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Themed Section: Spotlight on Small Molecules in Cardiovascular Diseases

REVIEW ARTICLE

Endoplasmic reticulum stress in the heart: insights into mechanisms and drug targets

Correspondence Xin Wang and Wei Liu, Faculty of Biology, Medicine and Health, The University of Manchester, M13 9NT, Manchester, UK, and Wei Xiao, State Key Laboratory of New-tech for Chinese Medicine Pharmaceutical Process, Lianyungang, China. E-mail: xin.wang@manchester.ac.uk; wei.liu@manchester.ac.uk; xiaowei1959@yahoo.com

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Shunyao Wang[1], Pablo Binder[1], Qiru Fang[2], Zhenzhong Wang[2], Wei Xiao[2], Wei Liu[1] and Xin Wang[1]

[1]Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK, and [2]State Key Laboratory of New-tech for Chinese Medicine Pharmaceutical Process, Lianyungang, China

The endoplasmic reticulum (ER) serves several essential cellular functions including protein synthesis, protein folding, protein translocation, calcium homoeostasis and lipid biosynthesis. Physiological or pathological stimuli, which disrupt ER homoeostasis and disturb its functions, lead to an accumulation of misfolded and unfolded proteins, a condition referred to as ER stress. ER stress triggers the unfolded protein response to restore the homoeostasis of ER, through activating transcriptional and translational pathways. However, prolonged ER stress will lead to cell dysfunction and apoptosis. Recent evidence revealed that ER stress is involved in the development and progression of various heart diseases, such as cardiac hypertrophy, ischaemic heart diseases and heart failure. Therefore, improved understanding of the molecular mechanisms of ER stress in heart disease will help to investigate more potential targets for new therapeutic interventions and drug discovery.

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Abbreviations

AAV, Adeno-associated virus; ASK1, apoptosis signal-regulating kinase 1; ATF4, activating transcription factor 4; Bip, immunoglobulin-binding protein; CaMKII, calcium/calmodulin-dependent protein kinase II; CHOP, CCAAT/enhancer-binding homologous protein; eIF2α, eukaryotic initiation factor 2α; ER, endoplasmic reticulum; ERAD, ER-associated degradation; ERO1, ER oxidoreductase-1; ERSE, ER stress response elements; GK, Ginkgolide K; GRP78, glucose-regulated protein 78; I/R, ischaemia–reperfusion; IRE1, inositol-requiring kinase 1; MI, myocardial infarction; PDI, protein disulphide isomerase; PERK, dsRNA-activated protein kinase-like ER kinase; PUMA, p53 up-regulated modulator of apoptosis; RIDD, IRE1-dependent decay of mRNA; RyR2, ryanodine receptor 2; SCN5A, sodium channel α-subunit 5; SERCA2a, sarco/ER Ca2+ ATPase 2a; SERCA3f, sarco/ER Ca2+ ATPase isoform 3f; sXBP1, spliced-XBP1; TUDCA, tauroursodeoxycholic acid; UPR, unfolded protein response; XBP1, X-box binding protein 1

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Introduction

The endoplasmic reticulum (ER) is a multifunctional intracellular organelle and the primary site of the secretory pathway. It is essential for protein synthesis, protein folding, protein translocation, calcium homoeostasis and lipid biosynthesis. The concentration of proteins and the protein synthesis rate are extremely high within the ER lumen (Stevens and Argon, 1999). The homoeostasis within the ER lumen must be carefully maintained for folding proteins properly. Perturbations of this homoeostasis by physiological or pathological stimuli lead to an accumulation of misfolded and unfolded proteins, a process known as ER stress. ER stress activates complex signalling pathways that deal with the misfolded and unfolded proteins, referred as the unfolded protein response (UPR). UPR activates transcriptional and translational pathways to reduce the rate of general translation and increase the expression of ER resident protein chaperones and protein foldases. ER-associated degradation (ERAD) is also activated by UPR to clear irreparably misfolded proteins. However, if the UPR fails to reduce ER stress and restore homeostasis, ER stress causes cell dysfunction and apoptosis. Recently, ER stress has received substantial attention and is thought to play an essential role in the development and progression of many human diseases, including cardiovascular diseases, diabetes mellitus, neurodegenerative diseases and liver diseases (Oyadomari *et al.,* 2002; Lindholm *et al.,* 2006; Minamino and Kitakaze, 2010). This review focuses on the molecular mechanisms of ER stress in cardiovascular diseases and the potential therapeutic targets in the process of ER stress.

UPR signalling pathway

In normal conditions, the ER-resident transmembrane proteins, ATF6, inositol-requiring kinase 1 (**IRE1**) and **PERK** (dsRNA-activated protein kinase-like ER kinase), are bound with the ER chaperone, immunoglobulin-binding protein (Bip)/glucose-regulated protein 78 (GRP78), to maintain their inactive state (Lee, 2005). When unfolded proteins accumulate in the ER, Bip dissociates from those three sensors to initiate their activity (Bertolotti *et al.,* 2000). The activated UPR then regulates downstream effectors to increase folding and handling efficiency by up-regulation of ER chaperones, reduce ER workload through attenuation of translation and eliminate unwanted proteins *via* induction of ERAD.

