ROLE OF ENDOPLASMIC RETICULUM STRESS AND UNFOLDED PROTEIN RESPONSES IN HEALTH AND DISEASE

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ABSTRACT

Unfolded proteins and other conditions affecting endoplasmic reticulum (ER) homeostasis cause ER stress. The cell reacts to ER stress by activation of the unfolded protein response (UPR), which induces profound changes in cellular metabolism including general translation attenuation, transcriptional upregulation of molecular chaperone genes and activation of ER-associated degradation. Moreover, ER stress is involved in causing many diseases inducing apoptosis like osteoporosis, diabetes, inflammatory responses, cancer, neurodegenerative diseases (Alzheimer's disease & Parkinson's disease) etc.

KEYWORDS: Alzheimer's disease & Parkinson's disease.

INTRODUCTION

Recent developments have highlighted the new insights toward the endoplasmic reticulum (ER) stress regulated pathways, and how they have serious impact on various diseases, which have attracted immense attention toward this organelle coordinating several functions essential for cell survival.\textsuperscript{[1]} It is a site for protein synthesis, protein folding, synthesis of
lipids and sterols, maintenance of calcium homeostasis and post translational modifications of proteins.\(^2\) ER shows high sensitivity toward alteration in calcium homeostasis and perturbations in its environment. Thus, Ca\(^{++}\) ionophores that reduces calcium levels from the ER lumen, inhibit glycosylation, chemical toxicants, oxidative stress and/or accumulation of misfolded proteins in the ER can all disrupt ER function, resulting in the development of ER stress.\(^3\) On the other hand, ER stress can be represented as perturbation arising from the failure in execution of functions assigned to ER or hindered working capacity of this organelle. To escape such adverse conditions, stress sensor pathways are activated by ER, which is known as unfolded protein response (UPR) via complex signaling network of Protein kinase R-like endoplasmic reticulum kinase-eukaryotic translation-initiation factor 2α (PERK-eIF2α), inositol-requiring enzyme 1α X-box-binding protein 1 (IRE1-XBP1), activating transcription factor 6-CREBH (ATF6-CREBH) transducers. This network of signaling activates the changes in the expression of hundreds of genes for cellular homeostasis restoring. For example, global protein synthesis arrest, via halting of general translation, promote up the ER chaperons expression that causes enhancement in protein folding for quality control maintenance of proteins while the misfolded proteins get degraded through the ER-associated degradation (ERAD) and autography. 'But, if ER stress prolonged, then UPR activates apoptotic signaling which may show progression through mitochondrial dependent or independent pathways'.\(^4\)

![Fig.1 Diagram showing effect of ER stress in cell death](image-url)
Three stress sensors are responsible for sensation of stress to ER, that is Protein kinase R-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1α (IREI) and activating transcription factor 6 (ATF6). These sensors induce UPR after the recognition of ER stress/misfolding of protein. During normalcy, ER-luminal domains of UPR transducers like IREI, PERK and ATF6 are associated with the glucose-regulated protein 78 (Bip/Grp 78), an HSP 70 family protein. Bip/Grp 78 maintains the homeostasis of these signaling transducers. When ER is subjected to stress, Bip/Grp78 is sequestered by unfolded proteins in ER, resulting in its dissociation from the UPR transducers and hence activation of these molecules.[5]

**ER Stress and Apoptotic Signaling**

Apoptosis, or programmed cell death, is essential for normal development and maintenance of tissue homeostasis, on the other hand, it is also a process by which physiological death of normal cells occur under stress or adverse conditions. Apoptosis is implicated in several human diseases like diabetes, hepatic disorders, neurodegenerative disorders like Alzheimer’s and Parkinson's disease etc. If ER stress gets prolonged, results into the apoptotic signaling activation through UPR.[6] During ER stress, calcium (Ca^{2+}) effluxes from the ER, increases cytosolic Ca^{2+} levels and disturb the mitochondrial membrane potential, which causes the release of cytochrome c and forms apoptosome complex with Apaf1 and caspase 9. Furthermore, this complex activates the executioner caspase like caspase 3 and caspase 7 which leads to apoptosis. ER membrane associated caspase 12 is also implicated in the activation of apoptosis through direct activation of caspase 3 or by caspase 9. CHOP/GADD 153, sensor of endoplasmic reticulum stress, is highly up regulated during endoplasmic stress.[5]

**ER Stress and Oxidative Stress**

Reactive free radicals, including both reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced in all cellular compartments and eventually results in protein damage.[7] ER provides an exclusive oxidizing environment to the protein to facilitate disulfide bond formation and the ROS produced as a result of this practice in ER alone contributes to 25% of ROS generated by the cell. Two enzymatic components protein disulfide isomerase (PDI) and endoplasmic reticulum oxidoreductase (ERO-1) are often implicated in promoting oxidative stress in ER compartment of the cell. Disulfide bond formation between thiol moieties is catalyzed by PDI through thiol-disulfide oxidation,
reduction and isomerization. PDI which is itself produced in the process is oxidized by ERO-1 via transfer of protein from reduced PDI to molecular oxygen that results in oxidative stress.\textsuperscript{[8]} This may imply that oxidation of multiple disulfide bonds would generate high levels of cellular ROS. Further, any invalid disulfide bonds so generated in the process are subsequently reduced by glutathione (GSH). This further weakens the reduced glutathione pool altering the redox environment within the ER.

