

## Review

### ER Stress and Disease: Toward Prevention and Treatment

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Secretory and membrane proteins are synthesized in ribosomes, then mature in the endoplasmic reticulum (ER), but if ER function is impaired, immature defective proteins accumulate in the ER. This situation is called ER stress: in response, a defensive mechanism called the unfolded protein response (UPR) is activated in cells to reduce the defective proteins. During the UPR, the ER transmembrane sensor molecules inositol-requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and RNA-dependent protein kinase (PKR)-like ER kinase (PERK) are activated, stress signals are transduced to the outside of the ER, and various cell responses, including gene induction, occur. In ER-associated degradation (ERAD), one type of UPR, defective proteins are eventually expelled from the ER and degraded in the cytoplasm through the ubiquitin proteasome system. Since ER stress has been reported to have relationships with neurodegenerative diseases, diabetes, metabolic syndromes, and cancer, it is the focus of increased attention from the perspectives of elucidating pathogenic mechanisms, and in the development of therapeutics.

**Key words** endoplasmic reticulum stress; unfolded protein response; neurodegenerative disease; diabetes; metabolic syndrome; cancer

#### 1. INTRODUCTION

Secretory and membrane proteins, such as hormones and transporters, respectively, account for as many as one-third of synthesized proteins. After these have been synthesized in the ribosomes, they are incorporated into the endoplasmic reticulum (ER) and then mature by receiving folding and various other modifications: proteolytic processing, N-linked glycosylation, disulfide bond formation, *etc.* Only the completed items are sent to the Golgi apparatus, which is the next modification organelle. Thus, the ER functions as a production factory for proteins *via* rigid product quality controls to maintain cell homeostasis.

When a stressor blocks ER function (for example, hypoxia and glucose deprivation during ischemia, or protein overload from a virus), the ER overflows with immature defective proteins. This situation is called ER stress. If it continues over a sustained period, apoptosis signals are initiated from the ER, resulting in apoptotic cell death. To respond to ER stress, cells activate three defensive mechanisms, collectively called the unfolded protein response (UPR), aimed at reducing the defective proteins. The first defense mechanism is the arrest of further protein synthesis in the ribosomes. Second is the repair of defective proteins by ER-resident molecular chaperone mobilization. Third is the elimination and degradation of defective proteins from the ER<sup>1)</sup> (Fig. 1). The final mechanism is called ER-associated degradation (ERAD).<sup>2)</sup>

Initially, the UPR was analyzed for the molecular yeast mechanisms of inositol-requiring enzyme 1 (Ire1), which acts as an ER stress sensor and transduces information by transfer outside the ER. After mammalian Ire1 homologs (IRE1) were identified in 1998,<sup>3,4)</sup> considerable attention was then focused on the mammalian UPR. Later, two ER stress sensors that do

not exist in yeast were identified,<sup>5,6)</sup> and at the same time the relationships of ER stress to various diseases were reported.<sup>7)</sup> ER stress and the UPR suddenly began to be highlighted. Beginning with a report in 1999 on the relationship between ER stress and Alzheimer's disease (AD),<sup>8)</sup> relationships were subsequently found between ER stress and Parkinson's disease (PD),<sup>9)</sup> polyglutamine disease,<sup>10)</sup> and other neurodegenerative diseases. Further relationships are indicated for many other diseases, including diabetes,<sup>11)</sup> metabolic syndrome,<sup>12)</sup> and cancer.<sup>13)</sup> Research related to ER stress-induced cellular dysfunction and disease pathogenesis has continued to expand explosively (Table 1). This review is a simple summary of the roles of ER stress in basic UPR and pathogenic mechanisms reported to date.

#### 2. ER STRESS SENSORS

The primary mammalian ER stress sensors, in addition to IRE1, include activating transcription factor 6 (ATF6) and double-stranded RNA-dependent protein kinase (PKR)-like ER kinase (PERK) (Fig. 2). Only IRE1 are conserved from yeast, whereas ATF6 and PERK are newly added during the evolutionary process.

