In vivo cellular adaptation to ER stress: survival strategies with double-edged consequences

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Summary

Disturbances to the balance of protein synthesis, folding and secretion in the endoplasmic reticulum (ER) induce stress and thereby the ER stress signaling (ERSS) response, which alleviates this stress. In this Commentary, we review the emerging idea that ER stress caused by abnormal physiological conditions and/or mutations in genes that encode client proteins of the ER is a key factor underlying different developmental processes and the pathology of diverse diseases, including diabetes, neurodegeneration and skeletal dysplasias. Recent studies in mouse models indicate that the effect of ERSS in vivo and the nature of the cellular strategies induced to ameliorate pathological ER stress are crucial factors in determining cell fate and clinical disease features. Importantly, ERSS can affect cellular proliferation and the differentiation program; cells that survive the stress can become 'reprogrammed' or dysfunctional. These cell-autonomous adaptation strategies can generate a spectrum of context-dependent cellular consequences, ranging from recovery to death. Secondary effects can include altered cell–extracellular-matrix interactions and non-cell-autonomous alteration of paracrine signaling, which contribute to the final phenotypic outcome. Recent reports showing that ER stress can be alleviated by chemical compounds suggest the potential for novel therapeutic approaches.

Key words: ER stress, Unfolded protein response, Cell fate, Development, Disorders

Introduction

In eukaryotic cells, secretory and membrane proteins are synthesized, assembled and secreted through a pathway composed of the endoplasmic reticulum (ER), Golgi, plasma membrane and intermediary transport vesicles. Folding and maturation of proteins is a primary function of the ER, which is equipped with a diverse array of molecular chaperones and folding-associated enzymes for protein folding and quality control. Protein folding requires a stable lumenal environment in the ER; any perturbation can lead to accumulation of misfolded proteins and induction of an ER stress response. We use the term 'ER stress signaling' (ERSS) to refer to the mechanisms by which protein load in the ER is sensed and the molecular consequence of the stress imposed on the ER in cells when the protein load exceeds normal limits: it includes the unfolded protein response (UPR; see Fig. 1A) and other downstream signaling events. Fig. 1B summarizes the possible consequences when a cell experiences different levels of ER stress. Readers are referred to recent reviews for a detailed description of the UPR (Ron and Walter, 2007; Scheuner and Kaufman, 2008). As briefly described below, ERSS pathways maintain homeostasis in cells, modulating the protein load in the ER by the sophisticated integration of controls that regulate gene expression at multiple levels to enhance protein folding, modulate translation, and facilitate the degradation of protein and mRNA.

The wide-ranging action of ERSS in controlling gene expression acts like a double-edged sword for the cell and for normal physiology. The ideal outcome is adaptation and survival – but at what cost? In this Commentary, we focus on our current understanding of the strategies that enable cells to adapt to and survive ER stress in vivo. In addition, we discuss the consequences of success or failure to adapt to and survive ER stress for normal development and physiology, as well as the associated implications for the pathology of disorders. Finally, we highlight the therapeutic potential of intervening with ER stress pathways as a means to minimize the pathology of these disorders.

