

## REVIEW ARTICLE

# The expanding roles of endoplasmic reticulum stress in virus replication and pathogenesis

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The endoplasmic reticulum (ER) is a cellular membrane organelle that plays important roles in virus replication and maturation. Accumulating evidence indicates that virus infection often disturbs ER homeostasis and leads to ER stress, which is associated with a variety of prevalent diseases. To cope with the deleterious effects of virus-induced ER stress, cells activate critical signaling pathways including the unfolded protein response (UPR) and intrinsic mitochondrial apoptosis, which have complex effects on virus replication and pathogenesis. In this review, we present a comprehensive summary of recent research in this field, which revealed that about 36 viruses trigger ER stress and differentially activate ER stress-related signaling pathways. We also highlight the strategies evolved by viruses to modulate ER stress-related signaling networks including immune responses in order to ensure their survival and pathogenesis. Together, the knowledge gained from this field will shed light on unveiling the mechanisms of virus replication and pathogenesis and provide insight for future research as well as antiviral development.

**Abbreviations:** ER, endoplasmic reticulum; UPR, unfolded protein response; PERK, pancreatic ER kinase (PKR)-like ER kinase; IRE1, inositol-requiring enzyme 1 (IRE1); ATF6, activating transcription factor 6 (ATF6); GRP78, glucose regulated protein 78; eIF2 $\alpha$ , the alpha subunit of eukaryotic translation initiation factor-2; ATF4, activating transcription factor-4; GADD34, growth arrest and DNA damage-inducible 34; PP1, protein phosphatase 1; XBP1, X box-binding protein 1; PDI, protein disulphide isomerase; DR5, death receptor-5; TRB3, tribbles-related protein 3; ERO1 $\alpha$ , ER oxidase 1 $\alpha$ ; ROS, reactive oxygen species; PVX, Potato virus X; CHIKV, Chikungunya virus; SFV, Semliki Forest Virus; SINV, Sindbis virus; LCMV, Lymphocytic choriomeningitis virus; ASFV, African swine fever virus; RV, Rubella virus; HEV, Hepatitis E virus; BMV, Brome mosaic virus; IPNV, Infectious pancreatic necrosis virus; HN, hemagglutinin-neuraminidase; CCHFV, Crimean-Congo hemorrhagic fever virus; DUGV, Dugbe virus; TULV, Tula virus; MHV, Murine hepatitis virus; IBV, Infectious bronchitis virus; PEDV, porcine epidemic diarrhea virus; SARS-CoV, Severe Acute Respiratory Syndrome Coronavirus; BVDV, Bovine viral diarrhea virus; DENV, Dengue virus; HCV, Hepatitis C virus; JEV, Japanese encephalitis virus; WNV, West Nile virus; HBV, Hepatitis B virus; HBx, HBV X protein; EBV, Epstein-Barr virus; HSV-1, Herpes simplex virus type 1; HCMV, Human cytomegalovirus; VZV, Varicella Zoster virus; RGNNV, Betanodavirus redspotted grouper nervous necrosis virus; IAV, Influenza A virus; CDV, Canine distemper virus; RSV, Human respiratory syncytial virus; SV5, Simian Virus 5; CVB3, Coxsackievirus B3; RRV, Rhesus rotavirus; HIV, Human immunodeficiency virus; MoMuLV, Moloney murine leukemia virus-TB; FrCas<sup>E</sup>, Neurovirulent mouse retrovirus; VSV, Vesicular stomatitis virus; VV, virus vaccinia virus; GFLV, Grapevine fanleaf nepovirus; YFV, Yellow fever virus; PKR, double-stranded RNA-dependent protein kinase (PKR); GCN2, general control non-repressible-2; MMP, mitochondrial membrane potential; MNV-1, murine norovirus; COS2, cyclo-oxygenase 2; gB, glycoprotein B; CLT, clotrimazole; DRACO, Double-stranded RNA activated caspase oligomerizer

**Keywords**

Antiviral therapy, apoptosis, ER-to-nucleus signaling pathway, unfolded protein response, virological treatments

**History**

Received 19 February 2013

Revised 28 May 2013

Accepted 7 June 2013

Published online 4 September 2013

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**Introduction**

The ER is an important cellular organelle that controls several critical aspects of cellular processes such as cellular proteins folding and post-translational modifications. It also plays pivotal roles in viral infection processes. As intracellular parasites, viruses must utilize the ER to complete some of

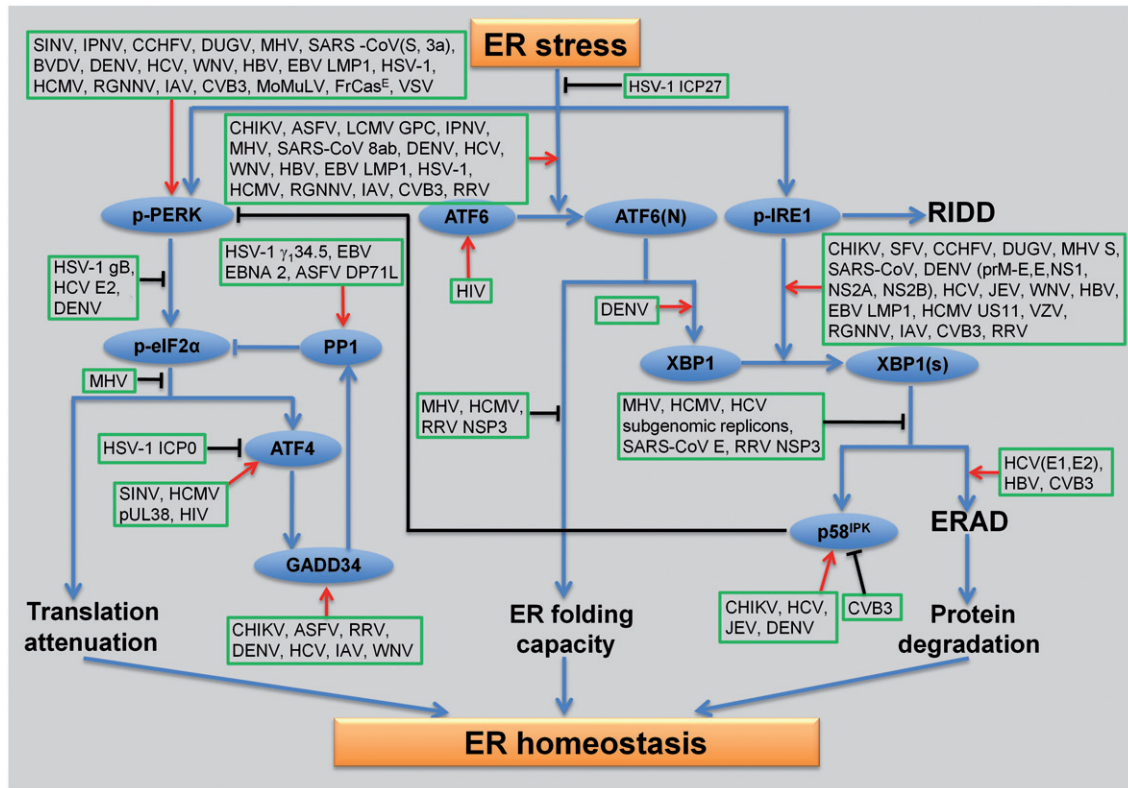


Figure 1. Modulation of the UPR by viruses. In virus-infected cells, three membrane transducers: PERK, ATF6, IRE1 are differentially activated to gain ER homeostasis. Arrows represent activation of the UPR components by virus infection; lines indicate inhibition of the UPR components by virus infection or inhibition between the UPR components.