IRE1 has a serine/threonine kinase region and a ribonuclease (RNase) region on the cytoplasmic side (C-terminus). When ER stress is sensed, IRE1 forms dimers or oligomers, and is activated by autophosphorylation, which expresses RNase activity to process mRNA.<sup>3,4)</sup> The X-box binding protein 1 (XBP1) is a molecule in which IRE1 RNase activity mediates splicing to promote proper translation. The splicing by IRE1 differs from conventional splicing *via* spliceosomes; it is a non-conventional splicing for cutting matured mRNA.<sup>14,15)</sup> XBP1 is a transcription factor with a basic leucine

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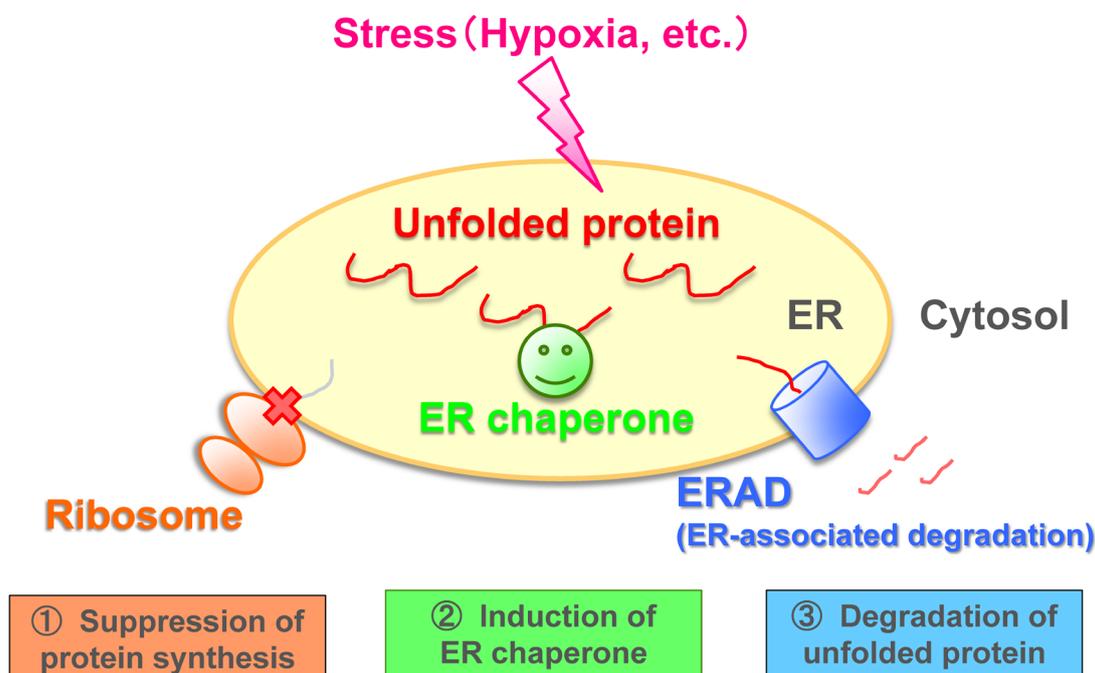


Fig. 1. Endoplasmic Reticulum (ER) Stress and Unfolded Protein Response (UPR)

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zipper (bZIP) domain, and it binds to the promoter region of genes related to ER chaperones, ERAD, ER membrane synthesis, and protein secretion to promote their transcription.<sup>16,17)</sup>

