

COMMENT

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How PERK kinase conveys stress signals to nuclear factor- κ B to mediate estrogen-induced apoptosis in breast cancer cells?

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Antihormone therapies using a selective estrogen receptor modulator or an aromatase inhibitor are standard strategies for treatment and prevention of estrogen receptor-positive breast cancer¹. However, acquired resistance to antihormone therapies is inevitable. Paradoxically, estrogen (E_2)-induced apoptosis was an unanticipated discovery in studies of long-term antihormone therapies that produced selective pressure on breast cancer cells to create selective cell populations vulnerable to E_2 -induced apoptosis *in vivo*² and *in vitro*^{3,4}. This finding is clinically relevant to low-dose E_2 treatment of aromatase inhibitor-resistant breast cancer, with a 30% clinical benefit rate⁵, and it is a mechanistic interpretation for the decrease in breast cancer incidence and mortality in the conjugated equine estrogen alone trial performed by the Women's Health Initiative⁶. All of these clinically relevant findings encouraged us to identify the key molecules involved in E_2 -induced apoptosis to improve the therapeutic effects of E_2 on endocrine-resistant breast cancer.

Accumulation of stress responses, including endoplasmic reticulum, oxidative, and inflammatory stresses, is a major mechanism of E_2 -induced apoptosis in long-term E_2 -deprived (LTED) breast cancer cells^{7,8}. Two cellular organelles—mitochondria and endoplasmic reticulum—participate in mediation of E_2 -induced stresses⁸. Mitochondrial dysfunction leads to the release of reactive oxygen species and impairs redox homeostasis⁸. Three sensors of unfolded protein response—protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring protein 1 alpha (IRE1 α), and activating transcription factor 6 (ATF-6)—are initially activated by E_2 ,

each having different functions in the endoplasmic reticulum stress^{8,9}. PERK attenuates protein translation, which is identified as an important mediator of E_2 -induced apoptosis⁸, whereas ATF-6 and IRE1 α are involved in endoplasmic reticulum-associated degradation of phospholipids⁹. The endoplasmic reticulum stress occurs prior to the oxidative stress after exposure to E_2 in LTED breast cancer cells⁸. Of note, inhibition of PERK kinase activity completely blocks oxidative stress¹⁰, indicating close crosstalk between these two stresses. Furthermore, a variety of inflammatory factors, such as interleukin-6, fatty-acid desaturase 1, and tumor necrosis factor alpha (TNF α), are activated by E_2 with different dynamics^{7,8}. Induction of TNF α expression peaks after 3 days of E_2 treatment and is confirmed to be an important factor that induces apoptosis in LTED MCF-7:5 C cells^{8,10}. Nevertheless, how E_2 induces TNF α is unknown and requires elucidation.

TNF α is well known to be a nuclear factor- κ B (NF- κ B)-dependent gene¹⁰. NF- κ B is a critical stress-responsive transcription factor. Activated sensors of endoplasmic reticulum stress and associated inflammatory responses can activate NF- κ B to modulate stress responses¹¹. However, literature contains no reports of activation of NF- κ B by E_2 to mediate stress-associated apoptosis in LTED breast cancer cells. This knowledge is very important to understand E_2 therapy for aromatase inhibitor-resistant breast cancer. Our recent study demonstrated that E_2 differentially modulates NF- κ B activity depending on the treatment time¹⁰. E_2 initially has significant potential to suppress NF- κ B activation; in other words, E_2 completely blocks TNF α -induced activation of NF- κ B. The lipid metabolism-associated transcription factor CCAAT/enhancer-binding protein beta (C/EBP β) is activated by E_2 , which is responsible for

