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Review Derleme

THE RELATIONSHIP BETWEEN CANCER AND ENDOPLASMIC RETICULUM STRESS-INDUCED APOPTOSIS

Kanser ve Endoplazmik Retikulum Stres İndüklü Apoptoz Arasındaki İlişki

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ABSTRACT

The endoplasmic reticulum (ER) plays a central role in critical cellular processes such as protein synthesis, folding, and lipid biosynthesis. ER stress is triggered by the accumulation of misfolded proteins and activates the Unfolded Protein Response (UPR) pathways to ensure cell survival. The UPR attempts to restore ER homeostasis through sensors such as PKR-like ER Kinase (PERK), Inositol-Requiring Enzyme 1 (IRE1), and Activating Transcription Factor 6 (ATF6). However, prolonged or severe ER stress can lead to cell death by triggering apoptosis. In cancer cells, factors such as hypoxia, nutrient deprivation, and oxidative stress in the tumor microenvironment contribute to chronic ER stress. Under these conditions, activation of the UPR supports cancer cell survival, while excessive ER stress can induce apoptosis. The role of ER stress and the UPR in cancer progression is complex due to their dual effects in both promoting tumor growth and triggering apoptosis. For instance, the IRE1α-X-Box Binding Protein 1 (XBP1) and PERK-Eukaryotic Translation Initiation Factor 2α (eIF2α)- Activating Transcription Factor 4 (ATF4) pathways can enhance survival and chemoresistance in cancer cells, while pro-apoptotic signals such as C/EBP Homologous Protein (CHOP) and c-Jun N-Terminal Kinase (JNK) can trigger cell death. Understanding this balance offers new targets for cancer therapy. Proteasome inhibitors and other agents that increase ER stress have therapeutic potential by inducing apoptosis in cancer cells. This review examines the molecular mechanisms of ER stress, the UPR, and ER stress-induced apoptosis, discussing how these processes can be manipulated in cancer cells and how new treatment strategies can be developed. Targeting ER stress pathways may represent a promising approach, particularly for chemoresistant tumors.

Keywords: *Endoplasmic reticulum stress, unfolded protein response, apoptosis, cell survival, cancer*

ÖZ

Endoplazmik retikulum (ER); protein sentezi, katlanması ve lipid biyosentezi gibi kritik hücresel süreçlerde merkezi bir rol oynar. ER stres, yanlış katlanmış proteinlerin birikmesiyle tetiklenir ve hücrenin hayatta kalmasını sağlamak için *Unfolded Protein Response* (UPR) yolaklarını aktive eder. UPR; PRKR benzeri ER kinaz (PERK), inozitol gerektiren enzim 1 (IRE1) ve aktive edici transkripsiyon faktör 6 (ATF6) gibi sensörler aracılığıyla ER homeostazını restore etmeye çalışır. Ancak, uzun süreli veya şiddetli ER stres, apoptozu tetikleyerek hücre ölümüne yol açabilir. Kanser hücrelerinde, tümör mikroçevresindeki hipoksi, besin yetersizliği ve oksidatif stres gibi faktörler kronik ER strese neden olur. Bu koşullar altında, UPR'nin aktivasyonu kanser hücrelerinin hayatta kalmasını desteklerken, aşırı ER stres apoptozu indükleyebilir. ER stres ve UPR'nin kanser progresyonundaki rolü, hem tümör büyümesini destekleyici hem de apoptozu tetikleyici etkileri nedeniyle karmaşıktır. Örneğin, IRE1α- X-Box Bağlayıcı Protein 1 (XBP1) ve PERK- Ökaryotik Translasyon Başlatma Faktörü 2α (eIF2α)- Aktive edici transkripsiyon faktör 4 (ATF4) yolakları, kanser hücrelerinde hayatta kalma ve kemorezistansı artırabilirken, C/EBP homolog protein (CHOP) ve C-Jun N-terminal kinaz (JNK) gibi pro-apoptotik sinyaller hücre ölümünü tetikleyebilir. Bu dengenin anlaşılması, kanser tedavisi için yeni hedefler sunmaktadır. Proteazom inhibitörleri ve ER stresini artıran diğer ajanlar, kanser hücrelerinde apoptozu indükleyerek terapötik potansiyele sahiptir. Bu derleme, ER stres, UPR ve ER strese bağlı apoptozun moleküler mekanizmalarını inceleyerek, kanser hücrelerinde bu süreçlerin nasıl manipüle edilebileceğini ve yeni tedavi stratejilerinin nasıl geliştirilebileceğini tartışmaktadır. ER stres yolaklarının hedeflenmesi, özellikle kemorezistan tümörler için umut verici bir yaklaşım olabilir.