ATF6 is a transcription factor of the cAMP response element-binding protein (CREB)/ATF family. In response to ER stress, ATF6 becomes free from the ER chaperone binding immunoglobulin protein (BiP), which is bound to the ATF6 ER luminal side (C-terminus).<sup>5,18)</sup> Furthermore, at steady state, ATF6 forms a disulfide bond in the ER lumen, whereas under ER stress conditions, it becomes a reduced form, into monomers that translocate to the Golgi apparatus.<sup>19)</sup> ATF6 undergoes two-step processing (called regulated intramembrane proteolysis, or RIP) by the site-1 protease (S1P) and site-2 protease (S2P) present in the Golgi, resulting in the release of the cytoplasm side (N-terminus) from the membrane.<sup>20)</sup> The separated ATF6 cytoplasmic side, including a bZIP domain, is translocated inside the nucleus and bound to the ER stress-response element (ERSE) to induce expression of the ER

chaperone and other genes.<sup>5)</sup>

PERK is a kinase; it forms oligomers and is activated by autophosphorylation in response to ER stress, such as IRE1. The activated PERK phosphorylates the eukaryotic initiation factor 2 $\alpha$  (eIF2 $\alpha$ ), which configures the ribosome to suppress protein translation.<sup>6)</sup> The PERK–eIF2 $\alpha$  pathway acts, prior to improvement of ER stress conditions *via* the ATF6 and IRE1 pathways, to reduce the unfolded protein load on the ER by temporarily inhibiting protein synthesis. The eIF2 $\alpha$  phosphorylation selectively promotes the translation of genes harboring an upstream open reading frame (uORF). The uORF is a reading frame that begins translation from 5'-upstream of the original mRNA coding region.<sup>21,22)</sup> There are a number of genes in which the original ORF or uORF is selected in response to cell differentiation, metabolism, and stress. Downstream of eIF2 $\alpha$ , the activating transcription factor 4 (ATF4) is induced.<sup>23)</sup> During ER stress, ATF4, whose translation is promoted by uORF, performs the transcriptional

### Biography

Dr. Masayuki Kaneko was born in Saitama, Japan, in 1974. He earned a Ph.D. (in Pharmaceutical Science) from Hokkaido University in 2004 under the supervision of Professor Yasuyuki Nomura (Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Hokkaido University). He has worked as a Research Associate at Nomura's laboratory from 2000 to 2005; as Lecturer and Associate Professor at the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Chiba Institute of Science (2005–2012); and as a Lecturer at the Department of Medical Therapeutics and Molecular Therapeutics, Gifu Pharmaceutical University (2012–2014). He moved to the Department of Biochemistry, Institute of Biomedical and Health Sciences, Hiroshima University in 2014 and became Associate Professor. He was awarded the 24th Encouragement of Young Investigator Award by the Japanese Pharmaceutical Society (2009), and the Pharmaceutical Society of Japan Award for Young Scientists (2012). He focuses on the molecular mechanisms and pathophysiological roles of transmembrane ubiquitin ligases he has identified. As representative of his work, he found the ubiquitin ligase HRD1 that can reduce amyloid  $\beta$  through ubiquitination of an amyloid precursor protein.



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Table 1. ER Stress and Diseases

Disease/Disorder	Cause	Literature
Alzheimer's disease	•Mutant presenilin inhibits the UPR sensors	8
	•A $\beta$ induces ER stress	29
	•HRD1 ubiquitinates and degrades APP	30
	•Increase in S-nitrosylated PDI in patient brains	30
Parkinson's disease	•Parkin mutation causes Pael-R accumulation in the ER	9
	•MPTP induces ER stress	31
	•Increase in S-nitrosylated PDI in patient brains	32
ALS	•Mutant SOD1 inhibits Derlin-1	33
Diabetes	•Dysfunctional PERK and eIF2 $\alpha$ induce $\beta$ cell apoptosis	11, 35, 36, 37
	•Insulin mutation induces ER stress in Akita mouse	38
	•WFS1 mutation activates ATF6 by HRD1 destabilization	41
Metabolic syndrome	•ER stress induces steatosis	42, 43
	•Chemical chaperone improves type 2 diabetes	12, 44
	•Chemical chaperone suppresses adipocytic inflammation	45
Cancer	•Inhibition of ER stress sensors reduces tumorigenesis	13, 47, 48, 49

