Oncology and Translational Medicine
DOI 10.1007/s10330-016-0201-1
2017, Vol. 3, No. 3, P131–P135

REVIEW ARTICLE

Roles of endoplasmic reticulum stress and apoptosis signaling pathways in gynecologic tumor cells: A systematic review

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Abstract

Efficient functioning of the endoplasmic reticulum (ER) is very important for most cellular activities, such as protein folding and modification. The ER closely interacts with other organelles, including the Golgi body, endosome, membrane, and mitochondria, providing lipids and proteins for the repair of these organelles. ER stress can be induced by various abnormal materials in the cell. ER stress is a compensatory intracellular environment disorder that occurs during areaction. ER can sense the stress and respond to it through translational attenuation, upregulation of the genes for ER chaperones and related proteins, and degradation of unfolded proteins by a quality-control system, but excessive ER activation can cause cell death. The Pubmed and Web of Science databases were searched for full-text articles, and the terms "endoplasmic reticulum stress / unfolded protein response / gynecologic tumor cell apoptosis" were used as key words. Thirty-five studies of ER stress and unfolded protein response published from 2000 to 2016 were analyzed. Stress triggers apoptosis through a variety of signaling pathways. Increasing evidence has shown that the ER plays an important role in tumor cell diseases. The present review discusses the molecular mechanisms underlying unfolded protein response and its ability to promote survival and proliferation in gynecologic tumor cells.

Key words: endoplasmic reticulum (ER); unfolded protein response (UPR); inositol-requiring-JNK (IRE1-JNK); caspase; CCAAT-enhancer-binding protein homologous protein (CHOP); gynecologic tumor cell

List of abbreviations: IRE1α: inositol-requiring enzyme 1α; UPR: unfolded protein response; XBP-1: X binding protein; PERK: protein kinase RNA-like ER kinase; GRP78: glucose-regulated protein 78; Tun: tunicamycin; CHOP: CCAAT-enhancer-binding protein homologous protein; ERSE: endoplasmic reticulum stress element; ASK1: apoptosis signal-regulating kinase 1; eIF-2alpha: eukaryotic translation initiation factor 2; IP3: inositol 1,4,5-trisphosphat; ERO1: endoplasmic reticulum oxidoreductin 1; TMEM214: transmembrane protein 214; GADD34: cofactor of eIF2.phosphatase; H1299: human hung carcinoma H1299 cells; SH-SY5Y: human neuroblastoma cells; JNK: c-Jun N-terminal kinase; VEGFA: vascular endothelial growth factor-A; SERCA: sarcoplasmic reticulum Ca^{2+}-ATPase; NAFLD: nonalcoholic fatty liver disease; ATF4: activating transcription factor 4; Pim-1: proviral integration moloney virus; PUMA: p53-upregulated modulator of apoptosis

Received: 23 October 2016
Revised: 19 December 2016
Accepted: 10 January 2017

The endoplasmic reticulum (ER) is the largest membrane-enclosed organelle in cells and is responsible for the synthesis, processing, and modification of proteins [1]. Conditions that interfere with ER function, such as virus infection and calcium homeostasis, lead to the accumulation and aggregation of unfold proteins, causing severe ER stress. The subsequent unfolded protein response (UPR) is a cellular attempt to lower the burden on the ER and restore ER homeostasis by imposing a general arrest in protein synthesis, upregulating chaperone proteins,

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and degrading misfolded proteins. An increasing number of studies has shown that persistent ER stress may largely result from an altered state of the UPR [2]. ER stress response / UPR signaling pathways are activated in primary solid tumors as a result of cell-intrinsic defects, such as dysregulation of protein synthesis, folding, and secretion, and also as a consequence of microenvironment changes. This article mainly describes the research progress of the gynecologic tumor cell apoptosis pathway and ER stress, which has provided important insights into the molecular mechanisms of ER stress-induced apoptosis.

ER stress signaling pathways

IRE1 signaling pathways

IRE1, which is activated in response to the accumulation of unfolded proteins, determines the splicing of a 26-nucleotide-long intron of mRNA encoding the transcription factor X box binding protein 1 (XBP1). The generated splicing variant, XBP1, acts as a transcription factor that moves to the nucleus and causes the transcription of genes coding ER chaperones in order to mitigate the stress [3-4]. Both IRE1 molecules respond to the accumulation of unfolded proteins in the ER, which activate their kinase and thus their RNase activities [5-6]. IRE1 and PERK are two type I transmembrane ER-localized protein kinase receptors that signal the UPR through a process involving homodimerization and autophosphorylation [7].