Anahtar Kelimeler: *Endoplazmik retikulum stresi, katlanmamış protein yanıtı, apoptoz, hücrenin hayatta kalması, kanser*

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INTRODUCTION

The endoplasmic reticulum (ER) is an organelle characterized by a membranous network structure, where the synthesis and folding of proteins destined for secretion outside the cell, as well as those integrated into the membrane, occur. It is also recognized as a critical quality control center for protein synthesis.[1] Additionally, the ER provides an optimal environment for the biosynthesis of lipids, steroids, and cholesterol.[2] In contrast to the cytosol, it contains a significantly higher concentration of Ca^{2+}.[3] All proteins intended for extracellular transport or incorporation into the membrane are delivered to the ER lumen by ribosomes situated on the cytosolic surface of the ER. Newly synthesized proteins arriving in the ER require posttranslational modifications—such as N-linked glycosylation, disulfide bond formation, hydroxylation, and lipidation—to achieve proper folding. To ensure the successful completion of each folding step, molecular chaperones, including Glucose-Regulated Protein 78 kDa (GRP78) and Glucose-Regulated Protein 94 kDa (GRP94), lectin-like proteins such as calnexin and calreticulin, and foldases, play essential roles in facilitating protein folding.[4] Protein folding is a very complex process cycle and the probability of error is quite high. Many extracellular factors such as inhibition of N-linked glycosylation, disruption of calcium homeostasis, hypoxia, oxidative stress, infections, ambient temperature, and nutrient deprivation affect the correct folding of proteins. These conditions, characterized as endoplasmic reticulum stress, diminish the protein folding capacity of the endoplasmic reticulum and result in the accumulation of unfolded proteins.[5]

Recent studies have highlighted the pivotal role of endoplasmic reticulum (ER) stress and the unfolded protein response (UPR) in the development and progression of cancer. In cancer cells, both intrinsic and extrinsic factors contribute to the activation of the UPR, which plays a critical role in oncogenic transformation, tumor survival, and disease progression. The growing recognition of the UPR's importance in tumorigenesis has generated significant interest in targeting this pathway for cancer therapy and exploring its therapeutic potential.[6] Research has demonstrated that intensifying pre-existing ER stress in cancer cells can overwhelm the ER stress response system, leading to the activation of pro-apoptotic components of the UPR. In this review, we will examine the mechanisms of ER stress, the UPR, and ER stress-induced apoptosis in the context of cancer cells.

Cancer is a group of diseases in which unregulated cell growth and proliferation occur continuously.[7] The mechanisms of formation and progression are not yet fully understood. Cancer cell proliferation is due to the ability to prevent programmed cell death, called apoptosis, and therefore the induction of apoptosis in cancer has been identified as a target for cancer therapy.[8] Various genetic and metabolic abnormalities present in tumors also generate detrimental microenvironments that induce sustained ER stress in tumor cells, ultimately impacting their functionality, destiny, and viability.[9] A prevalent trait of cancer cells is their capacity to locally disseminate or metastasize to other tissues in response to adverse environmental conditions, including hypoxia, glucose deprivation, growth factor insufficiency, lactic acidosis, oxidative stress, and amino acid deficiency.[10] Hypoxia in the tumor microenvironment is a common factor that disrupts ER homeostasis and causes ER stress.[11] Hypoxia restricts the function of the endoplasmic reticulum oxidoreductase 1 alpha (ERO1α) enzyme required for post-translational modification and prevents lipid desaturation.[12] At the same time, extreme hypoxia induces ER stress, while moderate hypoxia (1-5% O_2) has minimal effects on UPR activation.[13] Glucose deficiency interrupts the hexosamine biosynthetic pathway and also affects the production of ATP, which is necessary for protein folding.[14] Amino acid deficiency triggers eukaryotic translation ınitiation factor 2 alpha kinase 4, which leads to the phosphorylation of eukaryotic translation ınitiation factor 2α (eIF2α). This, in turn, activates the integrated stress response, which has been identified as a crucial adaptation mechanism in cancer cells.[15] Moreover, obesity is frequently linked to an increased cancer risk.[16] Saturated long-chain fatty acids like palmitate and stearate, which are abundant in high-fat diets, can alter the size, composition, and fluidity of the endoplasmic reticulum (ER) membrane. These changes might impact calcium reserves and disrupt protein glycosylation, ultimately resulting in ER stress.[17] Cancer cells generate lactic acid through aerobic glycolysis, which decreases the pH of the tumor microenvironment. This acidic environment can induce ER stress by disrupting intracellular calcium balance and/or leading to excessive production of reactive oxygen species (ROS). The overproduction of ROS can contribute to ER stress by affecting ER-resident calcium channels and by facilitating the creation of lipid peroxidation byproducts, which form stable adducts with ER-resident protein chaperones.[18,19] Alongside the challenging conditions in the tumor microenvironment, genetic alterations in cancer cells can also trigger ER stress.[20] UPR is activated in response to ER stress to reestablish ER homeostasis and assist the cell in adapting to stress and ensuring survival. Conversely, unresolved or chronic ER stress can result in cell death.[21] Normal cells typically do not