The cellular response to ER stress

First line of response to stressing the ER: activating ER stress sensors

Excess misfolded proteins in the ER bind to the chaperone BiP and titrate it away from the three ER stress sensors: inositol-requiring 1 (IRE1), PKR-like ER kinase (PERK) and activating transcription factor 6 (ATF6). Alternatively, it is suggested that misfolded proteins might bind with and activate IRE1 directly (Credle et al., 2005). In the presence of misfolded proteins and absence of BiP, these activated sensors elicit the UPR, a highly conserved protective feedback mechanism by which ER stress is relieved through transcriptional and translational controls that modulate the rate of translation and activate genes that enhance the protein folding and degradation capability of the ER. Misfolded proteins must be degraded to prevent triggering apoptosis. Soluble proteins can be ubiquitylated and subsequently degraded by 26S proteasomes; insoluble protein aggregates can be degraded by autophagy-mediated lysosomal degradation (Fujita et al., 2007; Kouroku et al., 2007; Ishida et al., 2009). An event unique to the UPR is the splicing of *Xbp1* mRNA by IRE1, which thereby generates the spliced form of XBP1 (XBP1^S), a potent transcription factor that activates genes encoding mediators of protein folding (such as chaperones) and protein degradation that help to restore ER homeostasis.
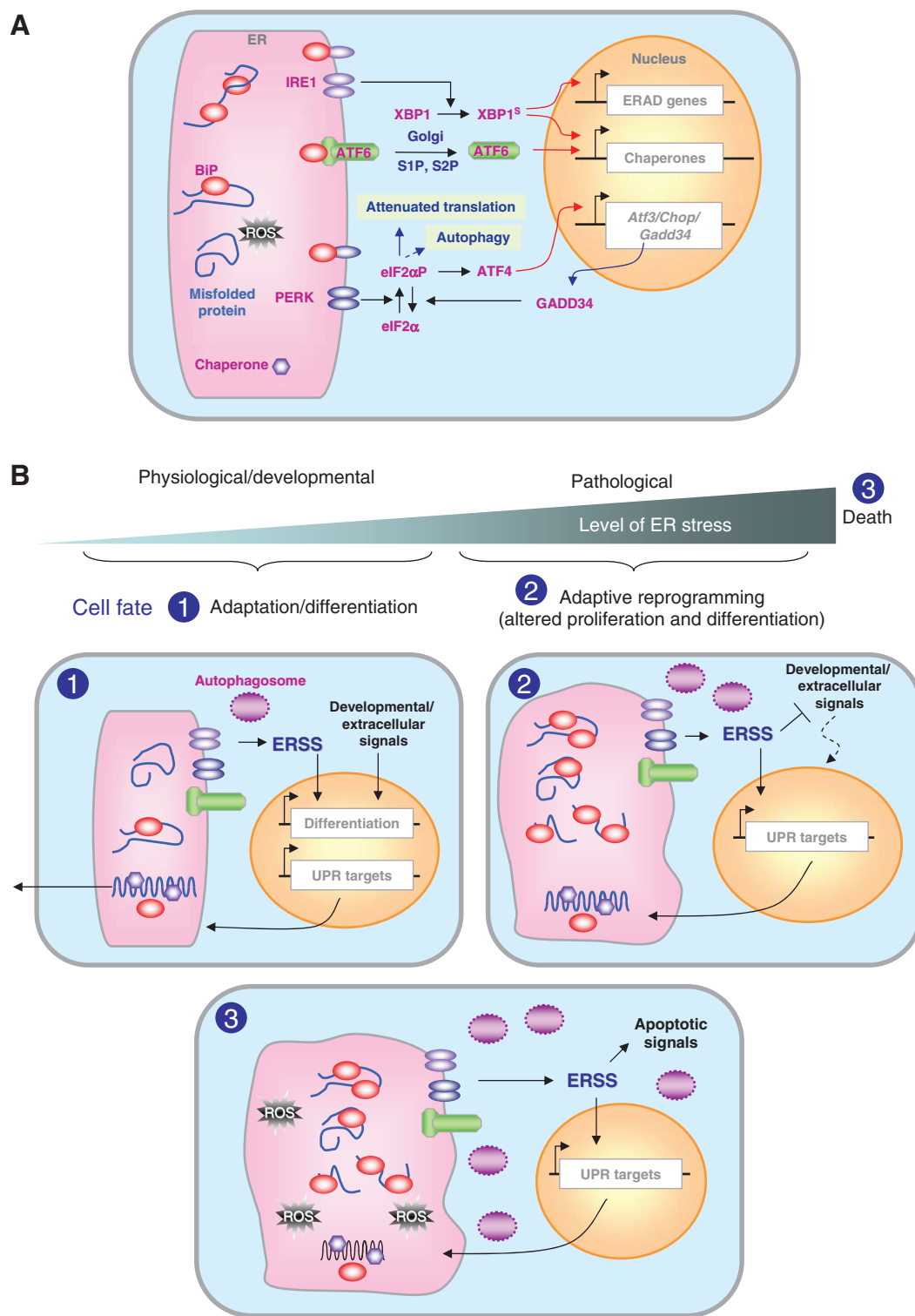
Fig. 1. Schematic diagram of ERSS and cellular adaptation to different levels of ER stress. (A) Misfolded proteins accumulating in the ER titrate BiP from the three stress sensors PERK, ATF6 and IRE1, leading to activation of the three respective pathways to alleviate protein-folding load in the ER. (B) The outcome of the cellular response to different levels of ER stress varies. (1) Physiological ER stress during development is mild and controllable. The cell can recover from and/or adapt to the protein-folding stress; it can also differentiate into a specialized cell type in a manner that depends on components of ERSS and appropriate developmental signals. (2) In severe pathological ER stress, ERSS is initially an adaptive response, but, if stress remains unresolved, perhaps because of continuous and/or cyclical expression of mutant protein, it can lead to interference with developmental signals and disruption of cellular gene expression patterns. The cell can then display altered proliferation and differentiation status, and become dysfunctional. (3) Under conditions of extreme stress, an apoptotic signal is triggered and becomes dominant, leading to cell death. This can occur through excessive production of reactive oxygen species (ROS) caused by enhanced protein-folding activity, which relates to levels of CHOP, GADD34 and ERO1α expression.

Attenuation of translation

One of the early responses to ER stress is the modulation of translation, a highly conserved adaptation mechanism (Yamasaki and Anderson, 2008). Phosphorylation of eukaryotic initiation factor 2α (eIF2α) by PERK reduces protein translation and shifts the translation machinery to favor alternative modes of initiation, including selective translation of activating transcription factor 4 (ATF4) (Harding et al., 2000). Phosphorylated eIF2α also activates the conversion of microtubule-associated protein 1 light chain 3 (LC3-I), an essential protein for autophagy, to LC3-II and hence promotes autophagosome formation (Fujita et al., 2007; Kouroku et al., 2007), as well as the formation of stress granules in which mRNAs are sequestered for silencing and/or degradation (Yamasaki and Anderson, 2008). Whether any specific types of mRNA are sequestered and whether there is ER-stress-induced microRNA expression in stress granules is not known.