their life cycles including virus entry, viral protein synthesis and modifications, genome replication and virus assembly (He, 2006; Inoue & Tsai, 2013). During productive viral infection, viruses hijack the host translation apparatus to produce a large amount of viral proteins accumulated in the ER lumen. Some viruses even utilize the ER as their replication sites such as Hepatitis C virus (HCV) (Moradpour et al., 2007) and African swine fever virus (ASFV) (Galindo et al., 2012). These activities of alien pathogens may disrupt the homeostasis of the ER and lead to a rapid accumulation of malformed and unfolded proteins inside the ER lumen. Consequently, cells face great challenges and stress, so called ER stress, which may cause a wide variety of prevalent diseases such as neurodegenerative diseases, renal disease, liver disease and cancer (Hetz, 2012). To alleviate the detrimental effects of ER stress, cells evolved an ER-to-nucleus signaling pathway termed the unfolded protein response (UPR), which functions to restore the ER homeostasis (Bernales et al., 2006; Schroder & Kaufman, 2005). However, if under unsolved or intense ER stress, cells would fail to regain the ER homeostasis and instead, initiate the intrinsic apoptotic cascades, which have been thought to primarily associate with virus pathogenesis (Galluzzi et al., 2008). Here, we give a comprehensive description about the dynamic relationship between ER stress and virus infection with emphasis on how UPR and apoptosis influence viral replication and pathogenesis as well as the strategies employed by viruses to cope with ER stress. In particular, we discuss the current developed ER stress-related antivirals including broad-spectrum therapy by targeting ER

stress signaling pathways and specifically targeted antiviral therapy by disturbing the functions of ER stress-triggering viral proteins.

## Virus, ER stress and the UPR

### Overview of ER stress-mediated UPR

In mammalian cells, the ER stress is sensed and mediated by three ER transmembrane receptors: pancreatic ER kinase (PKR)-like ER kinase (PERK), inositol-requiring enzyme 1 (IRE1) and activating transcription factor 6 (ATF6). In resting cells, these three sensors are maintained in inactive states through interactions with the ER resident chaperone glucose regulated protein 78 (GRP78); while when unfolded or misfolded proteins accumulate in the ER lumen, GRP78 dissociates from these three transducers, resulting in their activation and initiation of the UPR (Figure 1).

#### PERK-eIF2 $\alpha$ pathway

PERK is a type I ER-resident transmembrane kinase (Bernales et al., 2006; Lin et al., 2008). When it senses ER stress, PERK undergoes oligomerization and autophosphorylation to form active PERK, which then phosphorylates the  $\alpha$  subunit of the eukaryotic translation initiation factor-2 (eIF2 $\alpha$ ) to reduce global protein synthesis and thus relieve the ER stress (Harding et al., 2000). However, this limited amount of active eIF2 $\alpha$  selectively increases the translation of activating transcription factor-4 (ATF4), a pro-survival transcription factor that facilitates cell survival through activating

genes involved in amino acids biosynthesis and transport, stress response, redox reactions and protein secretion (Harding et al., 2000). One target gene of ATF4 is growth arrest and DNA damage-inducible 34 (GADD34), a protein phosphatase 1 (PP1) regulatory subunit that recruits PP1 to dephosphorylate eIF2 $\alpha$  and derepresses PERK-eIF2 $\alpha$ -mediated translation attenuation, thereby constituting a negative feedback loop in the UPR (Novoa et al., 2001).

#### *IRE1-XBP1 pathway*

The IRE1-XBP1 pathway is a highly conserved UPR branch in eukaryotic cells, which starts with activation of IRE1, a dual-activity enzyme harboring a serine-threonine kinase domain and an endoribonuclease domain (Sidrauski & Walter, 1997; Szegezdi et al., 2006). When IRE1 dissociates from GRP78, it undergoes oligomerization, which in turn activates its kinase and endonuclease activities. Activated IRE1 removes a 26-nucleotide intron from X box-binding protein 1 (XBP1) mRNA to form a spliced XBP1 (XBP1(s)), which is subsequently translated into a basic-zipper (bZIP) transcription factor that activates the transcription of genes that enhance the ER protein-folding capacity, phospholipid biosynthesis and ER-associated protein degradation (ERAD) (Lee et al., 2003; Shaffer et al., 2004). XBP1(s) is also able to activate the HSP40 family member P58<sup>IPK</sup>, which can bind PERK and inhibit its activity to phosphorylate eIF2 $\alpha$  (Yan et al., 2002). IRE1, by itself, can degrade ER-bound mRNAs through the regulated IRE1-dependent decay (RIDD) pathway to reduce protein translation and limit unfolded protein load in the ER lumen (Hollien et al., 2009).

#### *ATF6 pathway*

ATF6 is a bZIP transcription factor but is initially synthesized as a type II transmembrane protein having an ER stress-sensing luminal domain, transmembrane domain and a cytosolic N-terminal domain (Walter & Ron, 2011). In response to ER stress, ATF6 is packaged in ER-derived transport vesicles and translocates into the Golgi complex, where two specific proteases cleave its transmembrane domain and liberate its active N-terminal DNA binding domain, ATF6 (N) (Ye et al., 2000). ATF6 (N) then translocates into the nucleus and activates genes that improve the ER folding capacity such as ER chaperones, protein disulphide isomerase (PDI) as well as XBP1 (Yoshida et al., 2001). Overall, these three branches function to remedy ER stress by reducing the flux of newly synthesized polypeptides into the ER, degrading ER-localized proteins and expanding the ER folding capacity.

### **Induction of ER stress and UPR by virus infection**

Emerging evidence indicates that a large number of viruses are capable of eliciting ER stress during their infection, which is summarized in Table 1. Currently, 35 animal and 1 plant viruses, which belong to 18 virus families with the majority being RNA viruses, have been reported to trigger ER stress indicators including inducing ER chaperones and activating three UPR sensors. 19 viruses have been reported to induce the expression of ER chaperone proteins (Bip, GRP78,

GRP94, calnexin, calreticulin), which might be caused by accumulation of malformed and unfolded viral proteins inside the ER lumen. These viruses include Potato virus X (PVX), Chikungunya virus (CHIKV), ASFV, Infectious pancreatic necrosis virus (IPNV), Tula virus (TULV), Porcine epidemic diarrhea virus (PEDV), Bovine viral diarrhea virus (BVDV), Dengue virus (DENV), Japanese encephalitis virus (JEV), Hepatitis B virus (HBV), Hepatitis E virus (HEV), Herpes simplex virus type 1 (HSV-1), Canine distemper virus (CDV), Human respiratory syncytial virus (RSV), Simian virus 5 (SV5), Coxsackievirus B3 (CVB3), Human immunodeficiency virus (HIV), Moloney murine leukemia virus-TB (MoMuLV), and Neurovirulent mouse retrovirus (FrCas<sup>E</sup>) (Table 1). Intriguingly, upon virus-triggered ER stress, the three UPR transmembrane sensors are differentially activated: 19, 17 and 14 viruses have been reported to activate the PERK-eIF2 $\alpha$ , IRE1-XBP1 and ATF6 branches, respectively (Table 1 and Figure 1), implying that cells prefer to initiate the PERK-eIF2 $\alpha$  pathway in response to virus infection, presumably because the PERK-eIF2 $\alpha$ -mediated global translational attenuation can efficiently restrict virus replication by preventing the synthesis of viral and cellular proteins crucial for virus life cycle (Baltzis et al., 2004; Pena & Harris, 2011). Nonetheless, it should be noted that at least two other eIF2 $\alpha$  kinases, double-stranded RNA-dependent protein kinase (PKR) and general control non-derepressible-2 (GCN2) could be activated upon virus infection to restrict virus replication by preventing protein translation (Berlanga et al., 2006; Garcia et al., 2007). Hence, the role of PERK in translational attenuation could change depending on the virus.