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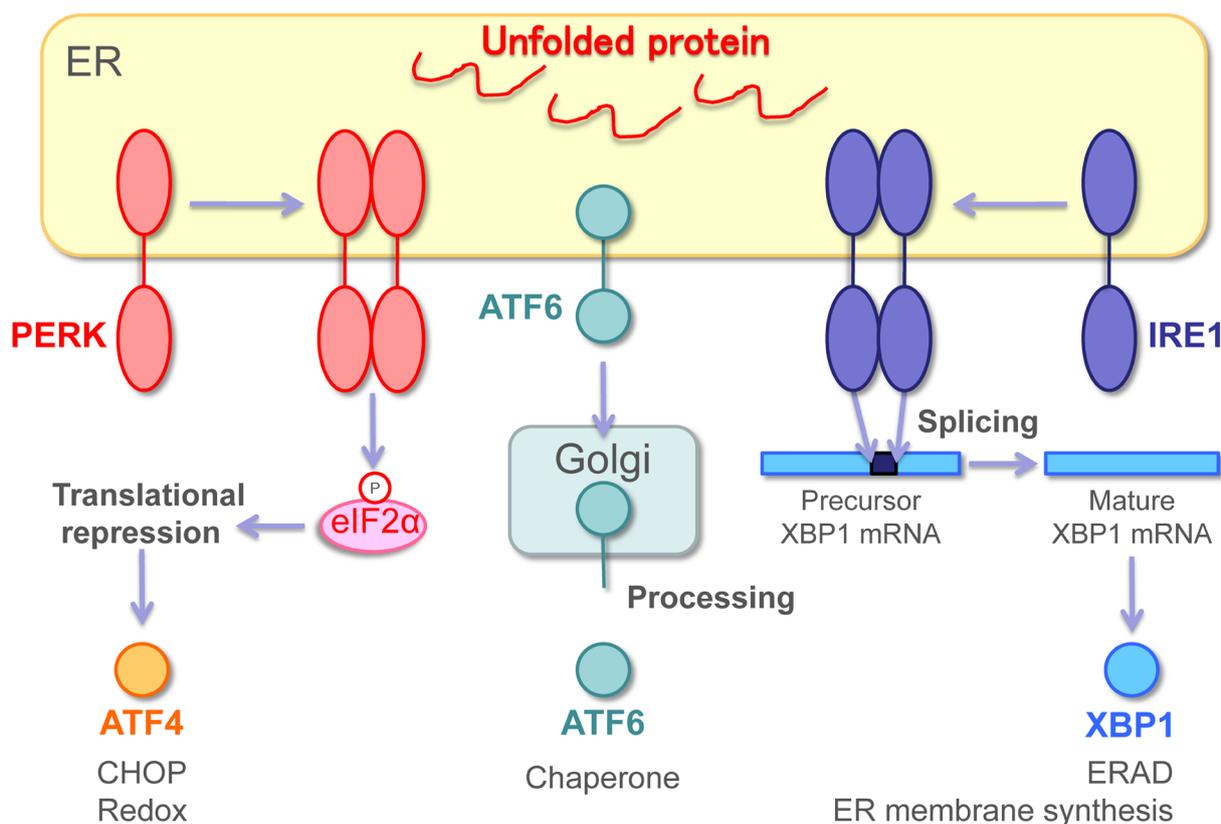


Fig. 2. Activation of ER Stress Sensors and Transcription Factors

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induction of genes, CCAAT/enhancer binding protein (C/EBP) homologous protein (CHOP), a transcription factor implicated in apoptosis<sup>23)</sup> and is related to amino acid metabolism<sup>24)</sup> and oxidation/reduction.<sup>25)</sup>

### 3. ER-ASSOCIATED DEGRADATION (ERAD)

ERAD is a system for the degradation of proteins in the ER

lumen and ER membrane through the ubiquitin–proteasome system in the cytoplasm; it is one of the defense mechanisms for ER stress that is conserved from yeast<sup>2)</sup> (Fig. 3). The eventual defective proteins, recognized by molecular chaperones, are discharged to the cytoplasm from the ER through a protein channel called the dislocon. The defective proteins undergo ubiquitination in the cytoplasm by the cooperative action of ubiquitin activating enzyme (E1), ubiquitin binding en-

