

Mechanistic Progress of Estrogen-induced Apoptosis in Estrogen-deprived Breast Cancer Cells

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Abstract

The laboratory discovery of estrogen-induced apoptosis has been translated to treat antihormone resistant patients and to reduce the incidence of breast cancer in postmenopausal hysterectomized women with estrogen replacement therapy (ERT). The key step is the selection pressure exerted by long-term antiestrogen therapy or over 5 years of menopause to specific breast cancer cell populations that will be vulnerable to estrogen-induced apoptosis. However, the mechanisms underlying estrogen-induced apoptosis are currently unclear. At the cellular level, estrogen-induced apoptosis is dependent upon the estrogen receptor (ER), which can be completely blocked by antiestrogen ICI 182,780 or 4-hydroxytamoxifen (4-OHT). Knockdown of ER alpha, but not ER beta, through specific small interfering RNAs effectively blocks estrogen-induced apoptosis, indicating that the ER alpha subtype participates in apoptosis. Further examinations demonstrate that estrogen-induced apoptosis is due to accumulation of endoplasmic reticulum stress, inflammatory responses, and oxidative stress, which, in turn, activate the intrinsic mitochondrial pathway and the extrinsic death receptor pathway to complete the process. This contrasts with paclitaxel, which causes G₂ arrest with immediate apoptosis. These stress responses are modulated by glucocorticoids and the c-Src inhibitor to block estrogen-induced apoptosis, but the mechanism for estrogen action is through a genomic pathway rather than a non-genomic pathway. In the nucleus, estrogen activates classic ERE-regulated endogenous genes, but the ERE transcriptional pathway does not directly participate in the estrogen-induced apoptosis *in vitro* or *in vivo*. Simultaneously, estrogen activates a non-classic transcriptional pathway involving the interaction of ER with transcription factors such as activator protein-1 (AP-1), which may regulate proliferation, stress responses, or apoptosis. Investigation of how AP-1 modulates the stress responses to trigger estrogen-induced apoptosis will ultimately uncover the mechanisms underlying estrogen-induced apoptosis.

Keywords: Estrogen; Estrogen receptor; Estrogen-induced apoptosis; Breast cancer

Introduction

Estrogen (E₂) plays a pivotal role in the development and progression of breast cancers [1]. As a result, blockade of E₂ signals through either aromatase inhibitors (AIs) or selective estrogen receptor modulators (SERMs) is an important therapeutic strategy to treat or prevent estrogen receptor (ER) positive breast cancers [2]. These endocrine therapies have significantly improved breast cancer survival [3-6]. Paradoxically, long-term endocrine therapy generates selection pressure for cell populations that evolve from acquired resistance to eventually expose a vulnerability that is expressed as E₂-induced apoptosis *in vitro* [7,8] and *in vivo* [9-11]. All of these laboratory data with MCF-7 cells provide the scientific rationale for the subsequent finding that high dose (30 mg daily) or low dose (6 mg daily) E₂ produces a 30% clinical benefit rate in patients failing aromatase inhibitor therapy [12]. Further, E₂-induced apoptosis has been used to explain lower incidences of breast cancer and mortality of postmenopausal women in their 60s undergoing E₂ replacement therapy (ERT) [13]. A recent study addresses the hypothesis that women taking traditional hormone replacement therapy (HRT) comprising of E₂ plus medroxyprogesterone acetate (MPA) may have an increased risk of breast cancer, as MPA may act as a glucocorticoid and block E₂-induced apoptosis in E₂-deprived breast cancer cells [14]. All of these observations indicate the clinical implications of E₂-induced apoptosis. However, mechanisms of E₂-induced apoptosis are currently unclear. This review will focus on the progress of mechanistic discoveries in E₂-induced apoptosis in breast cancer cells.

Evolution of Estrogen-induced Apoptosis is Selected by

Antiestrogen Pressure

Laboratory findings with the MCF-7 breast cancer cell line grown in athymic mice first described tamoxifen-stimulated growth as a new mechanism of drug resistance to a therapeutic intervention [15]. However, discovery that re-transplantation of tamoxifen-stimulated tumors into successive generations of athymic mice over 5 years results in the selection of a resistant tumor cell population that is killed by physiological levels of E₂ [9-11], which results in the new biology of E₂-induced apoptosis [16].