### **Characteristics of ER stress-triggering viruses and viral proteins**

Four pieces of information could be inferred from these published data about the characteristics of ER stress-triggering viruses and viral proteins, which could be useful in predicting the outcome of virus infection and unveiling the underlying pathogenic mechanisms. First, ER stress-triggering viruses are usually those that have cytopathogenic or virulence effects. For the same type of viruses, the avirulent strain triggers no or mild ER stress such as the nonvirulent mouse retrovirus F43 (Dimcheff et al., 2004), noncytopathic mild Dugbe virus (DUGV) (Rodrigues et al., 2012), and the attenuated HSV-1 strain KOS (Mao et al., 2001), suggesting that ER stress is associated with viral virulence and pathogenesis. One exception is Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) lacking the envelope (E) gene, which is attenuated *in vivo* but elicits higher UPR than SARS-CoV with E gene (DeDiego et al., 2011). Second, the ER-tropic viruses that exploit the host ER as an integral part of their life cycle are prone to trigger ER stress, such as *Flaviviridae* viruses (HCV, BVDV and JEV), *Asfarviridae* virus ASFV, and *Coronaviridae* viruses, which use the ER as the primary site of glycoprotein biosynthesis, genome replication and even particle assembly (de Haan & Rottier, 2005; Galindo et al., 2012; Knoops et al., 2008; Leyssen et al., 2000; Oostra et al., 2007). Third, viruses that modify the ER in order to create a compartment suitable for virus replication are also inclined to induce the ER stress. For example, DENV,

Table 1. Interconnection between of ER stress and virus infection.

Virus	Impact of virus infection on ER stress		References
	UPR (life)	Apoptosis (death)*	
<i>Alphaflexiviridae</i> (RNA) PVX	Induces BiP, PDI, calreticulin, and calmodulin Viral proteins: PVX TGBp3	Unknown	UPR alleviates ER stress-related apoptosis. Ye et al., 2011
<i>Alphavirus</i> (RNA) CHIKV	Induces BiP, Hsp90, GADD34 and p58 <sup>IPK</sup> , presses eIF2 $\alpha$ phosphorylation Viral proteins: CHIKV nsP4	Unknown	Unknown Rathore et al., 2013
SFV	Activates IRE1-XBP1 Viral proteins: SFV envelope glycoproteins	Leads to loss of MMP <sup>+</sup> , cytochrome <i>c</i> release and activation of CHOP, caspase-3, -8, -9 and -12	Activated IRE1-XBP1 branch could promote SFV-induced apoptosis. Barry et al., 2010; Urban et al., 2008
SINV	Activates IRE1-XBP1 and PERK, induces translation of ATF4	Induces CHOP, activates apoptosis	Activated UPR limits SINV replication. Nivitchanyong et al., 2009; Perry et al., 2012; Rathore et al., 2013
<i>Arenavirus</i> (RNA) LCMV	Activates ATF6 Viral proteins: LCMV glycoprotein precursor (GPC)	Unknown	Activated ATF6 branch promotes virus replication and cell viability. Pasqual et al., 2011
<i>Asfarviridae</i> (DNA) ASFV	Induces calnexin and calreticulin, activates ATF6 and GADD34	Activates caspase-3, -9 and -12, inhibits CHOP	Activated ATF6 pathway facilitates virus replication. The early activation of caspase-3 is required for virus exit. Andrés et al., 1998; Galindo et al., 2012; Netherton et al., 2004
<i>Bimaviridae</i> (RNA) IPNV	Induces GRP78, activates ATF6 and PERK-eIF2 $\alpha$	Induces PERK-mediated CHOP transcription, MMP loss and activates caspase-3 and -8 Viral protein: IPNV VP5.	ER stress leads to IPNV-infected cell death. Hong et al., 2002; Hong et al., 2005; Huang et al., 2011
<i>Bunyaviridae</i> (RNA) CCHFV	Activates IRE1-XBP1 and PERK	Induces PUMA, Noxa, CHOP and Bax	Activated ER stress and apoptosis contributes to virus pathogenesis. Rodrigues et al., 2012
DUGV TULV	Induces XBP1 and PERK Induces GRP78 transcription Viral proteins: TULV glycoproteins	Induces no apoptosis Induces CHOP and JNK pathways, activates caspase-12, -8 and -3	Unknown ER stress leads to TULV-infected cell death. Rodrigues et al., 2012 Li et al., 2005
<i>Coronavirus</i> (RNA) MHV	Modulates three UPR branches Viral protein: MHV spike (S) protein Unknown	Unknown	MHV modulates the UPR to facilitate its replication. Bechill et al., 2008; Versteeg et al., 2007
IBV	Induces GRP78 Viral protein: PEDV E and N	Induces CHOP/GADD153 and Bak, activates Mcl-1	Apoptosis facilitates virus release. Zhong et al., 2012
PEDV	Activates PERK-eIF2 $\alpha$ and IRE1-XBP1 Viral proteins: SARS S, 3a, 8ab and E	Induces apoptosis Viral protein: SARS-CoV E	PEDV E might protect cells from apoptosis. Xu et al., 2013a; Xu et al., 2013b
SARS			SARS-CoV E reduces apoptosis, which might limit virus production and dissemination. Chan et al., 2006; DeDiego et al., 2011; Minakshi et al., 2009; Sung et al., 2009

(continued)