IRE1α is a transmembrane kinase (Groenendyk *et al.,* 2010), and it is activated by homodimerization and autophosphorylation after release from Bip/GRP78. Activated IRE1α cleaves X-box binding protein 1 (XBP1) mRNA to initiate translation of transcriptionally active spliced-XBP1 (sXBP1). Active sXBP1 binds to a variety of UPR-target gene promoters to up-regulate a range of ER stress response elements (ERSE) to restore ER homoeostasis and promote cytoprotection.

ATF6α is a 90 kDa ER transmembrane protein under normal conditions but activated ATF6α translocates from ER to the Golgi to be cleaved by site-1 and site-2 protease. The cleaved N-terminal of ATF6α, a 50KDa fragment, migrates from the cytosol into the nucleus to combine with several b-Zip transcription factors and ERSE for transcriptional induction of several UPR related genes, including CCAAT/enhancer-binding homologous protein (CHOP), Bip and XBP1 (Haze *et al.,* 1999). In addition, there is an isoform of ATF6 called ATF6β. ATF6β is nonessential for transcriptional induction of ER chaperones (Wu *et al.,* 2007) and may even inhibit ATF6α activity (Thuerauf *et al.,* 2004). Recently, ATF6β has been shown to play a pro-survival role in chronic ER stress through induction of Wfs1 (Odisho *et al.,* 2015).

PERK is also activated by homodimerization and autophosphorylation. The activated PERK phosphorylates Ser^{51} on the α-subunit of the **eukaryotic initiation factor 2α** (eIF2α) to prevent the formation of translational initiation complexes, which leads to attenuation of cap-dependent protein translation (Bertolotti *et al.,* 2000). This transient translational arrest helps to recover ER homoeostasis through the reduction of protein synthesis. Meanwhile, eIF2α phosphorylation also induces the translation of the mRNA encoding activating transcription factor 4 (ATF4) to decrease unfolded proteins level in the ER through activation of various UPR genes (Figure 1).

ER stress-induced apoptosis

When the UPR fails to correct the protein-folding defect for the recovery of ER homoeostasis, the apoptotic signalling pathway is activated. Although all of the UPR sensor proteins are involved in ER stress-induced apoptosis, it is unclear how the cell decides to commit to death in response to excessive ER stress.

IRE1α mediates apoptosis by interaction with the adaptor molecule TNF-receptor-associated factor 2 (TRAF2) and **apoptosis signal-regulating kinase 1** (ASK1), which leads to the activation of **JNK** and **p38** (Urano *et al.,* 2000; Nishitoh *et al.,* 2002). p38 activates CHOP through phosphorylation of its transactivation domain (Wang and Ron, 1996). Both p38 and JNK can phosphorylate the proapoptotic protein **Bax** to induce its activity (Kim *et al.,* 2006). The association of IRE1α and TRAF2 has also been suggested to induce the activation of caspase-12 (Nakagawa *et al.,* 2000; Saleh *et al.,* 2006). Caspase-12 activates **caspase-9** to induce the activation of **caspase-3**, which leads to apoptosis. In addition, the IRE1α/TRAF2 complex can also recruit the IκB leading to the activation of NF-κB (Kaneko *et al.,* 2003), linking ER stress and inflammation. Recently, the regulated IRE1-dependent decay of mRNA (RIDD) was shown to promote degradation of a number of mRNAs encoding ER-targeted proteins to reduce a load of incoming proteins during ER stress (Han *et al.,* 2009; Hollien *et al.,* 2009). Under irremediable ER stress, prolonged activation of RIDD degrades mRNAs encoding prosurvival proteins to induce apoptosis (Hollien and Weissman, 2006; Maure *et al.,* 2013). The activation of RIDD requires IRE1 phosphotransfer activity (Son *et al.,* 2014) but the mechanisms involved are poorly understood and require further investigation. Furthermore, IRE1α also acts as an essential factor in calcium homoeostasis disruption induces apoptosis *via* the **inositol**-1,4,5-trisphosphate (IP_3) receptor (Son *et al.,* 2014).