The IRE1-JNK pathway is an important signaling pathway that helps cells survive via the UPR. First, the endoribonuclease activity of IRE1 cleaves XBP-1 mRNA, converting it into a potent transcriptional activator that, in turn, induces the gene expression of proteins involved in protein degradation [8–9]. Second, recent studies have shown that IRE1 links ER stress to the activation of JNK signaling pathways. Specifically, IRE1 binds to TRAF2 and through its kinase activity couples ER stress to the activation of JNK [10]. The activation of JNK by ER stress requires the presence of ASK1 [11]. IRE1 recruitment of protein kinase adapter TRAF2 (tumor) can be caused by permanent ER stress, which activates ASK and JNK protein kinase and initiates the apoptotic cascade [12]. The IRE1/XBP-1 pathway is required for efficient protein folding, maturation, and degradation in the ER and suggests the existence of subsets of UPR target genes, as defined by their dependence on XBP-1. ASK1 oligomerization promotes conformational changes induced by an IRE1-TRAF2 interaction, which also promotes Thr845 intermolecular phosphorylation in the ASK1 activation cycle (IRE-TRAF2-ASK1).

PERK signaling pathway

Oligomerization and ER stress increase PERK protein-kinase activity; PERK phosphorylates eIF2 on serine residue 51, inhibiting the translation of messenger RNA into protein. On one hand, protein translation and synthesis can be inhibited by phosphorylation of eIF2a to relieve ER pressure. On the other hand, phosphorylation of eIF2a can selectively activate the translation of ATF4, increase the synthesis of its binding partner, and affect amino acid metabolism. Inhibiting eIF2-GDP in the eIF2-GTP energy cycle can inhibit translation and reduce protein synthesis [13]. A previous study showed that activation and phosphorylation of PERK selectively enhance its affinity for the non-phosphorylated eIF2 complex. This switch is correlated with a significant change in the protease sensitivity pattern, indicating a major conformational change in the PERK kinase domain upon activation. Although it is dispensable for catalytic activity, PERK's kinase insert loop is required for substrate binding and eIF2alpha phosphorylation *in vivo*. A previous study revealed a novel mechanism for eIF2 recruitment by activated PERK and for unidirectional substrate flow in the phosphorylation reaction [14].

Previous studies showed that CHOP directly activates GADD34, which promotes ER client protein biosynthesis. Thus, impaired GADD34 expression reduces client protein load. Furthermore, mice lacking GADD34-directed eIF2alpha dephosphorylation (such as $CHOP^{-/-}$ mice) are resistant to renal toxicity of the ER stress-inducing drug tunicamycin. CHOP (promoter regions have four cis-response elements) can induce the expression of apoptosis proteins such as GADD34, ERO1, and DR5. ER transmembrane receptors detect the onset of ER stress and initiate the UPR to restore normal ER function. Recent studies have shown that prolonged stress or failures in the adaptive response are likely mediated by increases in target proteins, such as oxidase ERO1a (ER lumen protein), which transfer selectrons to molecular oxygen during disulfide bond formation [15]. Therefore, excessive expression of CHOP can lead to the generation of reactive oxygen species (ROS), ER stress, and apoptosis. In general, CHOP-GADD34-ERO1 partially mediates the ER stress signaling pathways that regulate cell apoptosis [16].

Caspase signaling pathway

It is generally thought that caspase-12, an ER outer membrane protein, is a specific apoptosis molecule involved in ER stress. Murine caspase-12 has an ER-associated proximal effect or activates procaspase-9 to cleave procaspase-3, leading directly to apoptosis [17]. However, human caspase-12 has no similar function because its gene is disrupted by a frame shift, resulting in a premature stop codon. In addition, human caspase-12

also contains amino acid substitutions in sites critical for caspase activity [18]. In contrast, human caspase-4 is specifically cleaved under ER stress, suggesting that it may be a functional ortholog of mouse caspase-12 in ER stress-induced apoptosis [19].

Recent studies have shown that caspase-4 participates in reactions in the human neuronal cell line SH-SY5Y [20]. Additionally, caspase-4 plays an important role as an innate immune effect or for discriminating between pathogenic and nonpathogenic bacteria [21]. Yamamuro showed that caspase-4 directly activates caspase-9 [22]. Reddy found that in contrast to the UPR, GRP78 overexpression does not result in G1 arrest or depletion of topoisomerase [23].

Some previous studies showed that a subpopulation of GRP78 can exist as an ER transmembrane protein as well as co-localize with caspase-7. A GRP78 mutant with no ATP binding domain failed to bind procaspase-7 and lost its protective effect against etoposide-induced apoptosis [23]. Li identified a critical mediator of ER stress-induced apoptosis, human transmembrane protein 214 (TMEM214) [24]. Over expression of TMEM214 induced apoptosis, whereas knockdown of TMEM214 inhibited ER stress-induced apoptosis, suggesting that TMEM214 is essential for ER stress-induced apoptosis by acting as an anchor for recruitment of procaspase-4 to the ER and its subsequent activation [24].