KÜ Tıp Fak Derg 2025;27(1):111-119
Doi: 10.24938/kutfd.1607213

undergo persistent ER stress. However, in cancer cells, the challenging conditions of the tumor microenvironment promote chronic ER stress and sustained activation of the UPR, which are crucial for tumor survival. While the aggravation of pre-existing ER stress may have minimal effects on normal cells, if homeostasis cannot be achieved in cancer cells by activation of the UPR, it can trigger apoptosis through both mitochondria-dependent and -independent pathways. If the programmed cell death, called apoptosis, cannot occur, cancer cell proliferation occurs.[22] Therefore, targeting apoptosis induction has emerged as a strategy for cancer therapy.[8] Apoptosis can be triggered through two primary pathways: the intrinsic and extrinsic pathways.[23] The intrinsic pathway involves mitochondria-mediated apoptosis, characterized by the release of cytochrome c and the activation of caspase-9, which in turn activates effector caspases such as caspase-3. The extrinsic pathway, on the other hand, is mediated by death receptors. It activates the Fas-associated death domain (FADD), leading to the formation of the death-inducing signaling complex, which encompasses downstream caspases like caspase-8, -7, -6, and -3.[24] Furthermore, studies have demonstrated that the buildup of unfolded proteins in the endoplasmic reticulum and the resulting cellular stress response also play a role in inducing apoptosis.[5]

Endoplasmic Reticulum Stress and the Unfolded Protein Response

In a cell, an increase in ER protein load is observed under conditions such as the result of differentiation processes or changes in physiological conditions. If the ER protein load increases beyond the ER folding capacity, ER stress arises from the accumulation of misfolded proteins in the ER.[4] In mammalian cells, three ER membrane-bound proteins-activating transcription factor 6 (ATF6), ınositol-requiring enzyme 1 (IRE1), and PRKR-like ER kinase (PERK)-act as sensors for ER stress.[25] Under normal proteostasis conditions, GRP78 binds to these sensors, keeping them in an inactive form.[21] When ER stress occurs, GRP78 exhibits increased affinity for misfolded or unfolded proteins and subsequently dissociates from ER stress sensors. This dissociation triggers the UPR, an adaptive mechanism designed to restore ER homeostasis through various processes, including the activation of ER stress sensors, transcriptional reprogramming, mRNA degradation, global attenuation of translation, the removal of misfolded proteins via the ER-associated protein degradation (ERAD) system, and the recycling of misfolded proteins and cellular materials through the induction of autophagy.[9] The UPR serves as a pro-survival response aimed at reducing the accumulation of unfolded proteins and reestablishing normal ER function.[26] However, if the stress persists and cannot be mitigated, the protective response may shift to a pro-apoptotic signal,[1] resulting in a three-stage response:

Stage 1: Initiation of ER Stress-Induced Apoptosis

PERK, ATF6, and IRE1 are key mediators in the initiation of UPR.