There are evidences suggesting that ROS may be generated when accumulation of unfolded proteins in the ER elicit Ca\textsuperscript{2+} leakage into the cytosol through inositol triphosphatase receptor (IP3R). The perturbed calcium levels in cytoplasm evoke influx of Ca\textsuperscript{2+} in the nuclei and mitochondria,\textsuperscript{[5]} resulting in the generation of ROS. Since protein folding and refolding in the ER lumen are highly energy dependent processes. ATP depletion consequential to protein misfolding may cause mitochondrial oxidative phosphorylation stimulation to increase the production of ATP and ROS. In addition, ER transmembrane protein NADPH oxidase complex and Nox4 may also be involved in superoxide anion and hydrogen peroxide production.\textsuperscript{[9]}

**ER Stress and Inflammatory Responses**

The very first response of the immune system to infection or tissue injury is known as inflammation. That helps in protection of the human body from various diseases.\textsuperscript{[10]} ER stress and inflammatory responses are concerned in the pathogenesis of various diseases such as, respiratory, cardiovascular, diabetes, neurodegenerative, cancer and other metabolic diseases. Inflammatory reactions are induced by unfolded protein response, via three arms of UPR, that are PERK, IRE1 and ATF6, which in turn induces the signal transducer nuclear factor NF-κB activation.\textsuperscript{[11]} NF-κB generally be located in the cytoplasm, present in inactive form by its association with IkB protein, that prevents its nuclear translocation and activation. The activation of NF-κB is caused by acute and chronic stress based upon proteosome degradation of IkB. Several studies have shown that IkB is degraded by IRE1α and thus causes nuclear translocation and activation of (NF-κB), whereas PERK activates NF-κB through translational suppression of IkB. Furthermore, AP1 is activated through IRE1, AP1 is a transcription factor which may induce the expression of tumor necrosis factor (TNF), granulocyte macrophage colony-stimulating factor (GMCSF), interleukin (IL)-8, keratinocyte growth factor (KGF) and some of the cytokine receptors.\textsuperscript{[12]} Moreover, UPR through ATF6 has been concerned in the activation of acute phase response (APR), generated
instantaneously after infections, inflammations, tissue damage etc. Concentration of acute phase protein increases in serum after the onset of mentioned conditions, eventually resulting in fever, pathological and neurological changes.\[13\]

**ER Stress and Diabetes Mellitus**

Diabetes mainly comprises of two forms,\[14\] the leading cause of morbidity and mortality, which decreases both life expectancy and life quality of affected people. β-cell mass reduction, due to increased β-cell apoptosis and defective β-cell regeneration, is the major cause of diabetes mellitus.\[15\] The main characteristic feature of type I diabetes is a severe lack of insulin production due to the destruction of pancreatic β-cells. β-cell loss is a result of an autoimmune-mediated process, in which a chronic inflammation known as insulitis results in destruction of β-cells. The process is mediated by cytokines and other factors released by or expressed on the surface of immune cells invading the islets, that causes secondary pathways activation inducing cell death in target β-cells. Type 2 diabetes is a result of reduced capacity of the β-cells to secrete adequate amount of insulin to stimulate utilization of glucose by peripheral tissues.\[16\] As secretory capacity β-cell worsens, glucose tolerance deteriorate and fasting glucose levels gradually increases culminating in overt hyperglycemia.\[17\] Accumulating evidence suggest that loss of β-cell in type 1 and type 2 diabetes is caused via stress responses regulated by key transcription factors and gene networks. There are diverging up-stream proapoptotic signals in every form of diabetes mentioned above, depending upon the NF-κB and activation of transcription (STAT)-1 in type 1 diabetes and other signaling molecules, in type 2 diabetes. These early signaling pathways may converge downstream into common execution pathways, such as ER stress, mitochondrial dysfunction and production of reactive oxygen species (ROS).\[18\] ER stress may also serve as a connecting link between obesity and insulin resistance in liver and fat, rising the intriguing possibility that this cellular response being a familiar mechanism for both β-cell malfunction and defected signaling of insulin in type 2 diabetes.\[5\]