ER stress in gynecologic tumor cells

GRP78, a dominant regulator of the ER stress response, is increased in a variety of cancer types [25–26]. GRP78 was originally identified as a protein whose expression levels are regulated by the amount of available glucose, where hypoglycemia or aglycemia represent a strong stimulus for increased GRP78 expression [27]. Hypoglycemia, often combined with hypoxia and acidosis, represents microenvironmental conditions that are frequently present in tumor tissues and are closely aligned with increased levels of GRP78. Elevated GRP78 is among the critical pro-survival mechanisms of tumor cells to withstand and thrive under such detrimental microenvironmental conditions [28]. Over expression of GRP78 is commonly detected in malignant breast cancer tissue and is correlated with poor prognosis for these patients. Expression of GRP78 and XBP-1 was observed in 76% and 90% of breast cancers [29]. In fact, over expression of GRP78 in breast tumors predicts resistance to doxorubic in treatment in these patients [30]. Interestingly, increased expression of GRP78, ameans for tolerating low-level chronic ER stress and thriving under sub-optimal microenvironmental conditions, provides for generally increased pro-survival robustness that extends to chemotherapy, a stress condition that could not have been anticipated by evolution. It would be highly desirable to block GRP78 expression in a tumor-specific manner to reduce the ability of tumor cells to survive and proliferate in the absence of optimal nutrient supply and to increase the efficacy of cancer therapy [31].

Angiogenesis is crucial to many physiological and pathological processes, including development and cancer cell survival. PERK can also facilitate tumor growth by upregulating vascular endothelial growth factor (VEGF) and thereby inducing angiogenesis in tumors [32]. Ghosh showed that IRE1a, PERK, and ATF6a powerfully regulate VEGFA mRNA expression under various stress conditions [33]. In Ire1a2/2 and Perk2/2 mouse embryonic fibroblasts and ATF6a-knockdown HepG2 cells, induction of VEGFA mRNA by ER stress is attenuated compared to that in control cells. Embryonic lethality of Ire1a2/2 mice occurred because of a lack of VEGFA induction in labyrinthine trophoblast cells of the developing placenta. Rescue of IRE1a and PERK in Ire1a2/2 and Perk2/2 cells, respectively, prevents VEGFA mRNA attenuation. Another study showed that induction of VEGFA by IRE1a, PERK, and ATF6 involves activation of the transcription factors, spliced-XBP-1, ATF4, and cleaved-ATF6, respectively [33].

Lei revealed possible mechanisms underlying the association of ANKRD1 with cisplatin response [34]. Cisplatin-induced apoptosis in ovarian cancer cell lines was found to be associated with ER stress, as evidenced by the induction of glucose-regulated protein 78 (GRP78), growth arrest- and DNA damage-inducible gene 153 (GADD153), and increased intracellular Ca^{2+} release. The level of sensitivity to cisplatin-induced apoptosis was associated with ANKRD1 protein levels and poly (ADP-ribose) polymerase cleavage. COLO 316 ovarian cancer cells, which express high ANKRD1 levels, were relatively resistant to cisplatin, and ER stress-induced apoptosis, whereas OAW42 and PEO14 cells, which express lower ANKRD1 levels, are more sensitive to ER stress-induced apoptosis [34].

Zhou investigated anti-cancer activity in human breast cancer cell lines and explored the underlying mechanism of this action [35]. The results showed that treatment with AMP dose-dependently inhibited cell viability and induced apoptosis in MCF-7 and MDA-MB-231 breast cancer cells without cytotoxicity in human normal breast epithelial cells MCF-10A. Additionally, AMP dose-dependently triggered ROS generation in both breast cancer cells. The ROS scavenger N-acetyl-L-cysteine strongly attenuated AMP-induced ROS production, along with cell growth inhibition and apoptosis. Furthermore, AMP was observed to activate ER stress, as evidenced by the up-regulation of ER stress-related proteins, including GRP78, p-PERK, p-eIF2, cleaved-ATF6, and CHOP,

while knockdown of ATF6 or PERK markedly down-regulated AMP-induced CHOP expression. Blocking of ER stress using 4-phenylbutyric acid not only down-regulated AMP-induced GRP78 and CHOP expression, but also significantly decreased AMP-induced cell growth inhibition and apoptosis, whereas the ER stress inducer thapsigargin had opposite effects. Additionally, N-acetyl-L-cysteine inhibited AMP-induced ER stress by down-regulating GRP78 and CHOP expression. In contrast, blocking of ER stress using CHOP siRNA decreased AMP-induced ROS production and cell apoptosis [35].

Conclusion

Tumor cells are often exposed to intrinsic and external factors that alter protein homeostasis, resulting in ER stress. To cope with this, cells evoke an adaptive mechanism to restore ER proteostasis known as the UPR. Previous studies demonstrated that ER stress and UPR signaling are involved in gynecologic tumor diseases (ovarian and breast cancer). However, in tumor diseases, the roles of ER stress and UPR in cancer remains unclear, and different components are known to be involved and may be promising targets for future anticancer therapy through the activation of thepro-apoptotic GRP-78/IRE-1/XBP-1/CHOP signaling pathway.

Acknowledgment

We thank Dr. Yajun Chen of the Department of Clinical Laboratory, Nanjing Obstetrics and Gynecology Hospital Affiliated to Nanjing Medical University, China, who helped us in revising the paper in English.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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DOI 10.1007/s10330-016-0201-1
Cite this article as: Liu KS, Fang WM, Sun EH, *et al*. Roles of endoplasmic reticulum stress and apoptosis signaling pathways in gynecologic tumor cells: A systematic review. Oncol Transl Med, 2017, 3: 131–135.