PERK

PERK is a type I transmembrane protein with a cytosolic domain and kinase activity that phosphorylates eIF2α and slows down translation.[3] Accumulation of unfolded proteins by upregulation of UPR genes leads to attenuation of protein translation via the PERK pathway.[27] With the onset of ER stress, GRP78, which is bound to PERK, is sent to the ER lumen to assist in folding, and then PERK activation begins with trans autophosphorylation and dimerization of the cytosolic domain of PERK. Activated PERK phosphorylates the serine at position 51 of eIF2α. Phosphorylated eIF2α inhibits general protein translation within the cell, reducing the load of nascent proteins in the ER and thus helping cell survival.[1,4] However, while general protein translation is attenationed, translation of specific mRNAs such as activating transcription factor 4 (ATF4) continues. Thus, by activating PERK, the protein load of the cell against ER stress is reduced and unfolded proteins are corrected.[1,4] ATF4, a member of the basic leucine zipper (bZIP) transcription factor family, supports cell survival by activating genes related to stress response, redox reactions, amino acid metabolism and protein secretion.[28] However, not all genes regulated by ATF4 contribute to cell survival. Specifically, the transcription factor C/EBP homologous protein (CHOP), whose expression is heavily reliant on ATF4, has been shown to trigger apoptosis.[29] Consequently, while the initial activation of PERK is protective and essential for cell survival under mild ER stress (Fig. 1), it also induces CHOP, which plays a crucial role in shifting from pro-survival to pro-apoptotic signaling. Upon entering the nucleus, ATF4 promotes the transcription of genes such as CHOP, growth arrest and DNA-damage-inducible 34 (GADD34), activating transcription factor 3 (ATF3), as well as genes involved in amino acid transport, glutathione biosynthesis, and resistance to oxidative stress.[4] The second target of PERK is to phosphorylate NFE2 Like BZIP transcription factor 2, a transcription factor that induces genes involved in antioxidants, detoxification of enzymes, immune signaling, and cell growth.[3]

ATF6

ATF6 is a cytosolic domain type II transmembrane protein containing ER luminal domain that binds to bZIP and GRP78. In the cell, it is normally found in an

KÜ Tıp Fak Derg 2025;27(1):111-119
Doi: 10.24938/kutfd.1607213

inactive form together with GRP78 in the ER membrane. When ER stress occurs in the cell, GRP78 is separated from ATF6 to facilitate folding into the lumen. After that, ATF6 is then transported to the Golgi, where it is cleaved by site 1 and site 2 proteases, respectively, and converted to the active form. Once activated, ATF6 is translocated to the nucleus and activates target genes. These target genes include GRP78, GRP94, protein disulfide isomerase (PDI), CHOP, X-box binding protein 1 (XBP1), and calreticulin.[1,4] XBP1 plays a crucial role in IRE1 signaling, thereby connecting ATF6 to pro-survival signaling through IRE1. While ATF6 is generally considered to transmit signals that promote cell survival and alleviate ER stress, its overexpression can also lead to the induction of CHOP mRNA expression (Fig. 1).

IRE1

IRE1 is a dual-function enzyme with both serine-threonine kinase activity in its cytoplasmic region and a C-terminal endoribonuclease domain.[30] In mammals, there are two isoforms: IRE1α and IRE1β. While IRE1α is expressed throughout various tissues, IRE1β is specifically found in intestinal epithelial cells.[1] Upon activation, IRE1's endonuclease activity triggers the rapid degradation of mRNAs encoding membrane and secreted proteins via regulated IRE1-dependent degradation.[30] A critical, more selective role of this endonuclease activity is the removal of a 26-nucleotide intron from the XBP1 mRNA transcript, a process induced by ATF6. The resultant frame-shift splice variant, XBP1s, encodes a stable and active transcription factor. XBP1s regulate a range of targets, including ER chaperones and the HSP40 family member p58IPK.[31] Conversely, IRE1's kinase activity can initiate pro-death signaling by interacting with TNF receptor associated factor 2 (TRAF2), which subsequently leads to the activation of C-Jun N-terminal kinase (JNK) (Fig. 1).[32]

Stage 2: Commitment Stage of ER Stress-Induced Apoptosis

The UPR pathway is activated to overcome the accumulation of unfolded proteins and the resulting ER stress. If the amount of unfolded protein is too high and the stress continues, activation of the UPR is insufficient to cope with ER stress, and in such a case, ER stress leads to apoptosis. Apoptosis is an important mechanism that induces cell death in ER stress; however, the mechanisms regulating apoptosis induced by ER stress have not yet been fully elucidated.[4] PERK, ATF6 and IRE1 signaling pathways not only initiate pathways that provide survival functions, but also induce apoptosis pathways,[33] nevertheless they do not directly lead to cell death. They act by initiating the activation of downstream molecules such as CHOP, JNK, BCL2 apoptosis regulator (Bcl-2) family members, and caspases.[1,33] The commitment stage of ER stress-induced apoptosis centers on the mechanisms by which CHOP and JNK transmit the pro-apoptotic signal to the execution phase (Figure 1).