CHOP is a basic leucine zipper-containing transcription factor, which is regulated by ATF6 and PERK pathways. CHOP

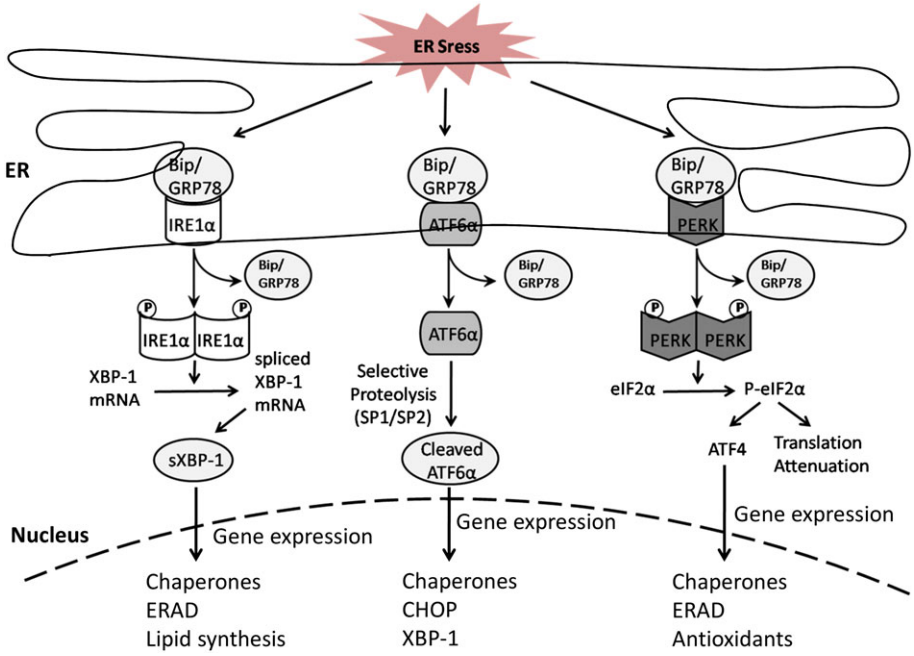

Figure 1

UPR signalling pathway. The three ER-resident transmembrane proteins, ATF6, IRE1 and PERK, associate with Bip in their inactive state under normal condition. In response to ER stress, these sensors are released and activated. Both IRE1 and PERK are oligomerized and autophosphorylated. Phosphorylated IRE1 catalyses the splicing of XBP1 mRNA leading to the generation of UPR-target transcription factor. Activated PERK phosphorylates eIF2α leading to general translational attenuation and increased expression of ATF4. ATF6 is cleaved by SP1/SP2, and the cytosolic fragment of ATF6 migrates to the nucleus. The downstream effectors of these three signalling pathways, in combination, induce the expression of proteins, which can help restore the ER protein folding capacity. ERAD is accelerated to remove terminally misfolded proteins.

can inhibit the expression of the anti-apoptotic protein Bcl-2 to induce apoptosis (McCullough *et al.,* 2001). In response to ER stress, CHOP also can directly activate the transcription of Bim leading to the induction of apoptosis (Puthalakath *et al.,* 2007). In addition, CHOP mediates the transcriptional induction of several genes that encode numerous proapoptotic proteins, such as GADD34, death receptor 5, ER oxidoreductase-1 (ERO1) and carbonic anhydrase VI (Malhotra and Kaufman, 2007). CHOP activates GADD34 to promote dephosphorylation of eIF2α reversing the translational attenuation (Novoa *et al.,* 2009). This accumulates unfolded proteins in the ER and increases the translation of proapoptotic proteins. The activation of ERO1 by CHOP promotes apoptosis by hyperoxidization of the ER and activation of IP_3 receptors (Li *et al.,* 2009a,b). Furthermore, recent evidence suggested that CHOP can interact with ATF4 to increase protein synthesis leading to ATP depletion, oxidative stress and cell apoptosis (Han *et al.,* 2013).

Recently, **Bcl-2 family proteins** have been suggested to induce apoptosis by calcium signalling during ER stress (Szegezdi *et al.,* 2009). In response to ER stress, proapoptotic Bcl-2 family proteins, Bak and Bax, undergo a conformational change in the ER membrane to release calcium into the cytoplasm (Scorrano *et al.,* 2003). Released calcium activates the calcium-dependent protease **m-calpain** to cleave procaspase-12 leading to caspase-12 activation (Nakagawa and Yuan, 2000). In addition, caspase-12 can also be activated by the translocation of Bim to the ER membrane in response to ER stress (Morishima *et al.,* 2004). In cardiomyocytes, a Bcl-2 family protein NIX, which localizes both to the ER and mitochondrial membrane, can induce apoptosis by modulating calcium in the ER in coordination with Bax and Bak (Diwan *et al.,* 2009). Additionally, Bax and Bak can directly associate with IRE1α to induce the activation of the IRE1α signalling pathway (Hetz *et al.,* 2006).

A recent study found that **calcium/calmodulin-dependent protein kinase II** (CaMKII) was activated by ER-released calcium and it acted as a unifying link between ER stress and mitochondrial apoptosis (Ozcan and Tabas, 2010). In response to oxidative stress, CaMKII was activated to mediate ER stress-induced cardiac dysfunction and apoptosis (Roe and Ren, 2013).