Modification of transcriptional and epigenetic controls

Rapid changes in transcriptional controls occur early during ERSS, with the induction of transcription factors such as ATF6, XBP1[S] and ATF4, which then upregulate the expression of activating transcription factor 3 (ATF3) and C/EBP homologous protein (CHOP). All the above factors are basic region/leucine zipper (bZIP) transcription factors that, through physical interaction, inhibit or alter the functions of other bZIP transcription factors, such as the binding of ATF6 to sterol regulatory element-binding protein 2 (SREBP2), which inhibits the transactivation activity of SREBP2 in lipogenesis (Zeng et al., 2004). This transcriptional reprogramming is accompanied by chromatin modification: for example, *BiP* transcription (mediated in part by ATF6) is associated with methylation and acetylation of histone H4 in the *BiP* promoter region (Baumeister et al., 2005). However, ER-stress-induced transcriptional repression of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene has been shown to be mediated by a combination of binding of ATF6 to the *CFTR* minimal promoter region, together with DNA methylation and histone deacetylation (Bartoszewski et al., 2008). This study suggests that transcriptional repression is part of the UPR, but the extent to which this form of regulation occurs in cells is still unknown.

Modulation of signaling cascades

ERSS can activate mitogen-activated protein kinases (MAPKs; including ERK1, ERK2, JNK and p38MAPK), which are known to regulate gene expression, differentiation and cell survival as part of cellular responses to various types of stress and extracellular signals. (Urano et al., 2000; Arai et al., 2004; Hung et al., 2004; Nguyen et al., 2004). In addition, IRE1 has been shown to activate nuclear factor κB (NF-κB) and JNK, which are key regulators of cell fate during stress stimulation (Urano et al., 2000; Hu et al., 2006; Han et al., 2009).

Components of ERSS play roles in normal development and physiology

There is increasing evidence that ER stress is triggered as a part of the normal development of different organ systems and tissues. During development, different cell types synthesize and secrete enzymes, antibodies, serum proteins and extracellular matrix (ECM) components, thereby placing a heavy protein load on the ER. In turn, this leads to physiological ER stress, which needs to be relieved by the UPR to re-establish cellular homeostasis. Several components of the UPR are essential for proper development in

mice. IRE1 is required for the placenta to support embryonic development (Iwawaki et al., 2009), and the IRE1-XBP1 pathway is needed for the differentiation of pancreatic exocrine and plasma cells, and adipocytes (Reimold et al., 2001; Zhang et al., 2005; Lee et al., 2005; Sha et al., 2009; Ma et al., 2009). Loss-of-function mutation of PERK results in symptoms of Wolcott-Rallison syndrome in both mice and humans, culminating in pancreatic and skeletal defects (Delepine et al., 2000; Harding et al., 2001; Zhang et al., 2002). *Perk*^{+/−} mice display defective glycemic control in the glucose tolerance test (Harding et al., 2001) and have oligodendrocytes that are hypersensitive to interferon-γ (IFNγ): exposure of these cells to IFNγ results in apoptosis and hypomyelination (Lin et al., 2005). Activation of the UPR also occurs in differentiating keratinocytes and stem cells (Cho et al., 2009; Sugiura et al., 2009). Thus, components of ERSS are involved in normal physiological management of enhanced secretory load.

Other types of stress response can interact with the UPR during development. For example, in hypoxic cartilage (an avascular tissue), phosphatase and tensin homolog (PTEN) and hypoxia-inducible factor 1α (HIF1α) collaborate with the UPR to maintain proper growth-plate development (Yang et al., 2008). Specifically, ATF4 is activated by both hypoxia and ER stress, and positively regulates chondrocyte proliferation and differentiation (Wang et al., 2009). In addition, chondrocyte-specific ablation of Site-1 protease (*S1P*) causes severe defects in cartilage growth and development (known as chondrodysplasia) (Patra et al., 2007), reflecting the upstream role of S1P in regulating all ATF6-related factors, including BBF2H7 (BBF2 human homolog on chromosome 7). Consistent with this, mice lacking BBF2H7 display less severe chondrodysplasia and ER dilation in chondrocytes owing to reduced expression of SEC23a (a target of BBF2H7 that is responsible for protein transport from the ER to the Golgi), which leads to reduced secretion of ECM proteins (Saito et al., 2009). Furthermore, expression of differentiation-related marker genes is perturbed in the growth plate of *Bbf2h7*-null mice, similar to that reported for the cartilage matrix deficiency (*cmd*) mutant, in which ER stress is activated in cartilage (Wai et al., 1998; Tsang et al., 2007). Thus, it is likely that establishment of both an ECM and an appropriate ERSS are required for proper chondrocyte differentiation and growth-plate cartilage development. In bone, the expression of PERK, ATF4 and another ATF6-like factor, old astrocyte specifically induced substance (OASIS), is required for osteoblast function; deficiency in any one of these factors results in osteopenia (Harding et al., 2001; Zhang et al., 2002; Yang et al., 2004; Wei et al., 2008; Murakami et al., 2009). Intriguingly, the expression of ATF4 in osteoblasts is also linked to insulin secretion and sensitivity, as it regulates the expression of the bone-specific 'hormone' osteocalcin (Yoshizawa et al., 2009). Thus, ERSS is part of the normal physiological program and many, if not all, cell types are well equipped to adapt to 'normal' levels of ER stress.