Table 1. Continued

Virus	Impact of virus infection on ER stress		References
	UPR (life)	Apoptosis (death)*	
<i>Flaviviridae</i> (RNA) BVDV	Induces GRP78, activates PERK-eIF2 $\alpha$	Induces CHOP, represses Bcl-2, activates caspase-12 Viral proteins: envelope glycoproteins Damages MMP	Jordan et al., 2002; Schweizer & Peterhans, 1999
DENV	Activates PERK-eIF2 $\alpha$ , ATF6 and IRE1-XBP1, induces GADD34 Viral proteins: DENV glycoproteins prM-E, E, NS1, NS2A, and NS2B	Activates PERK-eIF2 $\alpha$ -ATF4-CHOP pathway Viral proteins: HCV E1, E2 and core	Edgil et al., 2006; Limjindaporn et al., 2009; Pena & Harris, 2011; Umareddy et al., 2007; Yu et al., 2006
HCV	Activates three UPR branches. HCV sub-genomic replicons repress XBP1(s). Viral proteins: HCV E1, E2, NS2, NS4B, NS5A and core	Activates PERK-eIF2 $\alpha$ -ATF4-CHOP pathway Viral proteins: HCV E1, E2 and core	Benali-Furet et al., 2005; Bureau et al., 2001; Chan & Egan, 2005; Joyce et al., 2009; Li et al., 2009; Merquiol et al., 2011; Tardif et al., 2004; von dem Bussche et al., 2010
JEV	Induces calnexin, PDI, GRP78 and GRP94, activates IRE1-XBP1 Viral proteins: JEV glycoproteins prM, E, NS1, NS2A, NS2B and NS4B	Induces CHOP and ER stress-mediated cell apoptosis	Su et al., 2002; Wu et al., 2011; Yu et al., 2006
WNV	Activates XBP1 splicing, ATF6 proteolysis and eIF2 $\alpha$ phosphorylation Viral proteins: nonstructural proteins	Induces GADD34 and CHOP, activates caspase-3 Viral proteins: WNV nonstructural proteins	Ambrose & Mackenzie, 2011; Medigeshi et al., 2007
<i>Hepadnaviridae</i> (DNA) HBV	Induces GRP94, activates three UPR arms and ERAD pathway Viral proteins: HBx, S, and SHBs	Activates caspase-3 and -9 Viral protein: HBx	Arzberger et al., 2010; Kuo et al., 2012; Lazar et al., 2012; Li et al., 2007; Li et al., 2011
<i>Hepeviridae</i> (RNA) HEV	Interacts with Grp78, induces Hsp72, Hsp70B' and Hsp40. Viral protein: HEV ORF2	Activates PERK- eIF2 $\alpha$ -ATF4-CHOP pathway, but has no effect on apoptosis Viral protein: HEV ORF2	John et al., 2011; Yu et al., 2011
<i>Herpesviridae</i> (DNA) EBV	Activates three UPR branches Viral protein: EBV LMP1	Unknown	Bhende et al., 2007; Lee & Sugden, 2008a, 2008b; Sun & Thorley-Lawson, 2007; Taylor et al., 2011
HSV-1	Induces GRP78, activates ATF6 and PERK-eIF2 $\alpha$ -ATF4 at different infection stages Viral proteins: HSV-1 ICPO, glycoprotein B, $\gamma_1$ ,34.5 and Us11 Modulates three UPR branches Viral proteins: HCMV US2, US11, pUL37x1, and pUL38	Unknown	Boyce et al., 2005; Burnett et al., 2012; Cheng et al., 2005; He et al., 1997; Mao et al., 2001; Mulvey et al., 2007; Mulvey et al., 2003
HCMV	Modulates three UPR branches Viral proteins: HCMV US2, US11, pUL37x1, and pUL38	Inhibits apoptosis Virus proteins: HCMV pUL38, UL36, and pUL37x1	Buchkovich et al., 2008; Hegde et al., 2006; Isler et al., 2005; Keay et al., 1995; Qian et al., 2011; Sharon-Friling et al., 2006; Skaletskaya et al., 2001; Tirosh et al., 2005; Xuan et al., 2009
VZV	Unknown	Unknown	Carpenter et al., 2011

<i>Nodaviridae</i> (RNA) RGNNV	Activates IRE1-XBP1, induces CHOP Viral proteins: VZV gE and gI	Activates caspase-12, inhibits PERK-mediated Bcl-2 Viral proteins: RGNNV $\alpha$ and B2	Activated XBP1(s) might lead to VZV-caused ER expansion.	Su et al., 2011; Wu et al., 2010
<i>Orthomyxoviridae</i> (RNA) IAV	Activates three UPR branches Viral proteins: IAV hemagglutinin A	Induces caspase-3, -8, -9, -12, CHOP and GADD34	GRP78 facilitates virus replication at a middle replication stage.	Hassan et al., 2012; Roberson et al., 2012
<i>Paramyxoviridae</i> (RNA) CDV	Induces calnexin	Alters $Ca^{2+}$ homeostasis, induces calnexin and CHOP	CDV-induced apoptosis could eventually lead to the neurodegeneration.	Brunner et al., 2007, 2012
RSV	Induces GRP78 and calnexin	Virus proteins: CDV glycoproteins (F and H) Activates caspase-3 and -12 RSV NS1 and NS2 suppress early apoptosis.	RSV NS1 and NS2 suppress early apoptosis to facilitate virus replication. The RSV-induced apoptosis is a major cause of destruction of the lung epithelial in RSV-infected patients.	Bitko & Barik, 2001; Bitko et al., 2007
SV5	Induces GRP78, GRP94 and CHOP Viral protein: SV5 HN glycoprotein and V	Inhibits apoptosis Virus proteins: SV5 SH and V	Unknown	Sun et al., 2004; Watowich et al., 1991
<i>Picomaviridae</i> (RNA) CVB3	Induces GRP78, activates three UPR branches and ERAD; down-regulates p58 <sup>IPK</sup>	Induces CHOP-mediated apoptosis	ER stress mediates CVB3-induced apoptosis.	Zhang et al., 2010
<i>Reoviridae</i> (RNA) RRV	Modulates IRE1-XBP1 and ATF6 Viral proteins: RRV NSP3	Induces apoptosis, activates CHOP expression	Unknown	Martin-Latil et al., 2007; Trujillo-Alonso et al., 2011
<i>Retrovirus</i> (RNA) HIV	Activates ATF6 and ATF4, induces Bip	Triggers cytochrome c release, activates caspase-8, -9 and -3	Up-regulated ATF4 enhances HIV replication	Caselli et al., 2012; Lindl et al., 2007; Nie et al., 2002
MoMuLV	Activates PERK-eIF2 $\alpha$ , induces GRP78 and CHOP	Leads to altered $Ca^{2+}$ homeostasis, MMP dissipation, activation of caspase-3, -9, and -12	ER stress mediates MoMuLV-induced apoptosis.	Kim et al., 2004; Liu et al., 2004
FrCas <sup>E</sup>	Viral protein: MoMuLV gPr80 <sup>mv</sup> Induces GRP78, CHOP, calreticulin and PERK Viral proteins: FrCas <sup>E</sup> envelope protein pr85 <sup>env</sup>	Unknown	Unknown	Dimcheff et al., 2003
<i>Rhabdoviridae</i> (RNA) VSV	Activates PERK-eIF2 $\alpha$ Viral protein: VSV G	Activates caspase-12, caspase-8 and -9 Viral protein: VSV M	Activated PERK pathway inhibits VSV replication.	Baltzis et al., 2004; Gaddy and Lyles, 2005; Machamer et al., 1990

\*only viruses that affect intrinsic mitochondrial apoptosis have been listed;

†MMP, mitochondrial membrane potential.

VZV and JEV have been shown to stimulate ER proliferation and HCV has been reported to alter the ER structure (Carpenter et al., 2011; Hase et al., 1992; Moradpour et al., 2007; Umareddy et al., 2007; Yu et al., 2006). Fourth, the viral proteins that are synthesized, processed or located in the ER tend to trigger the ER stress, including PXV TGBp3, PEDV (E, N), the glycoproteins of viruses (SFV, LCMV, TULV, BVDV, DENV, JEV, VZV, SV5, HSV-1 and VSV) and ER-membrane-anchored or transmembrane proteins (SARS-CoV (3a, 8ab), HCV (NS4B, E2)) (Table 1). Although the precise mechanism remains elusive for most viral proteins, certain viral proteins could trigger ER stress via their interactions with ER chaperone GRP78 such as HCV E2 (Choukhi et al., 1998), VSV G (Machamer et al., 1990), DENV E (Limjindaporn et al., 2009), and SV5 hemagglutinin-neuraminidase (HN) glycoprotein protein (Watowich et al., 1991).