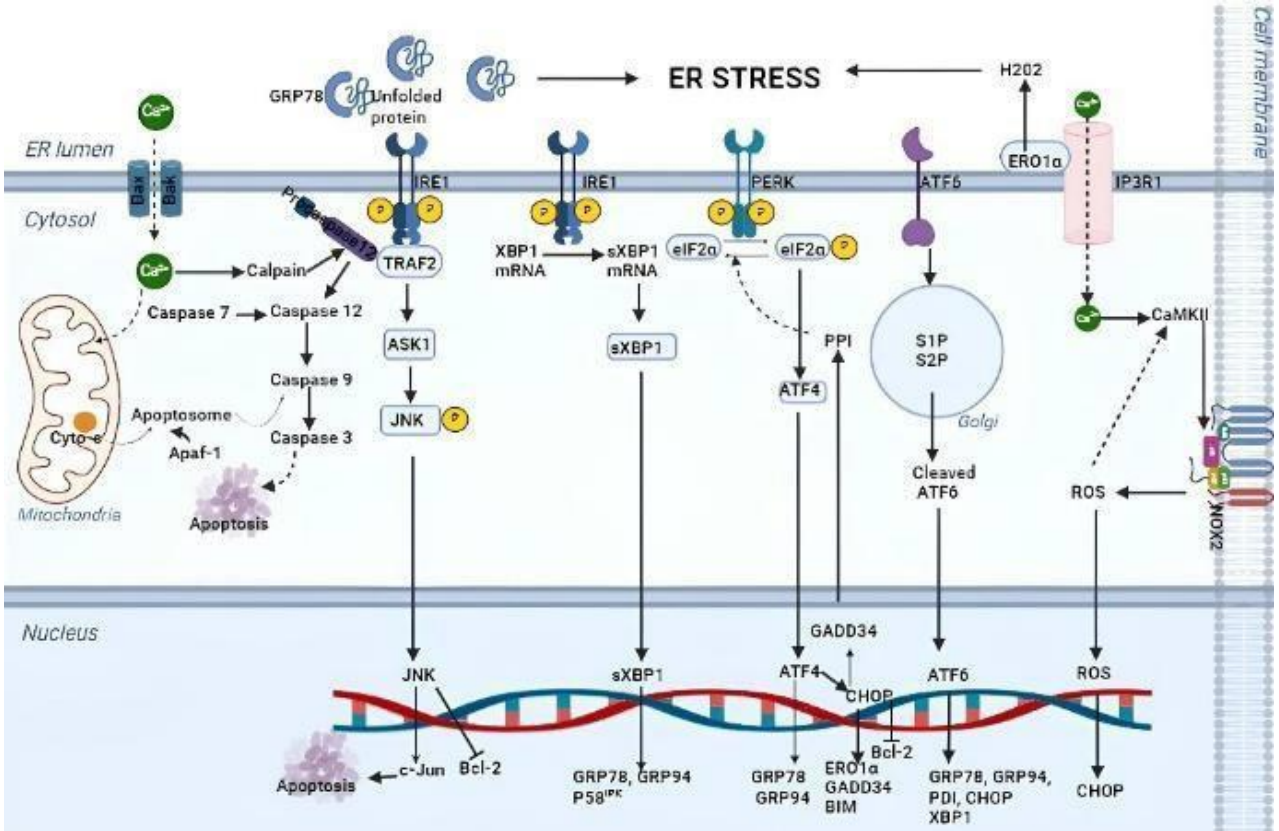

Figure 1: Molecular mechanism of ER stress-induced apoptosis

KÜ Tıp Fak Derg 2025;27(1):111-119
Doi: 10.24938/kutfd.1607213

CHOP

CHOP, a transcription factor, is a member of the C/ERB protein family. It has been shown that overexpression of CHOP leads to apoptosis. CHOP is expressed at very low levels in the resting state and its expression increases in ER stress.[33] While the transcription of CHOP is activated by PERK, ATF6 and IRE1 located in the ER membrane, the PERK-eIF2a-ATF4 pathway is required for CHOP. Activation of the PERK signaling pathway is a protective mechanism for cells by inhibiting protein synthesis in the early response to ER stress and regulates cell survival. However, at a later stage in the ER stress response, the PERK-eIF2a-ATF4 pathway is activated by ER stress and induces the expression of CHOP and regulates apoptosis.[33] For maximum induction of CHOP, all ER stress response pathways are required. In addition, expression of CHOP leads to the induction of proapoptotic proteins such as GADD34, ERO1α, death receptor 5, carbonic anhydrase VI and tribbles pseudokinase 3.[3]