ER stress and disease pathology: cause and effect

Although some ER stress is normal in some physiological contexts, abnormally high ER stress during growth and development can lead to pathogenic consequences. For example, raised levels of glucose and free fatty acids can induce the UPR and cell death in pancreatic β-cells during diabetes (Kharroubi et al., 2004; Hartman et al., 2004). Also, prolonged exposure to free fatty acids during liver steatosis (fatty liver) is associated with ER-stress-dependent

inhibition of apolipoprotein B100 secretion (Ota et al., 2008). In demyelinating diseases of the central nervous system (CNS), such as multiple sclerosis, IFNγ can induce ER stress in oligodendrocytes, leading to suppression of remyelination and cell loss (Lin et al., 2006; Lin et al., 2008). ER stress can also be directly elicited by the expression of misfolded proteins encoded by mutated genes. In the section below, we use key examples to explain how misfolded proteins can contribute to a clinical disease phenotype, and highlight studies of inherited skeletal dysplasias in humans and mice in which a link between ER stress and pathology is implicated (Table 1).

Mutated ER client proteins: ECM gene mutations in skeletal dysplasias

Skeletal dysplasias (also known as osteochondrodysplasias) are a group of more than 200 clinically distinct conditions, often lethal, that are caused by abnormal cartilage and/or bone development, and that commonly result in dwarfism and bone abnormalities. Many of these inherited disorders are associated with mutations in ECM components, including both collagens and non-collagenous proteins (Superti-Furga and Unger, 2007), and are inherited as an autosomal dominant trait. Conventional opinion is that the pathogenic mechanisms of these diseases involve either loss or gain of function of the mutated proteins. Loss-of-function mutations that cause mild clinical phenotypes have been attributed to haploinsufficiency for the gene product that occurs owing to instability of the mutant proteins or the degradation of mRNAs containing premature stop codons (nonsense mutations) (Chan et al., 1998; Bateman et al., 2003; Tan et al., 2008). Gain-of-function or dominant-negative effects arise when mutations result

in the synthesis and secretion of structurally abnormal proteins that interfere with ECM assembly and function (Cabral et al., 2003; Robinson et al., 2006). Causality of disorders arising from such mutations has conventionally been attributed to the presence of an abnormal ECM and impairment of structural integrity. However, the retention of such mutant proteins in the ER can induce ER stress, triggering ERSS that contributes to skeletal dysplasias; this connection is becoming increasingly obvious (Table 1). Indeed, protein misfolding and the intracellular accumulation, lumenal distension and/or dilation of the ER have long been observed as a hallmark of various diseases that are categorized as ER-storage diseases. These include certain skeletal dysplasias, such as osteogenesis imperfecta (OI), which is associated with mutations in the genes encoding collagen I (Kim and Arvan, 1998; Rutishauser and Spiess, 2002).

Metaphyseal chondrodysplasia, type Schmid (MCDS) is associated with heterozygous mutations in the *COL10A1* gene that affect the folding and trimeric assembly of the collagen X protein it encodes (Warman et al., 1993; Chan and Jacenko, 1998; Bateman et al., 2005). ER dilation was observed in the chondrocytes of a patient with MCDS (Wasylenko et al., 1980) and recent evidence indicates that the impact of ER stress is a major cause of the MCDS phenotype. Collagen X misfolding induces ER stress and the UPR directly in transfected cells (Wilson et al., 2005). This was confirmed by in vivo studies of transgenic mouse models in which the expression of mutant collagen X activated the UPR in hypertrophic chondrocytes (Ho et al., 2007; Tsang et al., 2007; Rajpar et al., 2009). This resulted in expansion of the hypertrophic zone and a phenotype resembling that observed in patients with MCDS (Ho et al., 2007) and in a porcine MCDS model

Table 1. Human autosomal dominant gene mutations associated with skeletal dysplasias that have signs of ER stress, and their relevant mouse models