### Predicted ER stress-triggering viruses and viral proteins

As viruses that modify or alter the ER tend to induce ER stress, we predict the following viruses might trigger ER stress and activate related signaling pathways. The *Picornaviridae* family virus Poliovirus, *Poxviridae* DNA virus vaccinia virus (VV), *Comoviridae* family virus Grapevine fanleaf nepovirus (GFLV) and *Bromoviridae* family virus Brome mosaic virus (BMV) as their infection can cause extensive cytopathic modifications to host ER and/or induce ER-derived virus vesicles (Bamunusinghe et al., 2011; Ritzenthaler et al., 2002; Suhy et al., 2000; Tolonen et al., 2001). Since viral proteins that are synthesized, processed or located in the ER tend to induce the ER stress, Yellow fever virus (YFV) envelope proteins, pre-membrane (prM) and envelope (E), might trigger the ER stress as these proteins accumulate in the ER (Ciczora et al., 2010). Based on the fact that some viral proteins can trigger ER stress through interacting with ER chaperones, the *Togaviridae* family virus Rubella virus (RV) glycoproteins E1 and E2 might trigger ER stress as these two proteins interact with ER chaperones calreticulin and calnexin (Nakhasi et al., 2001). Moreover, our assumptions could provide possible activating mechanisms for those experimentally validated ER stress-triggering viruses. For example, as the ER stress-triggering virus, CVB3 has been reported to modify the ER membrane permeability (van Kuppeveld et al., 1997), it is highly likely that this activity could be responsible for CVB3-induced ER stress. Given the facts that certain viral proteins trigger ER stress via their interactions with ER chaperone GRP78 and that the ER stress inducer, HEV ORF2 has been shown to interact with GRP78 (Yu et al., 2011), it is possible that this interaction could activate ER stress. Further efforts are required to examine these possibilities.

### Modulation of the UPR by viruses

The UPR has three major consequences to mitigate ER stress: attenuating global protein translation, degrading ER-localized proteins and expanding the ER folding capacity, some of which are beneficial to viral replication. For example, the ATF6-induced expression of chaperone proteins may

help viral proteins folding and prevent protein aggregation. The PERK-eIF2 $\alpha$ -activated ATF4 may help re-establish cell metabolism and resume protein translation. The IRE1-XBP1 pathway might facilitate virus replication by enhancing ER protein-folding ability and ER membrane biosynthesis (Ron & Hampton, 2004). In fact, activated ATF6 has been reported to promote the replication of ASFV (Galindo et al., 2012) and Lymphocytic choriomeningitis virus (LCMV) (Pasqual et al., 2011); GRP78 facilitates the replication of DENV (Limjindaporn et al., 2009), HCMV (Buchkovich et al., 2008), JEV (Wu et al., 2011) and RGNNV (Su et al., 2011; Wu et al., 2010) and activated ATF4 facilitates HIV replication (Caselli et al., 2012). The IRE1-XBP1 pathway has been activated by IAV to facilitate its own replication (Hassan et al., 2012). However, other UPR outcomes are detrimental for virus replication. The PERK-eIF2 $\alpha$ -mediated global translation attenuation is known as an antiviral response to restrict the replication of WNV (Ambrose & Mackenzie, 2011), DENV (Pena & Harris, 2011) and VSV (Baltzis et al., 2004). The IRE1-XBP1(s)-mediated ERAD pathway reduces intracellular HBV particles by degrading its envelope proteins (Lazar et al., 2012). In addition, the IRE1-XBP1 pathway inhibits the replication of Sindbis virus (SINV) and DENV through an unknown mechanism (Pena & Harris, 2011; Perry et al., 2012). To survive and propagate in host cells, viruses have evolved specific mechanisms to modulate the UPR (Figure 1).

### Subversion of the UPR by viruses

Although the PERK-eIF2 $\alpha$  pathway reduces global protein synthesis, some viruses are still able to translate their mRNAs regardless of high levels of phosphorylated eIF2 $\alpha$ , such as HCV (Robert et al., 2006) and SFV (Ventoso et al., 2006). These viruses contain a specialized internal ribosome entry site (IRES) that can efficiently recruit and assemble the initiation ribosome complex (Robert et al., 2006; Ventoso et al., 2006). Besides, some viruses have developed additional “smart” strategies to circumvent PERK-eIF2 $\alpha$ -mediated inhibitory effects and reset the cellular translational program for their own purposes (Table 1 and Figure 1). One salient strategy is to reduce the phosphorylated eIF2 $\alpha$  by activating eIF2 $\alpha$  phosphatase PP1 and inducing its regulatory subunit GADD34. The HSV-1  $\gamma_1$ 34.5, Epstein-Barr virus (EBV) nuclear antigens (EBNA 2) and ASFV DP71L proteins are GADD34 homologs that interact with PP1 and recruit its activity against eIF2 $\alpha$  (He, 2006; Rivera et al., 2007); while viruses like CHIKV, ASFV, RRV, DENV, HCV, Influenza A virus (IAV) and WNV activate the expression of GADD34 (Galindo et al., 2012; Hassan et al., 2012; Medigeschi et al., 2007; Merquiol et al., 2011; Pena & Harris, 2011; Rathore et al., 2013; Trujillo-Alonso et al., 2011). HCV and HIV might employ similar strategy to antagonize eIF2 $\alpha$  phosphorylation as HCV NS4B protein has been predicted to interact with PP1 and PP1 is required for HIV replication (Ammosova et al., 2003; Li et al., 2012). Another strategy involves regulation of the PERK activity. HSV-1 glycoprotein B(gB) interacts with the PERK luminal domain, which makes it refractory to acute ER stress (Mulvey et al., 2007). The *Alphavirus* CHIKV and *Flaviviridae* viruses (HCV, JEV and

DENV) could repress the activity of PERK via transcriptional up-regulation of its inhibitor, p58<sup>IPK</sup> (Merquiol et al., 2011; Rathore et al., 2013; Yu et al., 2006). Some viral proteins even function as a pseudo-substrate of PERK to sequester its activity such as HCV E2 protein (Pavio et al., 2003) and DENV has been proposed to encode a viral protein similar to HCV E2 to block the PERK-mediated eIF2 $\alpha$  phosphorylation (Pena & Harris, 2011).

The IRE1-XBP1 pathway is subject to modulation during the course of virus infection (Table 1 and Figure 1). HCV subgenomic replicons and HCMV infection suppress the IRE1-XBP1 pathway by inhibiting the transcriptional activity of XBP1(s) as well as transcriptional induction of EDEM presumably to enhance the synthesis of their viral proteins and persistent infection (Isler et al., 2005; Tardif et al., 2004). SARS-CoV E protein down-regulates the IRE1-mediated XBP1 splicing to reduce the ER stress (DeDiego et al., 2011). It remains to be elucidated whether viruses modulate the IRE1-mediated RIDD pathway.

Lastly, Rhesus rotavirus (RRV) NSP3 protein has been shown to impede the activation of two UPR branches, IRE1-XBP1 and ATF6, despite the fact that it induces XBP1 splicing and ATF6 proteolysis (Trujillo-Alonso et al., 2011). The three UPR branches are inhibited by MHV (Bechill et al., 2008) and HCMV (Isler et al., 2005). Although these two viruses induce XBP1 splicing, ATF6 proteolysis and PERK phosphorylation, they suppress the activation of their downstream targets. It is unclear about the specific mechanism by which these viruses regulate the UPR pathways as well as how viruses benefit from shutdown of these three UPR arms.

### Temporal regulation of the UPR by viruses

Viruses also differentially modulate the UPR at different infection stages and the prominent example is DENV (Pena & Harris, 2011). Very early DENV infection induces PERK-mediated eIF2 $\alpha$  phosphorylation but then suppresses that pathway. The IRE1-XBP1 and ATF6 pathways are activated during the mid and late infection, respectively, to help cells establish ER homeostasis and adapt to persistent ER stress without inducing apoptosis. HCV has also been shown to activate UPR in a temporal manner (Merquiol et al., 2011). There are two possible mechanisms that are responsible for the temporally modulated UPR by viruses. The first mechanism is related to differential expression of viral proteins at different stages and the typical example is HSV-1 (Burnett et al., 2012). During its early infection stage, only ATF6 proteolysis is induced but no up-regulation of its target chaperone proteins. Meanwhile, PERK-mediated eIF2 $\alpha$  phosphorylation and ATF4 expression are down-regulated. These effects might be modulated by its early gene product ICP0. As virus infection proceeds, these activated UPR indicators are totally aborted due to its viral proteins, VHS and ICP27, which degrade certain cellular mRNAs (Elgadi et al., 1999). At the final infection stage, these inhibitory effects are relieved and cells initiate apoptosis, which may facilitate virus particles release from host cells. The second mechanism by which the UPR is temporally regulated is associated with the stability of viral proteins. For example, CHIKV suppresses phosphorylation of eIF2 $\alpha$  at early infection stage due to

expression of its nsP4 protein; however, as nsP4 protein is not stable, cells recovers phosphorylation of eIF2 $\alpha$  at the late CHIKV infection stage (Rathore et al., 2013).