CHOP plays a role in another pro-apoptotic mechanism by directly inducing the expression of GADD34, which facilitates the dephosphorylation of the α subunit of eIF2. This dephosphorylation allows for the resumption of protein translation in stressed cells. Both ATF4 and CHOP, downstream of PERK, activate GADD34, which then interacts with protein phosphatase 1 (PP1) to dephosphorylate eIF2α. This process leads to increased protein translation, escalating ER protein loads, and further exacerbating ER stress and promoting apoptotic cell death.[34] GADD34, a growth arrest and DNA damage gene expressed during ER stress, mitigates the inhibition of protein translation. It binds to the α-isoform of PP1 and facilitates PP1-mediated dephosphorylation of eIF2α, creating a feedback loop that alleviates the transcriptional repression caused by PERK-dependent phosphorylation of eIF2α.[35] Although the exact mechanism by which GADD34 promotes apoptosis is not fully understood, its effect on eIF2α is likely significant. Additionally, CHOP promotes the expression of ERO1α, a gene coding for an ER membrane-associated enzyme involved in disulfide bond formation in newly synthesized proteins. ERO1α requires molecular oxygen to oxidize protein disulfide isomerase (PDI), which catalyzes these bond formations. Under prolonged ER stress, ERO1α may generate hydrogen peroxide (H_2O_2), creating a hyperoxidizing environment that can lead to apoptosis.[36] Elevated ROS levels in the ER lumen can activate the type 1 inositol 1,4,5-trisphosphate receptor (IP3R1), causing calcium leakage from the ER into the cytoplasm. This calcium release activates calcium/calmodulin dependent protein kinase II gamma (CaMKII), which can trigger various apoptotic signaling pathways. One notable outcome of the CHOP-ERO1α-IP3R1-CaMKII pathway is the induction of membrane-bound NADPH oxidase 2 (NOX2), which generates ROS.[37] The ROS produced by NADPH oxidase may create a positive feedback loop by further activating CaMKII and increasing CHOP expression. This interplay of high ROS levels and elevated CHOP expression can synergistically promote apoptotic cell death (Fig. 1).[38]

Bcl-2 Family Proteins

Bcl-2 family proteins are key regulators of apoptotic cell death. These proteins are located in the mitochondrial membrane and play a crucial role in controlling apoptosis by regulating the activation of caspases. The Bcl-2 gene family includes 20 members, encompassing both death-promoting and survival-promoting genes. Their products are categorized into two main functional classes: anti-apoptotic and pro-apoptotic proteins. Most anti-apoptotic proteins possess all four Bcl-2 homology (BH) domains. In contrast, the pro-apoptotic members of the Bcl-2 family are further divided into two subclasses. While pro-apoptotic Bcl-2 proteins such as BCL2 associated -2 like 11 (Bim), BCL2 associated agonist of cell death (Bad), Bcl-2 modifying factor (Bmf), Bcl-2 interacting killer (Bik), Blk proto-oncogene, Src family tyrosine kinase (Blk), phorbol pro-apoptotic Bcl-2 proteins such as -12-myristate-13-acetate-ınduced protein 1 (Noxa), Bcl-2 binding component 3 (Puma) have only one protective domain (BH3).[39] The Bcl-2 family of proteins is closely associated with the regulation of mitochondrial outer membrane integrity. As in mitochondrial-mediated apoptosis, ER stress-induced apoptosis is regulated by the Bcl-2 protein family. Although the role of Bcl-2 proteins in ER stress-induced cell death is well established, the mechanisms by which ER stress regulates these proteins remain less understood.[1] Bcl-2 family members Bax and Bak are required for apoptosis induced by ER stress and regulate both the release of proapoptotic factors from mitochondria and calcium release from the ER.[4] Bax and Bak affect apoptosis in two ways: The first way; Bax and Bak undergo conformational changes and oligomerization in the ER membrane during ER stress. This process results in the release of Ca^{2+} from the ER into the cytoplasm. The elevated Ca^{2+} concentration in the cytosol activates calpain, which in turn cleaves and activates procaspase 12. Activated caspase 12 then cleaves and activates procaspase 9, thereby initiating the caspase cascade. Additionally, the second pathway involves the uptake of cytosolic Ca^{2+} by the mitochondria. This Ca^{2+} uptake results in the depolarization of the mitochondrial inner membrane