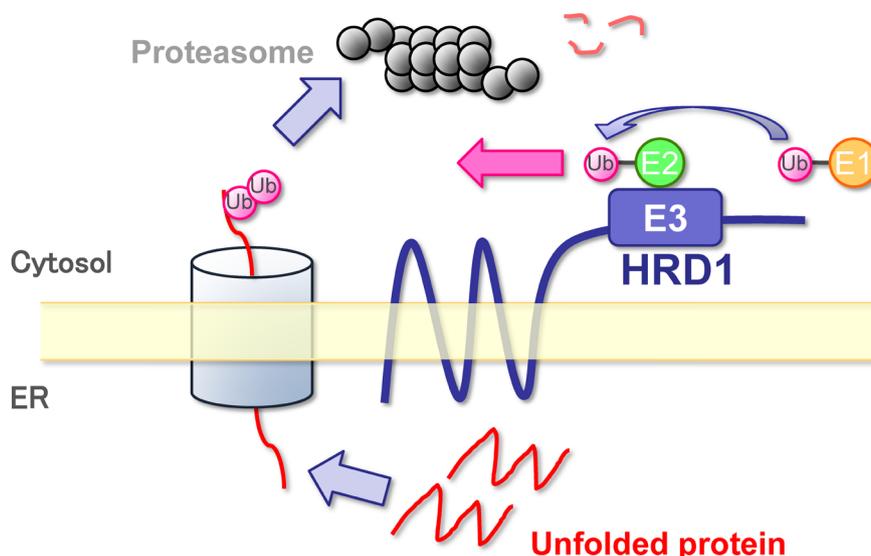


Fig. 3. ER-Associated Degradation (ERAD)

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zyme (E2), and ubiquitin ligase (E3). These poly-ubiquitinated proteins are quickly drawn from the ER membrane, and then recognized and degraded by proteasome.<sup>26)</sup> Of the enzymes involved in ubiquitination, the ubiquitin ligase plays the most important role in determining the recognition and degradation speed of a substrate protein. The classic HRD1 is an ER 5-transmembrane type ubiquitin ligase conserved from yeast, and its expression is induced by the UPR to regulate ERAD activity.<sup>27)</sup>

#### 4. NEURODEGENERATIVE DISEASE

AD, PD, and amyotrophic lateral sclerosis (ALS) are well-known neurodegenerative diseases. Their pathogenic mechanisms remain unclear, and their fundamental therapeutic methods have not yet been established. A commonly observed pathological finding of neurodegenerative disease is the accumulation of denatured proteins. A relationship with ER stress has been reported in many neurodegenerative diseases.<sup>28)</sup>

In 1999, it was reported that mutations of presenilin, one of the genes causing familial AD, suppresses the activation of IRE1 and other ER stress sensors, resulting in attenuated UPR, and making neurons more vulnerable to ER stress.<sup>8)</sup> Subsequently, it was also determined that amyloid  $\beta$  ( $A\beta$ ) deposited on the AD brain causes ER stress, thus contributing to cell toxicity.<sup>29)</sup> In addition, in another interesting report in understanding the pathology of AD, it was suggested that the ubiquitin ligase HRD1 involved in ERAD promotes ubiquitination and degradation of the amyloid precursor protein (APP), raising the possibility of reducing the  $A\beta$  level. Furthermore, the protein levels of HRD1 were significantly reduced in the cerebral cortex of AD patients.<sup>30)</sup>

Parkin (*PARK2*), one of the known genes that causes familial PD, is a ubiquitin ligase involved in ERAD. There is a hypothesis that parkin mutation causes the accumulation of a substrate, such as parkin-associated endothelin receptor-like receptor (Pael-R), in the ER, leading to ER stress, and resulting in ER stress-induced cell death of dopaminergic neurons in the substantia nigra.<sup>9)</sup> In addition, drugs which induce PD,

such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), cause ER stress, and the involvement of ATF6 activation in this process has been reported.<sup>31)</sup>