## Virus, ER stress and apoptosis

### Overview of ER stress-mediated apoptosis

Under acute or prolonged ER stress, cell apoptosis is activated marked by loss of mitochondrial membrane potential (MMP), release of cytochrome c from mitochondria into the cytosol and activation of caspases. Although little is known about how cells are committed to cell death, two ER stress sensors, IRE1 and PERK, are thought to mediate intrinsic mitochondrial apoptosis (Figure 2).

#### *IRE1 apoptosis pathway*

Under severe ER stress conditions, IRE1 exerts pro-apoptotic effects and functions as a critical factor controlling the commitment to cell death. The best-characterized mechanism is the IRE1-TRAF2-JNK pathway (Hotamisligil, 2010). Activated IRE1 interacts with the tumor necrosis factor receptor-associated factor-2 (TRAF2), which then recruits apoptosis-signal-regulating kinase (ASK1) to initiate a cascade of phosphorylation events that activates Jun amino-terminal kinase (JNK) (Urano et al., 2000). The pivotal mediators that link JNK to apoptosis are Bcl-2 family proteins, which include anti-apoptotic four Bcl-2 homology domain-containing (BH) proteins (Bcl-2, Bcl-X<sub>L</sub>, Mcl-1), pro-apoptotic three BH-containing proteins (Bax, Bak) and pro-apoptotic BH3-only proteins (Bad, Bim, Bid, Noxa, Puma) (Galluzzi et al., 2008). BH3-only proteins are necessary for Bax/Bak-mediated mitochondrial permeabilization and release of Ca<sup>2+</sup> from the ER into the cytosol but Bcl-2 promotes cell survival through sequestration of BH3-only proteins (Cheng et al., 2001). The released Ca<sup>2+</sup> can be taken up by mitochondria, resulting in MMP loss and subsequent efflux of cytochrome c from mitochondria to cytosol, which ultimately leading to apoptosis. JNK phosphorylates Bcl-2 to reduce its anti-apoptotic activity but phosphorylates BH3-only proteins to enhance its pro-apoptotic effects (Szegezdi et al., 2006). Moreover, prolonged activation of IRE1-mediated RIDD pathway may cause apoptosis by degrading mRNAs encoding essential cell-survival proteins (Hollien et al., 2009).

#### *PERK-eIF2 $\alpha$ -ATF4-CHOP pathway*

The activated PERK-eIF2 $\alpha$ -ATF4 pathway promotes apoptosis by inducing the expression of CHOP (also called GADD153), which exerts pro-apoptotic effects by up-regulating the expression of pro-apoptotic proteins BH3-only protein and down-regulating anti-apoptotic protein Bcl-2 (Hetz, 2012). In addition, CHOP triggers apoptosis through inducing the transcription of death receptor-5 (DR5) (Yamaguchi & Wang, 2004), tribbles-related protein 3 (TRB3) (Ohoka et al., 2005), GADD34 and ER oxidase 1 $\alpha$  (ERO1 $\alpha$ ) (Marciniak et al., 2004). DR5 is a critical mediator of ER stress-induced apoptosis in cancer cells and TRB3 promotes apoptosis presumably by binding to the pro-survival serine/threonine kinase Akt and reducing its kinase activity



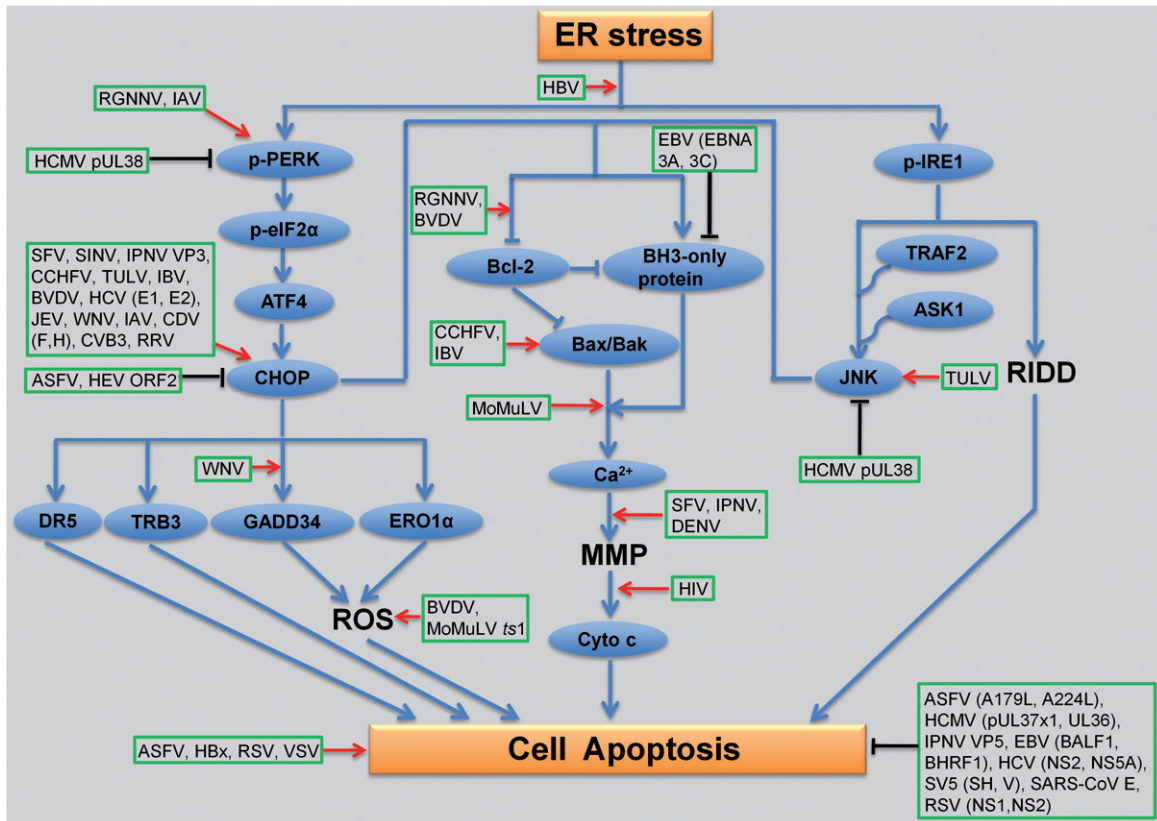


Figure 2. Modulation of apoptosis by viruses. Arrows indicate viruses and viral proteins that activate the components of apoptosis; lines indicate viruses and viral proteins that suppress the components of apoptosis; the left bottom box indicates the viruses that induce both ER stress and apoptosis but the causality between these two pathways is unknown; the right bottom box indicates the viruses that induce ER stress but repress apoptosis. HBV induces ER stress-mediated apoptosis but it is unknown which pathway contributes to apoptosis. Cyto c, cytochrome c.

