmic reticulum kinase, *PPARγ* peroxisome proliferator-activated receptor γ

MYC-driven breast cancer [22, 77]. Similarly, NF-κB is closely associated with IRE1 and XBP1 and contributes to endocrine resistance [23, 32]. As a result, inhibition of IRE1 and XBP1 signaling can reverse NF-κB–mediated endocrine resistance. In addition to targeting UPR branches, autophagy and the ubiquitin-proteasome system integrate with UPR to remove misfolded and/or short-lived proteins [23, 29, 82]. Therefore, the proteasome inhibitor bortezomib and autophagy inhibitor chloroquine can enhance the efficacy of antiestrogens in treating ER-positive breast cancer [108, 109]. Furthermore, emerging evidence has indicated that stabilization of ERα, MYC, and GRP78 is regulated by the deubiquitinases, which have potential to be considered as future therapeutic targets [110–112].

However, manipulating these fundamental biological responses for therapeutic purposes without causing severe side effects is still a formidable challenge [113]. Additionally, therapy-related stress creates a special microenvironment for transcription factors, including ERα, NF-κB, STAT3, and MYC, to exhibit paradoxical features: induction of either proliferation or apoptosis of breast cancer cells depending on the cellular context [15, 19, 21–23, 32, 36]. Compelling data suggest that the DNA landscape of ERα-binding sites is altered after antiestrogen treatment [114, 115], leading to functional alteration of ERα and different interactions of ERα with these stress-associated transcription factors [32, 36, 82, 116]. Given this complexity, more studies are needed to identify the mechanisms that fine-tune UPR for the purpose of breast cancer therapy under different circumstances [117]. Future exploitation of these novel data will facilitate inhibition of advanced breast cancer with fewer side effects.

3 Perspective

The discovery of E_2-induced apoptosis gives us new insights into recognition of transcriptional factor ERα. It is a molecule for the treatment to prevent proliferation of ER-positive breast cancer patients whereas it is also the initial site for triggering apoptosis by E_2 in endocrine-resistant breast cancer [1–3, 19]. Unexpectedly, ERα keeps its proliferative potential while cells undergo apoptosis [19]. Thus, ER remains a therapeutic target when endocrine-resistance occurs [94, 95]. Subsequent studies have found that the implications of ERα-associated transcription factors and related special microenvironment determine the final fate of breast cancer cells—proliferation or apoptosis [22, 23, 32, 77]. As a result, researchers are testing how novel therapeutic strategies precisely modulate these molecules to overcome antiestrogen resistance and improve the therapeutic effects of E_2-induced apoptosis on endocrine-resistant breast cancer.

Remarkably, abundant data demonstrate that ERα induces UPR in hormone-sensitive and hormone-insensitive breast cancer cells with different consequences depending on the duration and intensity of stress [19–21]. The UPR-associated proteins and related transcription factors form different regulatory networks in response to stress under hormone-sensitive or hormone-insensitive conditions. Even though similar UPR-associated proteins participate in stress responses under these two circumstances, there are preferences and selection on different UPR branches to perform the functions of proliferation or apoptosis. Compelling results support that IRE1α and XBP1 mainly contribute to cell proliferation and confer endocrine resistance, whereas sustained PERK activation is critical for E_2- or BHPI-induced apoptosis/necrosis of endocrine-resistant breast cancer cells [19, 21, 29, 77, 99, 100]. These findings create novel therapeutic opportunities for advanced ERα-positive breast cancer via modulating UPR-related signaling pathways.

The discovery of E_2-induced apoptosis not only has clinical relevance to treatment of AI-resistant breast cancer and reducing breast cancer incidence in postmenopausal women [33, 34, 118], but also a general principal has emerged to understand sex steroid-induced apoptosis in patients with long-term androgen-deprived prostate cancer [119, 120]. In line with this, UPR activation also contributes to the development of drug-resistance phenotypes of prostate cancer [121]. Recently, targeting UPR to overcome endocrine resistance of prostate cancer has become a therapeutic strategy similar to that for ER-positive breast cancer [76, 79]. Accordingly, precisely defining the paradoxical functions of UPR is critical for ensuring the efficacy of therapy for hormone-responsive cancers.

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Conflict of interest Ping Fan and V. Craig Jordan declared that they have no conflicts of interest that might be relevant to the contents of this article.

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Consent to participate Not applicable.

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Author contributions Not applicable.

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