While nitric oxide (NO) is known to induce ER stress, protein-disulfide isomerase (PDI) that works on protein disulfide bond formation in the ER has been identified as a NO target. When cysteine residues in the PDI enzyme active sites are *S*-nitrosylated by NO, the enzyme activity declines, resulting in the accumulation of defective proteins in the ER. Interestingly, PDI *S*-nitrosylation increases in the post-mortem brain of AD and PD patients.<sup>32)</sup>

ALS is a neurodegenerative disease that damages the motor neurons. Cu/Zn-superoxide dismutase (SOD1), a causative gene for ALS, is localized in the cytoplasm. If its gene mutations cause changes in protein structure, it binds with Derlin-1, an ERAD-related molecule. As a consequence, the ERAD system is impaired, and ER stress is elicited.<sup>33)</sup>

In addition, since polyglutamine protein found in Huntington's disease<sup>10)</sup> and prion proteins that cause Creutzfeldt-Jakob disease<sup>34)</sup> also induce ER stress, it would appear that many of the mutation proteins found in neurodegenerative diseases either directly or indirectly impair the ER function.

#### 5. DIABETES

Diabetes has been indicated to have a relationship with ER stress as well. Insulin, a peptide hormone, undergoes disulfide bond formation and processing in the ER to exert ligand activity. In pancreatic  $\beta$ -cells that produce insulin, the ER is developed to respond to insulin production increased by as much as approximately 100-fold during secretion.

Knockout (KO) mice for the ER stress sensor PERK exhibited apoptosis in  $\beta$ -cells from around 15 d after birth, causing the onset of insulin-deficient Type 1 diabetes.<sup>11)</sup> Furthermore, even with PERK target eIF2 $\alpha$  mutation (S51A)<sup>35)</sup> and PERK negative feedback factor *P58<sup>IPK</sup>*, which is induced downstream of PERK, KO mice<sup>36)</sup> exhibited a diabetic phenotype, suggesting that the PERK pathway plays an important role in the pathogenesis of diabetes. In Wolcott-Rallison syndrome,

concomitantly presented with juvenile Type 1 diabetes and osteogenesis imperfecta, PERK gene mutation has also been found to be a cause.<sup>37)</sup>

In the Akita mouse presented with early-onset diabetes, a mutation in cysteine residue (C96Y) that is necessary for the formation of disulfide bonds has also been found to be a cause. This insulin mutation appears to form an unfolded structure that accumulates in the ER and induces apoptosis. When CHOP that induces apoptosis downstream of PERK was knocked out in the Akita mouse, the onset of diabetes was delayed.<sup>38)</sup> This therefore supports the idea that ER stress contributes to  $\beta$ -cell death in diabetes.

Pancreatic  $\beta$ -cell-specific *Xbp1* conditional KO mice displayed impairment of proinsulin production and processing leading to decreased insulin secretion.<sup>39)</sup> *Atf6 $\alpha$*  KO mice fed with a high fat diet also exhibited impaired proinsulin production. Pancreatic  $\beta$ -cells suffered from ER stress in the *Atf6 $\alpha$* -deficient mice.<sup>40)</sup> These findings suggest that the three major UPR pathways are involved in ER stress of pancreatic  $\beta$ -cells.

Wolfram syndrome is an autosomal recessive genetic disease that initially presents alongside juvenile diabetes mellitus, followed by complications of optic atrophy and diabetes insipidus. WFS1 has been identified as a causal gene. Wild-type WFS1 stabilizes HRD1 to promote the degradation of ATF6 and to negatively regulate the ATF6 signal. In Wolfram syndrome, the mutation of WFS1 appears to reduce the HRD1 function, and to induce excessive activation of the ATF6 pathway.<sup>41)</sup>

## 6. METABOLIC SYNDROME

While various environmental factors are involved in the pathogenesis of metabolic syndromes, recent attention has focused on ER stress. ER stress activates inflammation and various stress signals, and induces glucose and lipid metabolic disorders in  $\beta$ -cells, hepatocytes, and adipocytes. Thus, ER stress appears to induce obesity, insulin resistance, steatosis, dyslipidemia, and other metabolic syndromes.