Genes (proteins)	Disorders	Mutations that cause ER dilation in patient cells	Examples of mouse model
COL1A1 and *COL1A2* (collagen I)	Osteogenesis imperfecta (OI). There are seven types of OI; severity varies greatly and can include perinatal lethality (Sillence et al., 1979; Rauch and Glorieux, 2004; Marini et al., 2007)	*COL1A1*: p.G1006V (Lamande et al., 1989; Cole et al., 1992) and p.G391R (Cole et al., 1990) *COL1A2*: a 4.5 kb deletion (Willing et al., 1988)	*Col1a1 Aga2* heterozygous mice display a phenotype ranging from postnatal lethality to growth-retarded adult resembling type II OI; increased expression of BiP, CHOP and Hsp47; ER dilation (Lisse et al., 2008)
COL2A1 (collagen II)	Collagenopathies of collagen II, such as a spectrum of spondyloepiphyseal dysplasias (SEDs) that range in severity (Nishimura et al., 2005)	Mutations in triple helical domain (Tiller et al., 1995; Fernandes et al., 1998; Weis et al., 1998; Mortier et al., 2000; Godfrey and Hollister, 1988; Vissing et al., 1989), C-propeptide (Zabel et al., 1996; Mortier et al., 2000) and splice donor site (Mortier et al., 2000)	Transgenic mice carrying p.R989C in the triple helical domain die at birth with symptoms similar to SED; increased expression of BiP; ER dilation; increased apoptosis (Gaiser et al., 2002; Hintze et al., 2008)
COL10A1 (collagen X)	Metaphyseal chondrodysplasia, type Schmid (MCDS) (MIM #156500); patients develop disproportionate dwarfism after birth (Lachman et al., 1988; Makitie et al., 2005)	An unidentified mutation that caused MCDS showed ER dilation (Wasylenko et al., 1980)	Mice carrying mutations p.P620fsX672 (13del) (Tsang et al., 2007), p.P620fsX621 (Cdel) (Ho et al., 2007) or p.N617K (Rajpar et al., 2009) display characteristics of MCDS and exhibit UPR induction
COMP (cartilage oligomeric matrix protein)	Multiple epiphyseal dysplasias (EDM) and pseudoachondroplasia (PSACH) (MIM# 177170), which can be thought of as a much more severe form of EDM1 (Briggs and Chapman, 2002)	EDM1: p.469insD (Delot et al., 1998; Delot et al., 1999) PSACH: p.G427E, p.469insDD (Delot et al., 1998; Delot et al., 1999), p.469delD, p.G427E (Hecht et al., 1998), p.D346N (Maddox et al., 1997), p.G465S (Cotterill et al., 2005)	p.T583M (Pirog-Garcia et al., 2007); homozygous mutant mice display a more severe dwarfism than the heterozygous, observed significantly at 9 weeks of age. Increased expression of BiP, phosphorylated eIF2α and CHOP; ATF6 cleavage
MATN3 (matrilin-3)	Patients with EDM display a continuum of clinical severity (Briggs and Chapman, 2002)	EDM1: *MATN3* p.R121W (Cotterill et al., 2005); intracellular accumulation of mutant protein; recombinant mutant protein was associated with the chaperone ERp72	p.V189D (Leighton et al., 2007); homozygous mutant mice display dwarfism, whereas heterozygous mice are phenotypically normal; increased expression of BiP and Grp94; ER dilation

(Nielsen et al., 2000). The same phenotype observed in these transgenic mice was recently reproduced in another strain of mice that ectopically express misfolded thyroglobulin in hypertrophic chondrocytes, thereby demonstrating the direct pathological role of ER stress in MCDS (Rajpar et al., 2009). Mouse models in which genes encoding other ECM components (*Matn3*, *Comp* and *Col1a1*) are mutated have also shown an association between ER stress and the pathogenesis of various skeletal dysplasias (Pirog-Garcia et al., 2007; Leighton et al., 2007; Lisse et al., 2008). These examples contribute to the growing body of evidence that establishes a major contribution of ER stress as an etiological factor in skeletal dysplasia caused by the synthesis of misfolded or unfolded secreted proteins.

Consequence of pathological ER stress: survival or death?

It is clear that the ability of a cell to adapt to ER stress, and the nature of its adaptation strategies, can have a crucial impact on cell fate and can determine the physiological outcome of ER stress in vivo. Table 2 summarizes conditions that can lead to pathological ER stress in different cell types as well as adaptation strategies of the different cell types. In the context of pathogenesis, activation of ER stress is often related to the induction of cell death in a PERK-eIF2α-ATF4-CHOP-dependent or caspase-dependent manner. It has been suggested that the PERK and IRE1 branches of the UPR determine cell fate following ER stress activation (Lin et al., 2009), and that the stabilities of UPR-target mRNAs and proteins [e.g. BiP, CHOP, and growth arrest and DNA damage-inducible protein 34 (GADD34)] can be differentially regulated for adaptation to different stress conditions (Rutkowski et al., 2006). Notably, the ATF4-CHOP branch of the UPR can be specifically inhibited by Toll-like receptor (TLR) signaling. Therefore, immune cells that express TLRs are protected from the detrimental effect of prolonged activation of ER stress during infection by pathogens that express TLR ligands (Woo et al., 2009). Furthermore, recent data suggest that the IRE1-XBP1 pathway can affect the balance between ER-associated protein degradation (ERAD) and autophagy. Specifically, deficiency of XBP1 led to impairment of ERAD and consequently increased autophagy for the clearance of misfolded proteins, hence delaying motoneuron degeneration in a mouse model of amyotrophic lateral sclerosis (Hetz et al., 2009). IRE1-dependent degradation of ER-localized mRNAs encoding chaperones, secreted proteins and membrane proteins can also modulate the ER protein-folding load and cell fate during ER stress (Hollien et al., 2009; Han et al., 2009).