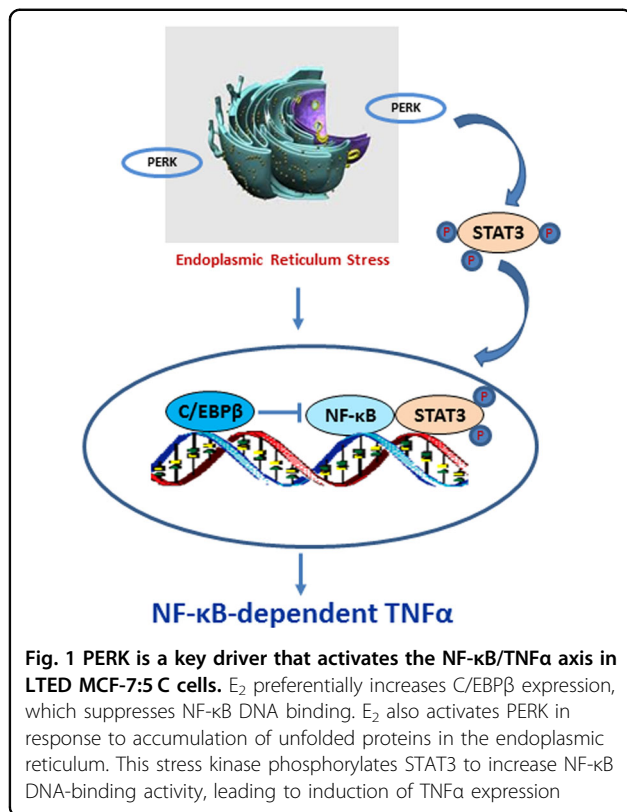
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suppression of NF- κ B activity in LTED MCF-7:5 C cells¹⁰. This result supports the existence of a trans-repressive relationship between ER α and NF- κ B¹². However, NF- κ B p65 DNA-binding activity is increased when E₂ treatment time is prolonged, leading to the induction of TNF α expression in LTED MCF-7:5 C cells¹⁰. Unlike the regulatory mechanism of TNF α , activation of NF- κ B by E₂ is independent of the canonical I κ B α signaling pathway¹⁰, suggesting novel modulation of NF- κ B directly in the nucleus. This delayed activation of NF- κ B by E₂ also indicates that some other factors are involved in overcoming the initial suppression of NF- κ B activity by ER α .

PERK is a key driver responsible for activation of NF- κ B after E₂ treatment¹⁰. This is a novel finding that further identifies mechanisms of E₂-induced apoptosis in LTED breast cancer cells. The basic biological function of PERK is to reduce unfolded proteins in the endoplasmic reticulum by phosphorylating the downstream signal eukaryotic translation initiation factor 2 alpha (eIF2 α)¹³. Although eIF2 α was reported to activate NF- κ B by decreasing the levels of I κ B α in mouse embryonic fibroblasts¹⁴, our results demonstrated that PERK kinase does not rely on eIF2 α phosphorylation to activate NF- κ B in LTED breast cancer cells¹⁰. Thus, how does this kinase in the endoplasmic reticulum activate nuclear NF- κ B under LTED conditions? Signal transducer and activator of transcription 3 (STAT3) is identified as a stress-

responsive mediator that is phosphorylated by PERK to increase NF- κ B DNA-binding activity¹⁰. Furthermore, a specific STAT3 nuclear translocation inhibitor remarkably decreases NF- κ B DNA-binding activity, suggesting that a DNA level interaction is sufficient for STAT3 to activate NF- κ B. These findings suggest that PERK kinase conveys stress signals from the endoplasmic reticulum to the nucleus through activation of STAT3 and NF- κ B under LTED conditions (Fig. 1).