Additionally, ER stress can also trigger necroptosis. Necroptosis is a regulated form of necrosis that involves the activation **of receptor-interacting protein kinase 1** (RIPK1), RIPK3 and **mixed lineage kinase domain-like protein** (MLKL) (Vanden Berghe *et al.,* 2015) and has been suggested as another important cell death mediator in cardiomyocytes. Studies using the RIPK1 inhibitor necrostatin-1 and RIPK3-deficient mice have shown that inhibition of necroptotic cell death has cardioprotective effects in mouse models of ischaemic injury (Koshinuma *et al.,* 2014; Zhang *et al.,* 2016). Interestingly, induction of ER stress by brefeldin-A, **thapsigargin** and tunicamycin triggers RIPK1 kinase-dependent necroptosis (Saveljeva *et al.,* 2015), and activation of GRP78 and eIF2α has been linked to necroptosis in macrophages/microglia after mouse spinal cord contusion (Fan *et al.,* 2015), further confirming the involvement of ER

stress in cardiomyocyte cell death and related pathologies (Figure 2).

ER stress in cardiac hypertrophy and heart failure

In failing hearts, the ER is overloaded and ER stress can be induced by enhanced protein synthesis, oxidative stress and hypoxia (Maron *et al.*, 1975). In 2004, GRP78 was first found to be increased in hearts from patients with heart failure, suggesting that UPR activation is induced in this condition (Okada *et al.*, 2004). In addition, extensive splicing of XBP1 was found in these patients and XBP1 has been shown to be a regulator of **brain natriuretic peptide** in cardiomyocytes (Sawada *et al.*, 2010). CHOP knockout mice developed less cardiac hypertrophy, fibrosis and cardiac dysfunction compared with wild-type mice after TAC, suggesting CHOP may also contribute to the transition from cardiac hypertrophy to heart failure (Fu *et al.*, 2010). The inhibition of GADD34 was lost after CHOP deletion, which leads to the increased phosphorylation of eIF2α and decreased global translation (Harding *et al.*, 2000). This could explain the mechanism of how CHOP deletion contributes to preventing the development of cardiac hypertrophy.

Heart failure can also be induced by protein accumulation. The Lys-Asp-Glu-Leu (KDEL) receptor is a retrieval receptor for ER chaperones in the early secretory pathway. Transgenic mice expressing a mutant KDEL receptor, which disturbs the recycling and protein quality control in the ER, showed aggregation of misfolded proteins, increased expression of CHOP and apoptosis in mutant hearts (Hamada *et al.*, 2004). Furthermore, rabbits immunized against **β-adrenoceptors** exhibited left ventricular dilation, systolic dysfunction and cardiomyocyte apoptosis in association with enhanced expression of GRP78 and CHOP (Mao *et al.*, 2007). These findings suggest that ER stress plays a critical role in dilated and autoimmune cardiomyopathy. In cultured rat ventricular myocytes, stimulation of β-adrenoceptors activated ER stress to induce apoptosis (Dala *et al.*, 2012). Antagonists of β-adrenoceptors can ameliorate ER stress to attenuate cardiac hypertrophy and heart failure (Ni *et al.*, 2011).

ASK1 plays an essential role in ER stress-induced apoptosis. ASK1 knockout mice exhibited less cardiac dysfunction and reduced cardiomyocytes apoptosis after 4 weeks TAC (Yamaguchi *et al.*, 2003). Meanwhile, neonatal cardiomyocytes with ASK1 deletion showed resistance to H_2O_2-induced apoptosis (Yamaguchi *et al.*, 2003). These results suggest that ASK1 could be involved in the development of heart failure. Mice with cardiac-specific and inducible overexpression of ASK1 showed greater TUNEL but no increase in myocyte or whole organ hypertrophy after 8 weeks TAC (Liu *et al.*, 2009). Thus, ASK1 is a key regulator of cardiomyocyte apoptosis but not hypertrophy. Additionally, a small molecule inhibitor of ASK1 can reduce cardiomyocyte