A similar story has also occurred over the past 20 years with the development of models to study antihormone resistance to aromatase inhibitors (AIs). The discovery that breast cancer cells, in particular MCF-7 cells, have been grown routinely in the medium with phenol red [17] that contains estrogenic activity revolutionized options to solve the question of what happens to hormone-responsive cells once starved of E₂ i.e. AI therapy. Wild-type MCF-7 cells grow with radio-immunologically undetectable levels of E₂ (Figure 1), whereas long-

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term E₂-deprived MCF-7 cells, MCF-7:5C undergo apoptosis by E₂ (Figure 1). Thus, both E₂ deprivation [7,8] and SERMs produce the same selective pressure on MCF-7 cells [9-11] to create selective cellular populations vulnerable to E₂-induced apoptosis [18]. A period of 5-10 years is required to accomplish the described selection process [19]. The apoptotic action of E₂ provides a new clinical treatment strategy for breast cancer patients following exhaustive antihormonal therapy [12,20,21]

Estrogen induces a delayed apoptosis in breast cancer cells

The estrogen receptor (ER) is the initial trigger for E₂ to induce apoptosis since antiestrogens ICI 182,780 and 4-hydroxytamoxifen (4-OHT) completely block apoptosis triggered by E₂ [22]. Contradictory to the traditional apoptosis mechanism caused by cytotoxic chemotherapy, E₂-induced apoptotic cells simultaneously undergo proliferation with an increased S phase of the cell cycle, resulting in an increased cell number [22-25]. The possibility that apoptotic impairment can be rescued by antiestrogen is a unique feature of E₂-induced apoptosis, which differs from the rapid (12 hour) chemotherapy-induced cytotoxic apoptosis [24]. E₂ exerts a dual function on E₂-deprived cells, with both initial proliferation and subsequent apoptosis [22]. In other words, there is not any detected apoptotic change in the first 24 hours after E₂ treatment [22], which gives researchers a possibility to reverse E₂-induced apoptosis by antiestrogen within 24 hours [24]. Activation of apoptotic genes appears after 48 hours treatment with E₂, and reaches a peak after 72 hours [22]. Our observations also indicate that insulin-like growth factor-1 receptor (IGF-1R)/phosphoinositide 3-kinase (PI3K) is a dominant growth driver after E₂ treatment in two E₂-deprived breast cancer cells [23,26], which activates Akt to promote cell growth [23, 26]. These data suggest that the higher rate of proliferation by E₂ might activate other pathways to trigger apoptosis.

Estrogen induces apoptosis through nuclear estrogen receptor alpha (ERα)

The original target site of E₂-induced apoptosis is ER alpha (ERα) [22,27], which can be completely blocked by the knockdown of ERα, but not ERβ, through small interfering RNA (siRNA) [28]. Oncogene c-Src functions as an important downstream signal of ERα in MCF-7:5C cells, which is activated by E₂ and demonstrates multiple levels of association with ERα [22]. A well-known function of c-Src is that

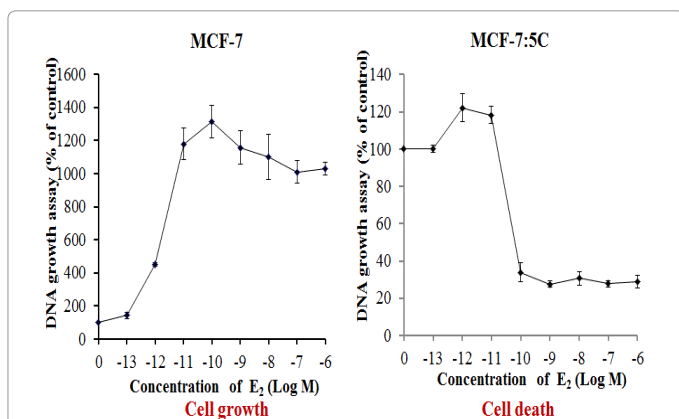


Figure 1: Different growth responses to E₂ between wild-type MCF-7 and MCF-7:5C cells. Wild-type MCF-7 cells were first transferred to E₂-free medium for 3 days. Then, MCF-7 and MCF-7:5C cells were loaded in 24-well plate respectively. After one day, cells were treated with different concentrations of E₂ as indicated. Cells were harvested after 7 days treatment. Cell viability was quantitated by determination of total DNA.