(Yamaguchi & Wang, 2004). GADD34 and ERO1 $\alpha$  mediate apoptosis by affecting the accumulation of reactive oxygen species (ROS) (Marciniak et al., 2004). The executioner of cell apoptosis by ER stress is a cohort of caspases including caspase-12, -3, -6, -7, -8 and -9 (Szegezdi et al., 2006).

#### Activation of apoptosis by ER stress-triggering viruses

Among 36 ER stress-triggering viruses, 23 viruses have been reported to induce or accelerate apoptosis, including Semliki Forest Virus (SFV), SINV, ASFV, IPNV, Crimean-Congo hemorrhagic fever virus (CCHFV), TULV, Infectious bronchitis virus (IBV), SARS-CoV, BVDV, DENV, HCV, JEV, West Nile virus (WNV), HBV, Betanodavirus redspotted grouper nervous necrosis virus (RGNNV), IAV, CDV, RSV, CVB3, Rhesus rotavirus (RRV), HIV, MoMuLV and Vesicular stomatitis virus (VSV) (Table 1 and Figure 2). This is not surprising given that their productive infection causes great damage to host cells. Among 23 apoptosis-inducing viruses, 5 viruses have been reported to trigger MMP loss and cytochrome c release into the cytosol including SFV, IPNV, DENV, HIV and MoMuLV. Four viruses (RGNNV, BVDV, CCHFV and IBV) might also induce MMP loss and cytochrome c release through regulating the upstream factors: Bcl-2, BH3-only proteins, Bax and Bak. 16 out of 23 viruses might induce apoptosis via the PERK-eIF2 $\alpha$ -ATF4-CHOP pathway including SFV, SINV, IPNV, CCHFV, TULV, IBV, BVDV, HCV, JEV, WNV, RGNNV, IAV, CDV, CVB3, RRV

and MoMuLV with WNV and HCV being shown to induce the expression of CHOP to promote apoptosis (Benali-Furet et al., 2005; Medigeshi et al., 2007). Although HEV infection activates the PERK-eIF2 $\alpha$ -ATF4-CHOP pathway in human cells H1299, it fails to induce apoptosis (John et al., 2011). Only TULV activates the IRE1-TRAF2-JNK-dependent cell apoptosis. The rest four ER stress-triggering viruses (ASFV, HBV, RSV and VSV) are able to sensitize cells to apoptosis but it is unclear whether these effects are mediated by ER stress (Bitko & Barik, 2001; Gaddy & Lyles, 2005; Galindo et al., 2012; Kuo et al., 2012).

#### Modulation of apoptosis by ER stress-triggering viruses

The ER stress-mediated apoptosis plays pivotal roles in virus pathogenesis (Table 1). For example, CDV-induced apoptosis may eventually lead to the neurodegenerative disease (Brunner et al., 2012). The RSV-induced cell death is a major cause of destruction of the lung epithelial in RSV-infected patients (Bitko & Barik, 2001). Apoptosis is also required for virus replication such as release and dissemination of virus particles. Activation of caspase-3 at the early stage of ASFV infection is required for virus exit (Galindo et al., 2012). Knockdown Mcl-1 in IBV-infected mammalian cells accelerates apoptosis and increases IBV progeny production and release, while knockdown Bak delays apoptosis and reduces the release of viral proteins and particles

(Zhong et al., 2012). However, premature cell apoptosis may function as a host defense response by limiting virus replication and pathogenesis. For example, the virus titers of WNV are significantly increased in CHOP-deficient cells that are resistant to ER stress-mediated apoptosis (Medigeschi et al., 2007). In addition, HBV has been shown to prevent apoptosis to facilitate the release and spread of infectious progeny (Arzberger et al., 2010).

To overcome the host resistance, viruses have evolved a battery of distinct strategies to subvert apoptosis to optimize their infection (Table 1 and Figure 2). One strategy is to modulate the expression and/or activity of components in the apoptotic pathways including the PERK-eIF2 $\alpha$ -ATF4-CHOP and IRE1-TRAF2-JNK. HCMV pUL38 protein has been reported to protect primary human foreskin fibroblasts from apoptosis by blocking the phosphorylation of PERK and activation of JNK (Xuan et al., 2009). ASFV infection down-regulates the transcription of CHOP, perhaps to prevent host cell death and prolong viral replication (Galindo et al., 2012; Galluzzi et al., 2008). Some viruses modulate apoptosis by targeting BH3-only proteins and Bcl-2 protein. EBV EBNA 3A and 3C proteins suppress the expression of BH3-only protein Bim and Noxa in B cells (Yee et al., 2011). PEDV E protein might suppress apoptosis by up-regulating anti-apoptotic Bcl-2 (Xu et al., 2013a) and further efforts are required to confirm it. Certain ER stress-triggering viruses inhibit apoptosis by modulating the activities of caspases. For example, ASFV A224L protein inhibits caspase-3 (Galluzzi et al., 2008) and HCMV UL36 protein blocks apoptosis by binding to the pro-domain of caspase-8 and inhibiting its activation (Skaletskaya et al., 2001). Other strategy involves encoding anti-apoptotic viral proteins that share significant sequence similarity with Bcl-2 family and these viral Bcl-2 proteins (vBcl-2s) preferentially bind to pro-apoptotic Bax and Bad and inhibit Bax/Bad-mediated apoptosis. For instance, IPNV VP5, ASFV A179L, HCMV pUL37x1 and EBV (BALF1, BHRF1) proteins exert anti-apoptotic effects presumably due to their Bcl-2 motif (Galluzzi et al., 2008; Hong et al., 2002). In addition, some viruses antagonize apoptosis by inducing the expression of chaperones or heat shock proteins, which could interfere with the functions of CHOP (Gotoh et al., 2004). HEV ORF2 protein has been suggested to prevent CHOP-mediated apoptosis through inducing Hsp70B', Hsp72 and Hsp40 (John et al., 2011). Besides, HCV (NS2, NS5A), SV5 (V, SH), SARS-CoV E and RSV (NS1, NS2) proteins have been reported to inhibit apoptosis through unknown mechanisms (Bitko & Barik, 2001; Bitko et al., 2007; DeDiego et al., 2011; Galluzzi et al., 2008; Sun et al., 2004).

### Why do viruses have distinct effects on the UPR and apoptosis?

There are two possible explanations with regards to the nature of viruses and the type of cells they infected. First, compared with DNA viruses, RNA viruses usually have relatively short replication period and can replicate to high titers before the infected cells undergo cell death. Hence, most ER stress-triggering RNA viruses (SFV, IPNV, CCHFV, TULV, SARS-CoV, DENV, JEV, WNV, RGNNV, IAV, CDV, RSV,

MoMuLV and FrCas<sup>E</sup>) tend to induce apoptosis; while ER stress-triggering DNA viruses (ASFV, EBV, HCMV) tend to protect the infected cells from apoptosis (Table 1 and Figure 2). Second, various types of cells adapt the ER capacity for different purposes and accordingly, may respond differentially to exogenous stimuli. For example, compared with epithelial cells, hepatocytes are specialized secretory cells that synthesize and secrete large quantities of proteins and thereby are more sensitive to ER stress (Hassan et al., 2012; Hetz, 2012). As expected, in HCV- or HBV-infected hepatocytes, the ATF6 pathway is preferentially activated to induce the transcription of chaperones; whereas in IAV-infected epithelial cells, ATF6 and its target chaperones are not activated (Hassan et al., 2012).