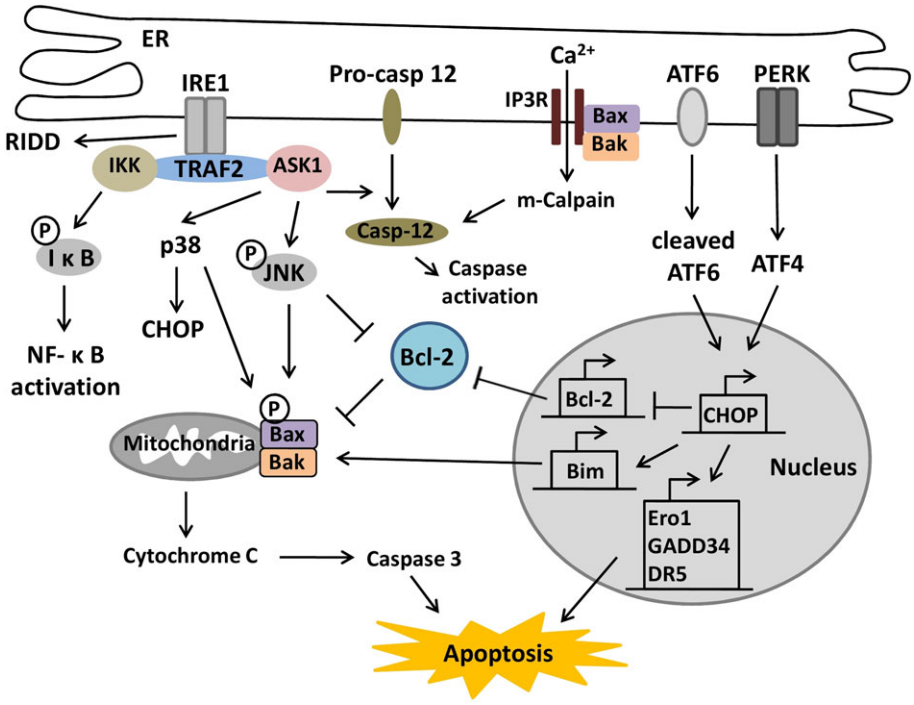

Figure 2

ER stress-induced apoptosis. Activated IRE1 recruits TRAF2 and ASK1 leading to the activation of JNK and p38 and also the release of procaspase-12 from the ER. IRE1/TRAF2 also recruits IκB for the activation of NFκB. In addition, IRE1 induces RIDD leading to apoptosis. During ER stress, Bax and Bak in the ER membrane undergo conformational change to release calcium into the cytoplasm, which activates m-calpain and caspase 12 leading to the activation of the caspase cascade. ATF6 and PERK activate CHOP to inhibit the expression of Bcl-2 and activate Bim leading to apoptosis. Activated CHOP increases the expression of GADD34, DR5 and Ero1 to induce apoptosis.

apoptosis and myocardial infarct size in a rat ischaemia/reperfusion model (Gerczauk *et al.,* 2012).

Prostatic androgen-repressed message-1 (PARM-1) is a transmembrane protein, which specifically expressed in hearts and skeletal muscles and predominantly localized in ER. Stimulation of PARM-1 and enhanced expression of GRP78 and CHOP were observed in Dahl salt-sensitive rats with cardiac hypertrophy and heart failure (Isodono *et al.,* 2010). In response to ER stress stimuli, cardiomyocytes with silencing PARM-1 showed enhanced apoptosis, repressed expression of ATF6 and PERK and increased induction of CHOP (Isodono *et al.,* 2010). These results indicate that PARM-1 act as an antiapoptotic protein to protect the heart from heart failure through regulating the expression of ATF6, PERK and CHOP.

Recently, PERK has been demonstrated to play a cardioprotective role in heart failure. The inducible cardiac-specific PERK knockout mice showed enhanced cardiac dysfunction, fibrosis and apoptosis compared with wild-type mice after TAC (Liu *et al.,* 2014a,b). In PERK knockout heart, the expression of CHOP was increased in response to TAC, which indicates that CHOP-induced apoptosis may contribute to heart failure. The ATPase **SERCA2a**, a key regulator of calcium homoeostasis in cardiomyocytes, was significantly reduced in PERK knockout mice after TAC (Mekahli *et al.,* 2011). Previous studies demonstrated that reduction of SERCA2a activity can induce ER calcium depletion leading to ER stress (Mekahli *et al.,* 2011) and disruption of the SERCA2 gene in cardiomyocytes causes ER stress and promotes heart failure (Liu *et al.,* 2011). Therefore, maintaining the expression of SERCA2a by PERK is probably an important mechanism to protect the heart from heart failure. In addition, the isoform **SERCA3f** was also up-regulated in human failing hearts (Dally *et al.,* 2009). Remarkably, overexpression of SERCA3f induces the increased expression of GRP78 and XBP1 splicing in cardiomyocytes (Dally *et al.,* 2009). Thus, SERCA3f may be involved in the mechanism(s) of ER stress leading to heart failure.

Chronic alcohol consumption is a risk factor of cardiac hypertrophy (Piano, 2002), and recent evidence has demonstrated that it can lead to ER stress. Friend virus-B-type mice chronically fed with alcohol showed the development of cardiac hypertrophy in association with increased expression of GRP78, CHOP and IRE1α (Li and Ren, 2008). In addition, mice with cardiac-specific overexpression of alcohol dehydrogenase exhibited enhanced expression of GRP78, IRE1α and CHOP compared with wild-type mice in response to chronic alcohol treatment (Li and Ren, 2008). Meanwhile, mice overexpressing **aldehyde dehydrogenase-2**, an enzyme which metabolizes acetaldehyde, showed less cardiac hypertrophy and significantly reduced expression of GRP78, IRE1α and CHOP (Li *et al.,* 2009a,b). Taken together, these results suggest that acetaldehyde may induce ER stress and cardiac hypertrophy in response to chronic alcohol consumption.