In summary, several stress-responsive transcription factors, including C/EBP β , NF- κ B, and STAT3, participate in stress responses to modulate E₂-induced apoptosis in LTED breast cancer cells. The regulatory relationship between C/EBP β and NF- κ B also suggests that E₂-induced apoptosis is closely associated with lipid metabolism. However, the crosstalk between stress responses and transcription factors is complex, depending on the cellular context and inflammatory microenvironment. Despite the fact that MCF-7:5 C and MCF-7:2 A cells are derived from the same parental MCF-7 cells under LTED conditions, NF- κ B is constitutively activated in MCF-7:5 C cells but not in MCF-7:2 A cells¹⁰, whereas MCF-7:2 A cells have a stronger antioxidant system than do MCF-7:5 C cells¹⁵. These different phenotypes lead to distinctive responses of MCF-7:5 C and MCF-7:2 A cells to E₂ exposure. For instance, the NF- κ B/TNF α axis is highly active in MCF-7:5 C cells but not in MCF-7:2 A cells¹⁰. Although E₂ activates PERK similarly in MCF-7:2 A and MCF-7:5 C cells^{8,15}, the NF- κ B/TNF α axis is not quickly activated in MCF-7:2 A cells in the same way as in MCF-7:5 C cells¹⁰. This suggests that PERK kinase alone is not sufficient to activate NF- κ B, depending on its interactions with other transcription factors. Furthermore, NF- κ B can function as a key mediator of oxidative stress¹¹. Ongoing studies are focused on how NF- κ B modulates oxidative stress in LTED breast cancer cells. These data will provide an important rationale for finding target molecules to improve the therapeutic effects of E₂-induced apoptosis on endocrine-resistant breast cancer.

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Conflict of interest

The authors declare that they have no conflict of interest.

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References

1. Jordan, V. C. & Brodie, A. M. Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer. *Steroids* **72**, 7–25 (2007).
2. Yao, K. et al. Antitumor action of physiological estradiol on tamoxifen-stimulated breast tumors grown in athymic mice. *Clin. Cancer Res.* **6**, 2028–2036 (2000).
3. Song, R. X. et al. Effect of long-term estrogen deprivation on apoptotic responses of breast cancer cells to 17 beta-estradiol. *J. Natl. Cancer Inst.* **93**, 1714–1723 (2001).
4. Lewis, J. S. et al. Intrinsic mechanism of estradiol-induced apoptosis in breast cancer cells resistant to estrogen deprivation. *J. Natl. Cancer Inst.* **97**, 1746–1759 (2005).
5. Ellis, M. J. et al. Lower-dose vs high-dose oral estradiol therapy of hormone receptor-positive, aromatase inhibitor-resistant advanced breast cancer: a phase 2 randomized study. *JAMA* **302**, 774–780 (2009).
6. Anderson, G. L. et al. Conjugated equine oestrogen and breast cancer incidence and mortality in postmenopausal women with hysterectomy: extended follow-up of the Women's Health Initiative randomised placebo-controlled trial. *Lancet Oncol.* **13**, 476–486 (2012).
7. Ariazi, E. A. et al. Estrogen induces apoptosis in estrogen deprivation-resistant breast cancer through stress responses as identified by global gene expression across time. *Proc. Natl Acad. Sci. USA* **108**, 18879–18886 (2011).
8. Fan, P. et al. c-Src modulates estrogen-induced stress and apoptosis in estrogen-deprived breast cancer cells. *Cancer Res.* **73**, 4510–4520 (2013).
9. Fan, P. et al. Integration of downstream signals of insulin-like growth factor-1 receptor by endoplasmic reticulum stress for estrogen-induced growth or apoptosis in breast cancer cells. *Mol. Cancer Res.* **13**, 1367–1376 (2015).
10. Fan, P. et al. Modulation of nuclear factor-kappa B activation by the endoplasmic reticulum stress sensor PERK to mediate estrogen-induced apoptosis in breast cancer cells. *Cell Death Discov.* **4**, 15 (2018).
11. Zhang, K. & Kaufman, R. J. From endoplasmic-reticulum stress to the inflammatory response. *Nature* **454**, 455–462 (2008).
12. Cvorc, A. et al. Distinct roles of unliganded and liganded estrogen receptors in transcriptional repression. *Mol. Cell* **21**, 555–564 (2006).
13. Harding, H. P., Zhang, Y. & Ron, D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* **397**, 271–274 (1999).
14. Deng, J. et al. Translational repression mediates activation of nuclear factor kappa B by phosphorylated translation initiation factor 2. *Mol. Cell Biol.* **24**, 10161–10168 (2004).
15. Sweeney, E. E., Fan, P. & Jordan, V. C. Mechanisms underlying differential response to estrogen-induced apoptosis in long-term estrogen-deprived breast cancer cells. *Int. J. Oncol.* **44**, 1529–1538 (2014).