### Virus, ER stress and immune responses

A growing body of evidence indicates that ER stress contributes to virus pathogenesis by modulating the immune responses in addition to causing apoptosis. VSV, HCV and SARS-CoV are able to inhibit the type I IFN signaling pathway by activating the PERK, which leads to the phosphorylation-dependent ubiquitination and subsequent degradation of the IFN  $\alpha$ -receptor subunit 1 (IFNAR1), thereby promoting immune evasion and virus pathogenesis (Liu et al., 2009; Minakshi et al., 2009). WNV has also been reported to induce ER stress and inhibit type I IFN signaling pathway to facilitate the escape from the host immune response and viral pathogenesis (Ambrose & Mackenzie, 2011). HCMV US11 protein activates the UPR to facilitate the degradation of class I major histocompatibility complex, leading to immune evasion (Tirosh et al., 2005). Moreover, ER stress is responsible for viral pathogenesis by interconnecting with the inflammatory responses. For example, HCV induces inflammatory responses by activating IRE1, which interacts with TRAF2 to phosphorylate JNK, leading to activation of inflammation mediators (Merquiol et al., 2011; Zhang & Kaufman, 2008). HCV NS4B and NS5A activate NF- $\kappa$ B via ER stress-elicited calcium depletion and ROS production (Gong et al., 2001; Li et al., 2009). Cho et al. found that HBV X protein (HBx) induces the expression of COS2 (cyclo-oxygenase 2), a key mediator of inflammation, through PERK-eIF2 $\alpha$ -activated ATF4 (Cho et al., 2011).

### Implications for antiviral therapy

Since the ER stress plays expanding roles in virus replication and pathogenesis, the ER stress mediated signaling pathways have become attractive targets for broad-spectrum antiviral therapy and considerable progress has been made in developing potential antiviral agents. The first class of antiviral targets involves the three UPR pathways. The PERK-eIF2 $\alpha$  pathway has been extensively investigated for antiviral development. Boyce and colleagues identified a small chemical compound salubrinal, a specific inhibitor of PP1/GADD34 complex and found it blocks HSV  $\gamma_1$ 34.5-mediated eIF2 $\alpha$  dephosphorylation and efficiently reduces HSV replication (Boyce et al., 2005). Salubrinal has also been shown to inhibit the replication of DENV through attenuating PP1/GADD34-mediated eIF2 $\alpha$  dephosphorylation (Umareddy et al., 2007). The clinically achievable reagent

glucose analog 2-Deoxy-D-Glucose, which induces the phosphorylation of eIF2 $\alpha$ , has been reported to suppress herpesvirus (KSHV) replication and serve as a novel anti-herpesviral therapy (Leung et al., 2012). Moreover, the IRE1-XBP1 pathway has become the target for antiviral development. Hassan et al. found that 3,5-dibromosalicylaldehyde, which is a specific inhibitor of IRE1 endoribonuclease, significantly blocks IAV replication (Hassan et al., 2012). The IRE1-XBP1 pathway agonist, WP1130, has broad antiviral effects against SINV, murine norovirus (MNV-1) and La Crosse virus (Perry et al., 2012). It remains to be determined whether ATF6 and its downstream genes (chaperone proteins) can be potential targets. It is noteworthy that the selection of the UPR antagonists or agonists depends on the positive or negative effects of the UPR on virus replication.

Other targets involve ER stress-mediated intrinsic apoptosis signaling pathways. Rana catesbeiana ribonuclease (RC-RNase) has been shown to inhibit JEV replication through activating caspase-3, -8 and -9 (Lee et al., 2011). Rider et al. developed a broad-spectrum antiviral therapy, dubbed Double-stranded RNA activated caspase oligomerizer (DRACO) that selectively induces apoptosis in double-stranded virus infected cells but has no harmful effects on uninfected cells and this DRACO has successfully been shown to eliminate 15 different viruses including DENV and HIV (Rider et al., 2011). Besides, Vaticanol B has been well-studied as an effective agent that protects against ER stress-induced apoptosis (Tabata et al., 2007). In addition, the components of apoptotic pathway, Bcl-2 families, JNK and CHOP have been investigated in pre-clinical trials (Tabas & Ron, 2011).

The ER stress-triggering viral proteins could become targets for specifically targeted antiviral therapy, such as CHIKV nsP4, SFV envelope glycoproteins, LCMV glycoprotein precursor (GPC), TULV glycoproteins, IPNV VP3, MHV S, SV5 HN, PEDV (E and N), SARS-CoV proteins (S, 3a, 8ab, E), BVDV envelope glycoproteins, DENV proteins (prM-E, E, NS1, NS2A, NS2B), HCV proteins (E1, E2, core, NS2, NS4B, NS5A), JEV (prM, E, NS1, NS2A, NS2B, NS4B), WNV nonstructural proteins, HSV-1 (ICP0, glycoprotein B,  $\gamma_1$ 34.5, Us11), HCMV (US2, US11, pUL37x1, UL36, pUL38), VZV (gE, gI), RGNNV ( $\alpha$  and B2), IAV hemagglutinin A, CDV (F, H), SV5 (V, SH), RRV NSP3, MpMuLV gPr80<sup>env</sup> and VSV M protein (Table 1). Special attention should be given to those viral proteins that can induce both the UPR and apoptosis signaling pathways such as HCV (E1, E2, core), HCMV (pUL37x1, pUL38) and SV5 V protein. Indeed, significant advances have been made in developing antivirals or vaccines against viral proteins: clemizol for HCV NS4B (Einav et al., 2010), vaccine vectors for HSV-1  $\gamma_1$ 34.5 (Shah et al., 2009), and Norakin for IAV hemagglutinin A (Ghendon et al., 1986).

## Conclusions and perspectives

As a virus replication site and factory to process viral proteins, the ER is required for virus replication and suffers greatly from these alien pathogen activities. Current research indicated that 36 viruses trigger ER stress and related signal

pathways during their activities. These ER stress responses play important roles in virus-induced apoptosis, immune evasion and inflammation, which have profound but complex implications in virus replication and pathogenesis. To combat with the deleterious effects of ER stress, viruses have developed elegant strategies to tune the ER stress-related pathways and these strategies differ dependent on the types of cells and viruses.

For the future directions, it is worth examining whether those predicted ER stress-triggering viruses can induce ER stress and modulate related signaling pathways. In addition, there are five open questions need to be addressed for future research. Firstly, for most ER stress-triggering viruses, less is known about the precise mechanism by which virus activates ER stress as much work is focused on detecting ER stress indicators during virus infection. Based on the published data (Table 1), the mechanisms may be related to the rapid synthesis and accumulation of viral proteins inside the ER lumen, the interactions of viral proteins with ER host factors including UPR sensors, the disturbance of ER membrane by virus replication. Secondly, for most ER stress-triggering viruses, it remains unclear about which viral protein(s) attributes to the occurrence of ER stress. Characterizing these proteins and elucidating the underlying mechanisms will undoubtedly help us understand the virus life cycle and pathogenesis. Most importantly, these viral proteins can be potential therapeutic targets to cure virus infection. Thirdly, little is known about how viruses activate the UPR transducers but selectively regulate their target genes. For example, ASFV activates ATF6 and its transcriptional targets calnexin and calreticulin but has no effects on XBP1 or GRP78 (Galindo et al., 2012). Fourthly, despite much is known about the regulation of ER stress by viruses, how virus-triggered ER stress regulates the intricate balance between pro-survival and pro-apoptotic effects and how these events results in cell death commitment remain enigmatic. Lastly, for most ER stress-triggering viruses, it remains to be determined to what extent virus-induced apoptosis can be explained by ER stress.

## Declaration of interest

This work was funded by National Nature Science Foundation of China (No. 31160034) and Nature Science Foundation of Jiangxi Province (No. 2010GZY0059) to L. Kong. The authors report no conflicts of interest.

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