In 2015, XBP1 was first shown to be a critical angiogenic factor for maintaining normal cardiac function in the early stage of cardiac hypertrophy, and its activity was inhibited by miR-214 and miR-30* (recently designated as the miR-30-3p family) in hypertrophic and failing hearts (Duan *et al.,* 2015). Further, XBP1 regulated the expression of **VEGFA** in cardiomyocytes, which is consistent with the binding of XBP1s to the promoter of VEGFA, in response to ER stress (Ghosh *et al.,* 2010). This finding suggests that XBP1 plays a cardioprotective role through promoting VEGF mediated-cardiac angiogenesis. Thus, miR-214 and miR-30* inhibit the expression of XBP1 and VEGFA in the progression from adaptive hypertrophy to heart failure.

A recent study showed that **4-phenylbutyric acid (4-PBA)** could prevent TAC-induced cardiac hypertrophy through attenuation of ER stress (Luo *et al.,* 2015). After mechanical unloading with a left ventricular assist device, heart failure patients showed reduced expression of ER stress markers and improved expression of Ca^{2+} cycling proteins (Castillero *et al.,* 2015). These findings indicate that mechanical unloading contributes to reverse remodelling of the failing heart in association with the restoration of ER homoeostasis.

ER stress in ischaemic heart disease

Ischaemia can lead to the impairment of protein folding in the ER, resulting in the activation of the UPR (Glembotski, 2008). Activation of the UPR has been observed in ischaemic heart disease, and ER stress appears to mediate the progression of ischaemic cardiomyopathy in a range of models from mice to humans (Azfer *et al.,* 2006; Szegezdi *et al.,* 2006). The increase in UPR activation by ischaemia is shown by early expression of XBP1, eIF2α and ATF6 with the consequent activation of ATF4 and GRP78 in cardiomyocytes to restore ER homoeostasis and efficient protein folding (Azfer *et al.,* 2006). However, under prolonged ischaemia and ischaemia–reperfusion (I/R) injury, persistent UPR activation leads to apoptosis by promoting the activation of JNK, cleaved caspase-3, caspase-12, **p53 up-regulated modulator of apoptosis (PUMA)** and CHOP contributing to the onset of heart failure (Thuerauf *et al.,* 2006).

The IRE1 branch of the UPR appears to have a protective role in ischaemia. *In vitro* and *in vivo* experimental models of ischaemia show a robust induction of XBP1 and GRP78 (Thuerauf *et al.,* 2006; Qi *et al.,* 2007). Importantly, increased expression of both XBP1 and GRP78 is also found in the ischaemic human heart (Sawada *et al.,* 2010). In addition, in mice subjected to I/R injury, cardiomyocyte-specific deletion of XBP1 shows an increase in myocardial infarct size, impairment in cardiac function and hypertrophic remodelling. Conversely, transgenic mice overexpressing of XBP1s, show reduced infarct size and significant improvement of cardiac function after I/R injury, further highlighting the protective role of XBP1 in cardiomyocytes (Wang *et al.,* 2014). Similarly, overexpression of **GRP94 (HSP90B1)**, a downstream target of XBP1, reduces cardiomyocyte cell death induced by calcium overload and simulated ischaemia (Vitadello *et al.,* 2003).

Activation of the PERK branch of the UPR is also observed under ischaemic conditions (Szegezdi *et al.,* 2006). Phosphorylation of eIF2α is an early event observed in cardiomyocytes after ischaemia *in vitro* and after I/R *in vivo* (Szegezdi *et al.,* 2006; Miyazaki *et al.,* 2011). PERK overexpression promotes cell survival under hypoxic conditions, and PERK down-regulation or the expression of a dominant-negative mutant

leads to decrease in cell viability (Lu *et al.,* 2004). However, when the activation of PERK is prolonged, cardiomyocyte cell death is triggered. In heart cells, persistent ER stress induced by ischaemia promotes the activation of the PERK/ATF4/CHOP axis (Mughal and Kirshenbaum, 2011). Furthermore, CHOP deficiency has been shown to reduce reperfusion injury in a mouse model of myocardial infarction (MI) (Terai *et al.,* 2005). Silencing of both CHOP and caspase-12 shows cardioprotective effects following exposure to hypoxia (Terai *et al.,* 2005). CHOP expression and cleavage of caspase-12 can both be inhibited by the activation of AMP-activated protein kinase (Nickson *et al.,* 2007), highlighting the importance of ER stress-induced apoptosis in hypoxic conditions. In addition, the pro-apoptotic member of the Bcl-2 family PUMA, also a downstream effector of PERK, is an important regulator of the ischaemia/reperfusion (I/R)-induced cell death under ER stress in cardiomyocytes. Overexpression of PUMA induces cell apoptosis in cardiomyocytes under ER stress, and PUMA deletion is protective after I/R injury *in vitro* and *in vivo* (Nickson *et al.,* 2007). Furthermore, I/R injury-associated apoptosis and myocardial dysfunction can be also alleviated by 4-PBA by attenuating the onset of ER stress through the inhibition of GRP78 expression and PERK phosphorylation (Jian *et al.,* 2016). Another downstream effector of PERK, Tribbles3, is elevated following myocardial infarction (MI) and cardiomyocyte-specific overexpression of Tribbles3 in mice shows increased pathological cardiac remodelling and apoptosis after MI (Avery *et al.,* 2010). Thus, sustained activation of PERK and, consequently, triggering of the ER stress-initiated apoptotic signalling mediates cell death in I/R myocardium, playing an overall negative role in the ischaemic heart.