ER stress promotes lipid synthesis and induces decreased lipoproteins, resulting in the development of steatosis. The overexpression of BiP in the liver of obese mice improves steatosis and promotes glucose utilization.<sup>42)</sup> In addition, if ER stress conditions continue, CHOP induction causes the suppression of the C/EBP  $\alpha$ , which is an important transcription factor in lipid metabolism. As a result, this inhibits the oxidation of fatty acids, the secretion of lipoproteins, and gluconeogenesis, and promotes the development of steatosis.<sup>43)</sup>

Low molecular weight compound chemical chaperones are known to be ER stress inhibitors. Chemical chaperones prevent the aggregation of defective proteins in the ER, and promote protein transport from the ER to the target location. In the *ob/ob* mouse with Type 2 diabetes, application of the chemical chaperone 4-phenyl butyric acid (4-PBA) and tauroursodeoxycholic acid (TUDCA) improved insulin resistance and glycemic control.<sup>44)</sup>

High fat diet-fed mice exhibit chronic inflammation in adipose tissue. This inflammation response implicates ER stress mediated by reactive oxygen species and inflammatory cytokines due to free fatty acids. In mice fed a high fat diet, the administration of 4-PBA or TUDCA attenuates the UPR activation and inflammation response in liver and adipose

tissue, suppresses weight gain, and improves insulin signaling.<sup>45)</sup> In other words, ER stress plays an important role in the dysfunction of adipose tissue associated with obesity. Indeed, subcutaneous adipose tissue of obese human subjects showed upregulation of several UPR stress-related proteins and activation of c-jun N-terminal kinase (JNK), which inhibits insulin action and activates proinflammatory pathways.<sup>46)</sup>

## 7. CANCER

The UPR is a mechanism activated to prevent apoptosis due to ER stress, whereas cancer abuses the UPR's ability to acquire stress resistance and thus promotes cell proliferation. In solid cancers, angiogenesis cannot keep up with this rapid proliferation, leading to hypoxia and glucose deprivation in the internal microenvironment, and resulting in the generation of persistent ER stress. To overcome these ER stress conditions and to adapt to the poor environment for proliferation, cancer cells constitutively activate the UPR while suppressing apoptosis. In *Perk*-deficient mice, tumorigenicity and the expression of angiogenesis genes are attenuated.<sup>47)</sup> Meanwhile, since CHOP is expressed downstream of constitutively activated PERK by way of the transcription factor ATF4, this should induce apoptosis. However, cancers ingeniously escape from apoptosis because they suppress CHOP expression by inducing the expression of P58<sup>IPK</sup> that inhibits PERK activation.<sup>48)</sup> The IRE-XBP1 pathway is also important for cancer proliferation under hypoxic conditions. *Xbp1*-deficient mice exhibit increased cancer cell death as a result of hypoxia and decreased proliferation.<sup>13)</sup> ATF6 also appears to contribute to the survival and resistance of cancers in dormancy through mammalian target of rapamycin (mTOR) signaling.<sup>49)</sup> In conclusion, while cancer uses the UPR to survive and proliferate, suppressing the UPR in cancer patients may be an effective novel treatment method.

## 8. CONCLUSION

To date, attention has focused on ER stress as a cause of disease that results in damage to an organism, based on a literal "stress." In recent years, however, functional analyses of ER stress sensors and their downstream genes have revealed that ER stress is positively involved in tissue differentiation and maturation. The UPR conserved from yeast was diversified in the process of evolution in multicellular organisms, and has developed as a variety of signaling pathways that affect cell differentiation and fate.<sup>50)</sup> In the future, if we are able to understand the complete picture of signaling pathways and physiological functions of UPR-related molecules, we can expect the elucidation of pathogenic mechanisms in diseases of unclear pathology, as well as the development of novel therapeutics.

**Conflict of Interest** The authors declare no conflict of interest.

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