A later stage of ERSS promotes cellular recovery: this is mediated in part by CHOP, which upregulates GADD34 to restore general protein synthesis (through dephosphorylation of eIF2α) and ER oxidase 1α (ERO1α) to enhance protein folding in the ER. CHOP also stimulates the production of reactive oxygen species, inducing oxidative stress that, when too strong, might lead to cell death (Marciniak et al., 2004; Song et al., 2008; Li et al., 2009). This ability of CHOP to promote cellular recovery or cell death (Zinszner et al., 1998) indicates that a fine balance determines the outcome of adaptation to and alleviation of ER stress; this balance might depend on the level of stress and the cellular context. The resolution might lie with the regulation of eIF2α-dependent translation, which is important in modulating ER protein-folding load and in managing oxidative stress (Back et al., 2009). The outcome is very cell-context dependent. Cells expressing mutant proteins that have a folding abnormality can experience cycles of stress activation and recovery that threaten their long-term survival, as observed in the pancreatic β-cells of *Akita* mice, which express a mutant form of insulin (Oyadomari et al., 2002). Whereas the ER stress caused by normal insulin secretion can be tolerated, sustained high expression of insulin due to insulin resistance leads to ER-stress-induced death of pancreatic β-cells in a CHOP-dependent manner (Song et al., 2008).

Table 2. In vivo adaptation to pathological ER stress by different cell types

Cell type	Stress-inducing agent	Related disease	Cellular consequence in mouse model	References
β-cell	(M) Insulin (*Akita* Cys96Tyr)	Diabetes	CHOP-mediated cell death	(Oyadomari et al., 2002)
	Elevated free fatty acid and glucose	Diabetes	ATF3-mediated cell death	(Hartman et al., 2004)
Chondrocyte	(M) Collagen II (p.R989C)	Spondyloepiphyseal dysplasias	Increased apoptosis in transgenic chondrocytes	(Gaiser et al., 2002; Hintze et al., 2008)
	(M) Collagen X (p.P620fsX672)	Metaphyseal chondrodysplasia, type Schmid	'Reprogramming' of transgenic hypertrophic chondrocytes: cell-cycle re-entry and de-differentiation	(Tsang et al., 2007)
	(M) COMP (p.T583M)	Multiple epiphyseal dysplasia 1 and pseudoachondroplasia	Increased apoptosis in transgenic chondrocytes	(Pirog-Garcia et al., 2007)
Fiber cell	(M) Collagen IV (Δex40)	Cataract	Defective fiber-cell terminal differentiation	(Firtina et al., 2009)
Hepatocyte	Elevated free fatty acid	Liver steatosis	Inhibition of apolipoprotein B100 secretion	(Ota et al., 2008)
Macrophage	Lipopolysaccharides	Pathogen invasion	Surviving prolonged ER stress with inhibition of ATF4-CHOP branch of UPR pathway	(Woo et al., 2009)
Osteoblast	(M) Collagen I (*Aga2*)	Osteogenesis imperfecta	Increased apoptosis in transgenic osteoblasts	(Lisse et al., 2008)
Oligodendrocyte	(M) Proteolipid protein 1 (*rsh* I186T)	Pelizaeus-Merzbacher disease	CHOP is anti-apoptotic and promotes recovery of oligodendrocytes	(Southwood et al., 2002)
	IFNγ	Multiple sclerosis	Myelinating activity and PERK-dependent survival	(Lin et al., 2006; Lin et al., 2008)
Schwann cell	(M) Protein zero (P$_0$) (S63del)	Charcot-Marie-Tooth 1B neuropathy	CHOP-mediated dysfunction (demyelination), but not cell death	(Pennuto et al., 2008)

(M), gene mutation.

In many instances, ER dilation is associated with disorganization of the ECM in patients and mouse models with skeletal dysplasias (Brodie et al., 1999; Bonnemann et al., 2000; Hicks et al., 2001; Hecht et al., 2004; Paupe et al., 2004). For mutations that affect the folding or secretion of collagen I and II, the relative contribution of ER stress to the disease outcome is difficult to assess because of the developmental requirement for these proteins and their roles as structural components of the ECM. For example, mice heterozygous for either a *Col1a1*- or *Col2a1*-null allele develop skeletal defects (Lohler et al., 1984; Harbers et al., 1984; Bonadio et al., 1990; Li et al., 1995; Sahlman et al., 2001) and, therefore, loss of collagen function is an integral part of the pathogenesis observed. By contrast, the effects of null mutations of *Comp*, *Col10a1* and *Matn3* in mice are subtle (Rosati et al., 1994; Kwan et al., 1997; Svensson et al., 2002; Ko et al., 2004; van der Weyden et al., 2006), facilitating evaluation of the impact of ER stress in skeletal dysplasia. In humans, the relative contribution of ER stress to the skeletal phenotypes that are associated with mutations in *COMP*, *COL10A1* and *MATN3* cannot be determined, because the effects of true null mutations of these genes are not known. In cultured chondrocytes or transfected cells, a moderately severe mutation (p.469delD) in cartilage oligomeric matrix protein (COMP) (Briggs et al., 1995), which is associated with the disorder pseudoachondroplasia, causes reduced cell viability in a dose-dependent manner. Affected cells retained other ECM proteins in the ER in addition to the mutant COMP protein, showed expansion of ER cisternae and induction of ER stress markers, and were unable to make a proper ECM (Dinser et al., 2002).