**ER Stress and Osteoporosis**

Osteoporosis, characterized by reduced bone strength and is a major health problem in aging population. Low bone mineral density (BMD), a hallmark to osteoporosis, has been related to ER stress. Signaling by PERK-eIF2α is necessary for an ordinary development of the postnatal growth, feasibility and function of pancreas as well as skeletal system.\[19\] Jie Liu et al. have studied that low BMD haplotype is distinct, due to associated single nucleotide
polymorphism, which is exhibited by increased phosphorylation of eIf2α during ER stress, as compared to alternate haplotype.\[^{[20]}\] A balance between osteoblasts (the mesenchymal stem cells derived bone forming cells) and osteoclasts (the bone resorption cells derived form hematopoietic stem cells) is very important for the normal functioning and development of bone.\[^{[21]}\] He et al. reported that blocking of dephosphorylation of eIf2α by Salubrinal causes an increased osteoblast differentiation. They also hypothesized that regulation of ER stress via ATF4 and eIf2α might be a fine system for antiosteoporosis.\[^{[22]}\] Diabetic patients are more susceptible to fractures mainly in the regions of upper extremities and hip in comparison to the non-diabetics subjects. Hyperglycemia and Insulinopenia causes low BMD, resulting in impairment of bone formation.\[^{[23]}\] In addition, it has been reported that diabetes itself induces expression of ER stress specific CHOP in osteoblast cells causing the progression of apoptosis. Consequently, a balance is distributed between the osteoblast and osteoclast cells which results in bone disorders and development of diabetic osteoporosis.\[^{[24]}\] According to some reports, the ER specific molecular chaperones like BiP (immunoglobulin heavy-chain binding protein) and PDI (protein-disulfide isomerase) obtained from osteoporosis patients get down-regulated in osteoblasts.\[^{[25]}\] These observations put forward the significance of ER stress to skeletal development osteoporosis and also for formulating the therapeutic approaches for skeletal diseases.

**ER Stress and Cancer**

It has been reported that high protein folding capacity of ER chaperon’s is required for cancerous cells so as to their increased growth rate and proliferation. Several stressful conditions such as, nutrient deprivation, hypoxia, pH changes or poor vascularization can limit up the growth of malignant cells, thereby activating the UPR. Both nutrient starvation in tumor cells as well as excess of nutrient under normal conditions causes ER stress.\[^{[26]}\] It is also been observed that the hypoxic conditions causes redox imbalance in cancerous cells leading to UPR activation. Grp78 known as ER chaperone protein, one of the most active components of cancerous cells gets over expressed in different cancer types, such as in lung, breast, prostate and colon cancers.\[^{[27]}\] Moreover, it has been studied that, those cells which do not express Grp78 are not capable of forming tumor. Jamora C et al. has been interpret it as a chaperone protein which makes the cancerous cells more adaptive against any hypoxic condition and as a resistance protein against anti-cancer therapy.\[^{[28]}\] Cell apoptosis, proliferation, invasion, immunity and inflammation, are regulated through glucose-regulated Grp78 induction, mainly in cancer systems.\[^{[29]}\] In essence, Grp78 increases the ER capability
for protein folding thereby reducing the cell apoptosis in cancer cells. PERK/eIF2α are crucial for regulation of tumor initiation and survival, thus facilitate adaptation in unusual situations like hypoxia and oxidative stress.\[^{30}\] In hypoxic condition, HIF1α (a transcription regulator) is stabilized and completely activates the whole branch of UPR, i.e., PERK, resulting in phosphorylation of elf2α, ATF4 and GADD34. The phosphorylation of elf2α inhibits general protein synthesis, but ATF4, a transcription factor, is related to cancer cell proliferation and survival against nutrient deprivation through amino acid synthesis.\[^{31}\] TP53 (Tumor suppressor gene), activated during diverse stressfull conditions, participate in a number of biochemical mechanisms including cell cycle arrest and apoptosis.\[^{32}\] TP53 regulation in debatable in ER stress. It has been discovered that ER stress stabilization of p53 activity inducing p53 mediated apoptosis. On the other hand, it's also been reported that downregulation of p53 by Gsk3β is induced by ER stress.\[^{33}\] A report for downregulation of p53 has been observed in response to ER stress mediated through aluminium in SHSY-5Y cells.\[^{34}\] Furthermore, according to a clinical setting, formation of tumor as well as the efficacy of therapy might be influenced by the ER stress capability to inhibit p53. It confers resistance to the inhibitory effect of ER stress on p53 functionally and may prove ruinous for tumor cells that hold on to wild type p53 gene. The kind of effect would facilitate the resistant power of cancerous cells to DNA damaging effect against the agents used in cancer treatment. In case, ER stress inhibition may serve as a strategy to enhance the effectiveness of therapy directed against progression of cancer.