KÜ Tıp Fak Derg 2025;27(1):111-119
Doi: 10.24938/kutfd.1607213

and the subsequent release of cytochrome C, which in turn activates caspase 9 (Fig. 1).[22]

JNK

JNK, one of the proteins involved in signal transduction, is another apoptotic pathway in ER stress. The functional significance of JNK activation within the ER stress pathway has not yet been fully elucidated.[22] It is reported that the IRE1-TRAF2-Apoptosis Signal-Regulating Kinase 1 (ASK1) complex has a direct effect on JNK activation. IRE1 triggers apoptosis by activating the JNK signaling pathway, independent of its role in XBP1. The IRE1-TRAF2 interaction also activates ATF3, a transcriptional repressor that triggers apoptosis. It is also known that JNK regulates Bcl-2 proteins by phosphorylation.[3,4]

Stage 3: Execution Stage of ER Stress-Induced Apoptosis

Caspases

Apoptosis is a critical mechanism for maintaining tissue homeostasis by selectively eliminating damaged, mutated, or potentially harmful cells, such as precancerous cells.[40] The central molecular players in the apoptotic process are caspases, a family of cysteine proteases essential for regulating the apoptosis signaling pathway.[41] Caspases are initially synthesized as inactive forms called procaspases and are activated in response to various cellular stresses, including oxygen deprivation. These inactive proforms are converted into their active enzyme states upon the induction of apoptosis.[4] Caspases are categorized into two main classes based on their role in the apoptotic signaling cascade. The first class, known as initiator caspases, includes caspases 8, 9, and 12. These caspases are crucial for triggering the caspase cascade, which typically leads to swift apoptosis. The second class consists of effector caspases, with key members being caspases 3, 6, and 7.[42]

Activation of caspases is necessary for apoptosis and the functions of caspases 12, 3, 6, 7, 8 and 9 have been shown in studies on ER stress. Caspase 12, in particular, is important as a key mediator of ER stress-induced apoptosis and can be activated by ER stress.[43] It is known that ER stress-induced caspase activation occurs in three ways. The first pathway is activated by calpains, which are cytoplasmic proteases activated by calcium.[4] After activation of caspase 12 in the ER, caspase 12 leads to activation of procaspase 9, caspase 9 leads to activation of caspase 3, and ultimately apoptosis occurs. The second pathway can be autoactivated in direct association with IRE1 and the adaptor protein TRAF2. The third pathway involves the translocation of caspase 7 to the ER in response to certain apoptotic stimuli, where it can directly activate caspase 12 (Fig. 1).[33]

Communication Between ER and Mitochondria

Both the ER and mitochondria can directly initiate signaling pathways that activate the caspase cascade, resulting in apoptotic cell death. Members of the Bcl-2 protein family are located not only in the outer mitochondrial membrane but also in the ER membrane. Anti-apoptotic Bcl-2 proteins can inhibit the activation of pro-apoptotic Bcl-2 proteins such as Bax and Bak. During ER stress, pro-apoptotic proteins such as Bax and Bak oligomerize, forming pores in the mitochondrial outer membrane and leading to its permeabilization. This permeabilization is crucial for apoptosis, as it allows the release of cytochrome c into the cytoplasm. Cytochrome c binds to Apaf-1 and procaspase-9 to form the apoptosome, a large complex that activates caspase-9. Once activated, caspase-9 initiates the caspase cascade, leading to mitochondria-mediated apoptosis by activating caspase-3, the main effector caspase responsible for cell destruction.[44] Conversely, prolonged ER stress can lead to apoptosis via caspase-12. Caspase-12, which is associated with the cytoplasmic side of the ER membrane, is activated in response to chronic ER stress and calcium leakage from the ER lumen. This activation triggers a rapid cascade involving calcium, calpain, caspase-12, and caspase-3, resulting in ER stress-induced apoptotic cell death (Fig.1).