The activation of the ATF6 branch of the UPR appears to have a cardioprotective role. Primary cardiomyocytes show ATF6 activation under hypoxia and nutrient deprivation conditions (Doroudgar *et al.,* 2009), and ATF6-inducible genes show cardioprotection under stress, including I/R. Consistent with this, cardiac-specific expression of active ATF6 in mice shows increased expression of ER stress-inducible mRNAs and proteins including GRP78 and GRP94 and protection against tissue damage, necrosis and apoptosis after I/R injury (Martindale *et al.,* 2006). Moreover, expression of a dominant-negative ATF6 or using ATF6-targeted miRNA significantly decreases the induction of GRP78 and increases cardiomyocyte death upon simulated reperfusion (Doroudgar *et al.,* 2009). Additionally, in mice after MI, inhibition of ATF6 activation impairs cardiac function and increased mortality, further demonstrating the protective role of ATF6 (Toko *et al.,* 2010). Also, ATF6 is protective in heart tissue, by induction of ERAD, promoting the degradation of terminally misfolded protein in the ER (Nakatsukasa *et al.,* 2008). The ability to clear misfolded proteins from the ER appears to be especially critical during ischaemic stress. One of the early ERAD components, Derlin-3, a retro translocation channel, is induced by ATF6 in the heart (Belmont *et al.,* 2010). Overexpression of Derlin-3 enhances the clearance of misfolded proteins and attenuates ER stress activation and caspase activity, protecting cardiomyocytes from ischaemia-induced apoptosis. Conversely, genetic deletion or knockdown of Derlin-3 impairs the clearance of misfolded proteins and shows an increase in cell death after simulated I/R (Belmont *et al.,* 2010).

The cardioprotective effects of the UPR can be attributed to the induction of ER chaperones and the consequent enhancement of protein folding (Glembotski, 2008). Hypoxia impairs disulphide bond formation resulting in oxidative protein misfolding in the ER in which protein disulphide isomerase (PDI), a marker of the UPR, plays a key role (Glembotski, 2008). In human hearts, PDI acts as a cardiomyocyte survival factor in ischaemic cardiomyopathy (Glembotski, 2008), and an increase of PDI is observed in the viable peri-infarcted myocardium. Adenoviral-mediated expression of PDI results in a significant decrease in infarct size and decrease in pathological remodelling with improvements in contractility and shows reduced cardiomyocyte apoptosis in the peri-infarct region in an *in vivo* mouse model of MI (Severino *et al.,* 2007).

ER stress in arrhythmias

Over the last decades, new evidence has emerged for the involvement of ER stress in arrhythmias. Two cardiac cation channels, **$Na_v1.5$** and **$K_v4.3$** were inhibited by activation of PERK (Gao *et al.,* 2013). In human heart failure, the gene for the α-subunit of cardiac $Na_v1.5$ channels (SCN5A), was abnormally spliced and resulted in truncated mRNA variants. The truncated mRNA variants translated into nonfunctional channel proteins and were trapped in the ER inducing the activation of UPR to down-regulate the protein expression of the full-length Na^+ channel (Gao *et al.,* 2013). In addition, inhibition of PERK prevented the degradation of full-length SCN5A mRNA and the reduction in Na^+ currents, suggesting that PERK activation could induce Na^+ current reduction through destabilization of full-length SCN5A mRNA to contribute to arrhythmic risk (Gao *et al.,* 2013). The effect of PERK activation was not specific to cardiac $Na_v1.5$ channels. Blocking PERK also prevented the down-regulation of the gene for the α-subunit of cardiac $K_v4.3$ channels (Liu and Dudley, 2015). Cardiac $K_v4.3$ channels contribute to the cardiac transient outward potassium current (I_{to}), which is the main contributor to the repolarizing phase 1 of the cardiac action potential. Therefore, PERK activation could reduce I_{to}, resulting in shortening of the cardiac action potential duration and phase 2 reentry (Liu and Dudley, 2015). Thus, inhibition of PERK may reverse the down-regulation of arrhythmogenic ion channels to prevent arrhythmias.