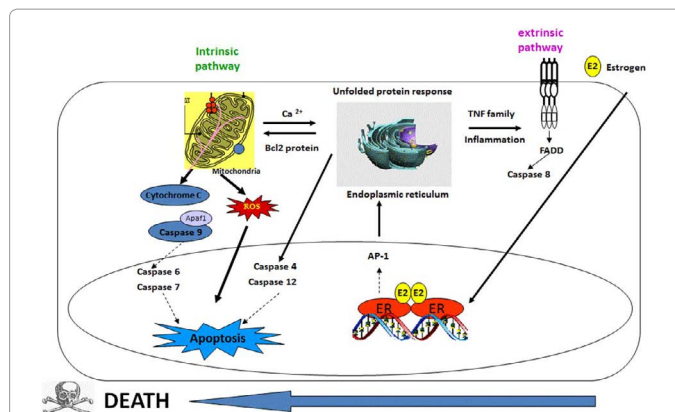


Figure 2: A schematic diagram of the mechanisms underlying E₂-induced apoptosis. (1) E₂ activates nuclear ER to modulate multiple transcriptional factors including AP-1 complex [27]; (2) unfolded protein responses are activated to reduce protein translation or increase protein degradation to reduce the burden of unfolded protein in the endoplasmic reticulum [22]; (3) failure to relieve endoplasmic reticulum stress induces apoptosis via crosstalk with mitochondria to increase reactive oxygen species (ROS) production or activate the mitochondrial pathway [22]; (4) the endoplasmic reticulum stress subsequently activates inflammation response and the extrinsic pathway of apoptosis [22,27]; (5) apoptosis can occur independent of the intrinsic and extrinsic pathways through activation of caspase 4, 12 [27].

it mediates the non-genomic pathway of E₂ in E₂-deprived breast cancer cells [22]. However, E₂-induced apoptosis is not through a non-genomic pathway [22]. This conclusion is supported by the evidence that synthetic macromolecules, estrogen-dendrimer conjugates (EDCs) that remain outside the nucleus [29] activate the non-genomic pathway while are unable to activate the genomic pathway, thereby not inducing apoptosis in MCF-7:5C cells [22]. All of these observations indicate that nuclear ERα plays an important role in E₂-induced apoptosis [22]. In the nucleus, E₂ activates classic ERE-regulated endogenous genes in MCF-7:5C cells [22,27], but the ERE transcriptional pathway does not directly participate in the E₂-induced apoptosis *in vitro* [22] or *in vivo* [30]. Our global gene array [27] data suggest that E₂ signaling can occur through a non-classic transcriptional pathway involving the interaction of ER with transcription factors such as activator protein-1 (AP-1), which may regulate proliferation, stress responses, or apoptosis [31].

Accumulation of stresses activates estrogen-induced apoptosis

The endoplasmic reticulum and mitochondria are two key organelles involved in E₂-induced apoptosis [22]. The sensors of unfolded protein response (UPR) and the oxidative stress sensor HMOX1 are activated by E₂ as initial adaptive responses in an attempt to sustain a balance in cell survival. A cross-talk exists between the endoplasmic reticulum and mitochondria to activate apoptosis cascades during E₂-induced apoptosis (Figure 2). UPR initially occurs after a few hours of treatment with E₂ [22]. Three sensors of endoplasmic reticulum stress, protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring protein 1 alpha (IRE1α), and activating transcription factor 6 (ATF6) are activated by E₂ [22,27]. PERK attenuates protein translation, which has been confirmed as an important inducer for E₂-induced apoptosis [22]; On the other hand, ATF-6 and IRE1 increase endoplasmic reticulum folding capacity by up-regulating the endoplasmic reticulum chaperones and the endoplasmic reticulum-associated protein degradation (ERAD) machinery [32,33]. The initial aim of UPR is to restore the normal function of the cell; however, if the damage is too severe to repair, the UPR ultimately initiates cell death

through activation of the apoptotic pathway [34]. Compelling evidence suggests that E_2 induces apoptosis through accumulation of stress responses, including endoplasmic reticulum stress, oxidative stress, and inflammatory stress [22,23,27]. Glucocorticoid and the c-Src inhibitor are able to modulate stress responses to block E_2 -induced apoptosis [14,22]. Currently, it is under investigation how E_2 activates the nuclear AP-1 complex to modulate the stress and apoptosis in E_2 -deprived breast cancer cells (Figure 2).

Conclusion and Future Direction

Despite the limitations of ER positive breast cancer cell lines [35], long-term estrogen-deprived MCF-7 cell models [7,8] are invaluable tools to uncover the mechanisms underlying E_2 -induced apoptosis (Figure 2). This scientific rationale has been utilized in clinical trials which have confirmed that E_2 can reduce the incidence of breast cancer in postmenopausal women [12,13]. Laboratory evidence for modification of apoptosis by E_2 through the glucocorticoid-like action of MPA provides an important rationale to change the traditional HRT strategy [14]. Principles have emerged for understanding and applying physiological E_2 therapy appropriately by targeting the correct patient populations [36,37]. However, new findings reflect that the rapid plasticity of hormone resistance occurs as a response to selection pressure [14,22,26], indicating that there is still long way to translate such treatment into bedside practice.

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