ER Stress And Cancer

Does ER stress trigger cancer cell survival or cell death by apoptosis? The effects of ER stress and UPR on tumor growth are controversial in many types of cancer.[45] It is reported in the literature that due to the rapid spread of malignant neoplasm, cancer cells are exposed to nutrient deprivation, poor vascularization and hypoxia; thus, GRP78, GRP94, PDI, ATF6, IRE1, XBP1, PERK and eIf2α genes are overexpressed in many types of cancer cells.[46] In addition, three branches of the UPR (IRE1α, PERK, and ATF6) have been shown to be highly activated in glioblastoma and a wide variety of human hematopoietic and solid tumors, including breast, stomach, colon, esophagus, lung, prostate, pancreas, and liver carcinomas.[47] GRP78 and GRP94, ER chaperones that support adaptation to ER stress, are also overexpressed in various cancer types.[48] It has been reported that the IRE1α -XBP1s pathway induces c-MYC signaling, thus enabling prostate cancer growth,[49] supports tumor progression in triple-negative breast cancer,[50] and in a study conducted with a human glioma cell line, genetic deletion of IRE1α inhibits angiogenesis and then reduces tumor growth when the IRE1 gene is injected into mice.[51] Additionally, PERK activation is reported to promote tumor growth in mouse breast cancer models[52] and increase chemoresistance in HT29 colon cancer cell lines.[53] It is suggested that ATF6 prevents

KÜ Tıp Fak Derg 2025;27(1):111-119
Doi: 10.24938/kutfd.1607213

DNA damage and cell death in colon cancer cells undergoing ER stress[54] and increased ATF6 gene expression may be a marker in colorectal carcinoma.[55] There are also studies showing that ER stress triggers cell death as well as proliferative effects in cancer cells. Proteasome inhibitors used as anticancer agents induce unresolved lethal ER stress[31] and induce cell death in cancer cells that are highly sensitive to ER stress.[25] PS-341, a di-peptidyl boronic acid derivative, has been shown to induce cell death in a number of cancer cell lines by binding to proteasome 26S,[56] while surprisingly, PS-341 was also found to induce topoisomerase-1 inhibitor-mediated apoptosis.[57] Another study reported that PS-341 could be a potential agent of ER stress-induced apoptosis through the induction of Bik and Bim.[58] It has been reported that tolfenamic acid application to colorectal cancer cells causes apoptosis via PERK-eIF2α-ATF4 pathway and induces suppression of cyclin D1 translation.[38] In another study conducted with colon cancer cells, it was shown that esculetin triggered ER stress-induced apoptosis by causing CHOP induction.[59] It has been shown that andrographolide administration causes cell death by increasing the expression of IRE1a, XBP1s and BAX genes in cancer cells.[60]

CONCLUSION

The ER plays a pivotal role in maintaining cellular homeostasis by regulating protein synthesis, folding, and lipid biosynthesis. However, under conditions of stress, such as hypoxia, nutrient deprivation, or oxidative stress, the ER's capacity to manage protein folding is overwhelmed, leading to the accumulation of misfolded proteins and the activation of the UPR. The UPR, mediated by sensors such as PERK, IRE1, and ATF6, initially acts as a pro-survival mechanism by attenuating protein synthesis, enhancing protein folding capacity, and promoting the degradation of misfolded proteins. However, prolonged or severe ER stress can shift the UPR from a protective response to a pro-apoptotic signal, ultimately leading to cell death. In cancer, the role of ER stress and the UPR is complex and context-dependent. Cancer cells often exploit the UPR to survive the harsh conditions of the tumor microenvironment, such as hypoxia and nutrient deprivation, which induce chronic ER stress. The activation of UPR pathways, including IRE1α-XBP1, PERK-eIF2α-ATF4, and ATF6, supports tumor growth, angiogenesis, and chemoresistance in various cancers. However, excessive ER stress can also trigger apoptosis through the activation of pro-apoptotic factors such as CHOP, JNK, and caspases. This dual role of ER stress in promoting both survival and death presents a unique therapeutic opportunity. Targeting ER stress pathways, either by exacerbating stress to induce apoptosis or by inhibiting pro-survival UPR signaling, holds promise for cancer therapy. Proteasome inhibitors, for example, have shown the potential to induce lethal ER stress in cancer cells, leading to apoptosis. Understanding the molecular mechanisms underlying ER stress-induced apoptosis and the UPR's role in cancer progression is crucial for developing novel therapeutic strategies. By manipulating these pathways, it may be possible to selectively target cancer cells while sparing normal cells, offering a promising approach for treating chemoresistant tumors and improving patient outcomes. Further research into the intricate balance between pro-survival and pro-apoptotic signaling in ER stress will be essential for translating these findings into effective cancer therapies.

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