In contrast to patients with pseudoachondroplasia, transgenic mice expressing a moderate level of p.469delD COMP protein showed only a very mild dwarfism (Schmitz et al., 2008). When bred onto a COMP-deficient background, the dwarfism became more severe, which correlated with increased intracellular retention of mutant proteins and apoptosis in chondrocytes (Schmitz et al., 2008). The authors suggested that the wild-type COMP protein modulated the phenotype by facilitating the secretion of mutant COMP proteins as heteropentamers, whereas the mutant COMP protein alone could not form secretable homopentamers.

The above examples indicate that mutations capable of causing ER stress and cell death in vitro do not necessarily induce cell death constitutively in vivo. The in vivo environment, in which other pro-survival pathways, such as the hypoxic stress response (Schipani et al., 2001) and autophagy (Settembre et al., 2008), are present, might enable cells to better adapt to and overcome ER stress.

Adapting to and surviving ER stress in vivo by reprogramming cellular function

The transcriptional and translational measures of ERSS undoubtedly alter both the transcriptome and proteome of the cell, and might thereby affect its differentiation status. For example, the induction of 'ectopic' bZIP factors during ER stress might affect the existing network of bZIP transcription factors, as well as the global epigenetic status, altering the transcriptional or developmental program. However, such 'reprogramming' not only might allow the cell to survive and adapt to ER stress, but also might interfere with normal cellular activity, which, in turn, impacts organ and/or tissue function. Therefore, surviving and adapting to ER stress might not be equivalent to achieving a full recovery of cellular homeostasis and tissue and/or organ function. Crucial to the outcome is whether a full recovery is achieved eventually.

There is emerging evidence that one cellular strategy of adaptation to ER stress is to shut down mutant gene expression by altering the transcriptional or differentiation program. Reprogramming of differentiation has been observed in two transgenic mouse models that express similar MCDS *Col10a1* mutations (13del and Cdel; see also Table 1) in hypertrophic chondrocytes (Ho et al., 2007; Tsang et al., 2007). In the 13del mice, as the chondrocytes begin to hypertrophy, misfolded collagen X is expressed and rapidly induces ERSS (Tsang et al., 2007). Instead of undergoing terminal differentiation and subsequent apoptosis (similar to wild-type hypertrophic chondrocytes), the 13del mutant cells survive. In addition, many of the mutant cells re-express pre-hypertrophic markers and can even re-enter the G1 phase of the cell cycle and become arrested, while transcription of both *Col10a1* and the 13del transgene is downregulated. Some of the mutant cells coexpress both pre-hypertrophic and terminal differentiation markers, suggesting that reprogrammed differentiation helps them to adapt to ER stress and recover. However, the reprogramming process also renders the mutant cells dysfunctional for extended periods before they recover from ER stress; they accumulate in the hypertrophic zone, resulting in retardation of longitudinal bone growth (Tsang et al., 2007). This is supported by the phenotype of a similar MCDS mouse model that expresses the p.N617K mutation of *Col10a1*. Retarded endochondral bone formation occurs due to disrupted expression of vascular endothelial growth factor (VEGF) in the ER-stressed hypertrophic zone, subsequently causing impaired vascular invasion of and chondroclast and osteoclast recruitment to the transition zone (Rajpar et al., 2009). The reprogramming of hypertrophic chondrocytes depends on the expression of misfolded protein and is cell autonomous, as normal hypertrophic chondrocytes in a 'chimeric' growth plate do not become reprogrammed (Tsang et al., 2007).

These findings have implications for the interpretation of the underlying causality of mutant phenotypes. For example, the chondrodysplasia phenotype of *Bbf2h7*-null mice has been attributed to perturbed ECM secretion and a consequentially abnormal ECM (Saito et al., 2009). The disruption in the pattern of markers that characterize differentiation states of chondrocytes in the growth plates of *Bbf2h7* mutants is reminiscent of the alterations that are found in the aggrecan mouse mutant, *cmd* (Wai et al., 1998), in which ER stress is triggered (Tsang et al., 2007). This might therefore indicate that chondrocyte differentiation is perturbed in the *Bbf2h7* mutant. In the light of the link between ER stress in chondrocytes and alteration of their differentiation program, the root causes of the phenotype of *Bbf2h7*-null mice are probably more complex.

Mutations that induce ER stress and cellular reprogramming in chondrocytes or osteoblasts, which secrete ECM proteins, probably also impair the protein synthesis and secretory capacity of these cell types. Defective secretion might be exacerbated by premature intracellular matrix assembly, as observed in pseudoachondroplasia chondrocytes in which mutant COMP interacts with collagen II, collagen IX and matrilin-3 (MATN3) to form a stable network inside the ER (Merritt et al., 2007). The abnormal ECM might in turn generate aberrant signals that contribute to the reprogramming process. These cases might involve a secondary extracellular effect that is highly context dependent and difficult to demonstrate in the background of cellular reprogramming, and contributes to the phenotypic variety of skeletal dysplasias. In homozygous mice expressing a 'mild' structural mutation of COMP (p.Thr583Met)

(Pirog-Garcia et al., 2007), ER stress was induced, proliferation of growth-plate chondrocytes was reduced and apoptosis was increased. The authors of this latter study noticed a similar effect on the proliferation and apoptosis of chondrocytes in mice carrying a cartilage-specific deletion of β1 integrin (Aszodi et al., 2003) and in α10-integrin-null mice (Bengtsson et al., 2005). Thus, it is possible that, in the *Comp* mutant, cell-matrix interaction is altered as a secondary effect of ER stress.