**ER Stress and Neurodegeneration**

A condition responsible for a no. of neurodegenerative diseases is the accumulation and deposition of misfolded proteins in living cells that affects the signaling systems, as well as neuronal connectivity and cell death.\[^{35}\] As neurogenerative disorders have multi-factorial causes that includes environmental factors, genetic predisposition, redox imbalance, neuroinflammation, glutamate-induced excitotoxicity, Ca++ levels disruption, mitochondrial dysfunction and accumulation of misfolded protein. It is to suggest the role of ER stress induced UPR activation, that is responsible for neurodegeneration related disorders. Misfolded proteins when gets accumulated act as a characteristic feature for a no. of neurogenerative diseases such as Alzheimer’s and Parkinson’s disease.\[^{5}\] UPS malfunctioning causes accumulation of misfolded proteins resulting in UPR induced by ER stress.\[^{36}\]
ER Stress and Alzheimer’s Disease
Alzheimer's disease is an irresistible neurodegenerative disease characterized by progressive rejection of cognitive functions resulting in dementia. The disease shows high deposition of senile plaques of β-amyloid proteins and intracellular neurofibrillary tangles containing hyperphosphorylated tau called neurofibrillary tangles (NFT). The β-amyloid proteins are formed by the cleavage of amyloid precursor proteins (APP) with the help of γ-secretase and β-secretase (BACE). IRE1 and PERK (ER stress sensors) are greatly affected by presenilin protein (an integral membrane protein and a part of the γ-secretase complex), is broadly expressed in both ER as well as Golgi apparatus. It has been observed that mutated form of presenilin decreases the phosphorylation of PERK-eIF2α pathway that leads to the protein accumulation in ER. Furthermore, increased levels of PERK and eIF2α in hippocampus neurons of AD brain has also been reported. In addition, IRE1 phases signaling inhibition induced by mutant presenilin 1, which as a result suppresses the ER chaperones transcription, such as GRP78 gets down-regulated in AD. Implication of XBP1 is also been reported in AD, that controls diverse cell type and context dependent transcriptional regulatory network. Modulation of IRE1 activity may cause reduced splicing of XBP1, thereby controlling the signaling to a pro-death response.

Generation of Aβ, being a characteristic of AD, is also associated with ER. Aβ is reported to persuade Ca$^{2+}$ discharge from ER stores. Though invasion of Ca$^{2+}$, plasma membrane or ER membrane is via calcium channels, which increased generation of Aβ by altering the metabolism showing a complicated association between AD and Ca$^{2+}$ dysfunction.

ER Stress and Parkinson's Disease
Parkinson's disease is characterized as degenerative disease with loss of dopaminergic neurons in the substantia nigra pars indicating that wild type protein may be causative reason for this disease, showing the role of α-synuclein in PD. A majority of the PD cases, up to 90%, are irregular and only 5-10% of cases show monogenic form of the disease. Recent investigations shown the inference of ER stress in the pathophysiology of PD and increased UPR activity has been observed in the affected regions of brain. It also been observed that deficiency of CHOP helps in protection of neonatal striatum from neurotoxicant 6-hydroxydopamine. Furthermore, some reports demonstrated that enforced expression of ER sensor proteins, ATF6 alpha and spliced XBP1, limits dopaminergic neuronal death.
induced by neurotoxins. These reports are indicative of the role of ER stress in the death and dysfunction of dopaminergic neurons exposed to neurotoxicant models of PD. Accumulating misfolded proteins such as α-synuclein and Parkinson-associated endothelin receptor-like receptor (Pael-R) has been reported to be a main cause of triggering post translational modifications, like phosphorylation and nitrosylation, of α-synuclein can cause misfolding and later nigra of PD patients. Smith et al reported that mutation of A53T in α-synuclein activates UPR leading to increased expression of CHOP and GRP78 and increased phosphorylation of eIF2α. Moreover, the authorized reports suggest that suppression of UPR through eIF2α phosphorylation inhibition defended the A53T mutant α-synuclein-overexpressing cells from cell death. It is been suggested that UPR mediates shift of the balance toward programmed cell death.\cite{49}

![Diagram showing the role of ER stress and unfolded protein response in health and disease](image)

**Fig.2** Representing the role of ER stress and unfolded protein response in health and disease

**CONCLUSION**

It may be now concluded that ER have a fundamental role in protein synthesis and folding. UPR forms the stable arm of ER stress signaling, which on facing stress gets activated. And when ER stress is prolonged, UPR assume adverse role, causing disruption of cellular homeostasis. On the whole, the existing examination designated the role of ER stress in health and diseases. Fig. 2 shows that how protein unfolding causes ER stress thereby activating UPR inducing health and disease related implications.
REFERENCES