Effects of ERSS on cell differentiation are not restricted to skeletal cells. In lens fiber cells, expression of either ectopic collagen IV isoforms or mutant collagen IV α-chain induces ER stress and impairs fiber cell differentiation, leading to cataract formation (Firtina et al., 2009); cell death is observed only in a group of ectopic collagen-IV-expressing fiber cells that experience prolonged UPR activation. ER-stress-inducing drugs result in de-differentiation in a differentiated thyroid cell line through a Src-mediated signaling pathway (Ulianich et al., 2008). Furthermore, it has been shown that, in a mouse model of Charcot-Marie-Tooth 1B neuropathy, CHOP expression causes Schwann cell dysfunction (demyelination or hypomyelination), but not cell death (Pennuto et al., 2008); these observations are similar to JUN-dependent Schwann cell de-differentiation after nerve injury (Parkinson et al., 2008; Gow and Wrabetz, 2009). These examples further implicate alteration of cell fate by reprogramming to a less differentiated state (which might be energetically more favorable than maintaining the differentiated state) as an adaptive strategy during ER stress. However, such a strategy to avoid or adapt to ER stress probably also impacts normal cellular function in vivo. Recognizing the complexity and double-edged outcome of implementing 'rescue' mechanisms and achieving cell survival in the face of ER stress enriches our understanding of the functional relationship between mutation and phenotype. This relationship is clearly the sum of the downstream consequences that result from the expression of unfolded proteins, which has an impact at both intracellular and extracellular levels.

Perspectives: therapeutic protection from ER stress

In the context of pathogenesis, if we can predict when ER stress will happen (and its consequence) before clinical symptoms of diseases associated with prolonged stress occur, it is possible that pharmaceutical prevention of ER stress or the enhancement of reprogramming signals will allow affected cells to quickly recover, ultimately improving clinical outcome. The recognition that ER stress contributes to disease pathology and that adaptation of cells to ER stress is an important determinant of clinical outcome offers new therapeutic possibilities. Chemical compounds are available that can alleviate ER stress, mainly through enhancing protein-folding capacity and/or degrading misfolded proteins. These compounds include chemical chaperones, such as 4-phenyl-butyrate (Ozcan et al., 2006; Yam et al., 2007; Datta et al., 2007), ER-chaperone inducers, such as BiP inducer X (BIX) (Kudo et al., 2008), and inducers of autophagy, such as rapamycin (Ravikumar and Rubinsztein, 2006; Kouroku et al., 2007; King et al., 2008). These compounds can be used alone or synergistically to enhance different aspects of protein homeostasis (Mu et al., 2008). Alternatively, chemical-genetic approaches to artificially induce the PERK (Lu et al., 2004) or IRE1 (Lin et al., 2007; Han et al., 2008) pathway promote the survival of cells in conditions of ER stress in vitro. Other possible approaches could include the development of novel drugs that target proteins such as GADD34

to fine-tune the recovery of translation of both mutant and normal proteins in cells under ER stress.

Pathological ERSS can also be reduced using gene-inactivating viral vectors, ribozymes or RNA interference to reduce or eliminate mutant gene expression. This has been demonstrated in vitro by the restoration of the bone-forming activity of mesenchymal progenitor cells from patients with OI through silencing the mutant *COL1A1* or *COL1A2* alleles or mRNAs (Dawson and Marini, 2000; Millington-Ward et al., 2002; Millington-Ward et al., 2004; Chamberlain et al., 2004; Chamberlain et al., 2008). In addition, a diet containing antioxidants might also help to reduce the deleterious effect of ERSS, as shown in a diabetic mouse model (Back et al., 2009). Although these approaches are promising, it is important to realize that the ER stress response is an essential cellular regulatory pathway and its manipulation in vivo could have unintended consequences. Crucial objectives for the future are to gain a better understanding of how the ERSS response intersects with and cooperates with other regulatory pathways – such as those that handle oxidative and hypoxic stress – in ameliorating ER stress, and to characterize the molecular mechanisms that underlie the reprogramming of cellular differentiation.

The potential of harnessing ERSS mechanisms to overcome various forms of pathological ER stress is clear. In addition, recent reports have linked antioxidants with enhanced cellular reprogramming in the generation of human induced pluripotent stem (iPS) cells and the repression of the mitochondrial/oxidative stress pathway in iPS cells (Prigione et al., 2010). The finding that ERSS influences differentiation plasticity raises the thought-provoking possibility that manipulation of ERSS mechanisms could be extended to facilitate reprogramming or reversing the differentiation of cells at multiple levels, even to the progenitor or pluripotent state.

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