In rats with diabetic cardiomyopathy, inhibition of PERK in association with reduced activity of calcineurin decreased arrhythmias (Liu *et al.,* 2014a,b). An *in vitro* study also demonstrated that PERK activation was required in the activation of calcineurin and the dissociation of the FK506 binding protein 1B, 12.6 kDa from the **ryanodine receptor 2 (RyR2)** (Liu *et al.,* 2014a,b). Thus, PERK activated calcineurin to facilitate degradation of FKBP-RyR2 complex leading to intracellular calcium accumulation, which might be a mechanism inducing arrhythmias. ER-dependent ion channel glycosylation could be another mechanism contributing to cardiac arrhythmias. A recent study indicated that only the fully glycosylated form of $Na_v1.5$ channel protein was trafficked normally (Mercier *et al.,* 2015). Hence, altered glycosylation during ER

stress might be involved in alterations of ion channels and induction of cardiac arrhythmias.

UPR as a therapeutic target in cardiac diseases

Pharmacological agents that directly modulate the UPR are emerging as promising tools towards effective treatment of cardiovascular diseases. It has been shown that salubrinal, an eIF2α phosphatase inhibitor, significantly increases GRP78 expression and appears to be protective against ER stress-induced cardiomyocyte apoptosis in a rat MI model (Li *et al.,* 2015). **SIRT1**-activating compounds such as resveratrol appear to have protective roles in cardiovascular disease (Hubbard and Sinclair, 2014). Interestingly, activation of SIRT1 prevents cardiomyocytes ER stress-induced apoptosis through eIF2α deacetylation (Prola *et al.,* 2017), supporting the therapeutic potential of SIRT1 activators for the treatment of cardiac pathologies associated with ER stress. However, it is important to consider that, as discussed above, the UPR response participates in both protective and proapoptotic responses and that very little is known about the mechanistic aspects of the switch from pro-survival to pro-apoptosis. In this context, the kinase inhibitor **sunitinib** can directly activate IRE1 with the consequent activation of XBP1 and reduction of ER stress. However, in patients with previous history of hypertension and heart disease, sunitinib appears to increase the risk for cardiovascular disease (Chu *et al.,* 2007). Recently, Ginkgolide K (1,10-dihydroxy-3,14-didehydroginkgolide, GK), a diterpene lactone isolated from the leaves of *Ginkgo biloba*, has been demonstrated to protect cardiomyocytes from ER stress-induced apoptosis both *in vitro* and *in vivo* (Wang *et al.,* 2016). In response to ER stress, GK can selectively activate the IRE1α/XBP1 pathway and inhibit the activation of RIDD and JNK (Wang *et al.,* 2016). Therefore, GK has a potential for treating cardiovascular diseases.

Pharmacological alleviation of ER stress can also be achieved by stabilizing and rescuing protein misfolding using chemical chaperones that mimic ER chaperones (Perlmutter, 2002). Two such compounds, 4-PBA and **tauroursodeoxycholic acid** (TUDCA), have been approved by the FDA for clinical use, and demonstrate the opportunity for pharmacological treatment. Oral administration of PBA in mice reduced ER stress and apoptosis and reduced pressure-overload cardiac hypertrophy, after TAC (Park *et al.,* 2012). Interestingly, PBA prevented **doxorubicin**-induced cardiac injury and **isoprenaline**-induced cardiac fibrosis, further highlighting its potential as a cardioprotective drug (Ayala *et al.,* 2012). Additionally, TUDCA appears to restore the reduced contractile function in mouse cardiomyocytes under oxidative stress (Guo *et al.,* 2009). Thus, by relief of ER stress, chemical chaperones can play a protective role against cardiac hypertrophy and, potentially, heart failure. Similarly, ROS-induced ER stress can be prevented by **curcumin** and **masoprocol** through GRP94 induction, reduced caspase-12 activation and preservation of PDI integrity (Pal *et al.,* 2010). However, it is important to note that PBA treatment unexpectedly led to a higher mortality, promoted cardiac hypertrophy and dysfunction in mice after TAC. This is in part explained by actions of PBA outside its role as a chemical chaperone (Ma *et al.,* 2016). Thus whereas alleviation of ER stress shows protection in the heart, 'off target' effects of the compounds used as chaperones have to be taken into consideration.

The increased expression of CHOP found in pressure-overloaded hearts also appears as an attractive target for inhibition of ER stress-induced apoptosis. As there are no direct pharmacological agents targeting CHOP, the indirect modulation of its activity appears as a promising therapeutic strategy. The statin atorvastatin, used for prevention of cardiovascular disease, was shown to decrease the expression of caspase-12 and CHOP and decrease cardiomyocyte apoptosis in a post-MI-induced heart failure model (Song *et al.,* 2011). The decrease in CHOP expression and phosphorylation was also observed after treatment with **SP600125**, a JNK inhibitor, which prevents CHOP up-regulation under cyclic stretching in cardiomyocytes (Cheng *et al.,* 2009). Similarly, angiotensin AT_1 receptor antagonists reduce apoptosis and cardiac hypertrophy by attenuation of ER stress-mediated apoptosis. **Telmisartan** prevented the increase in GRP78, CHOP, caspase-12 and p-JNK in rats after abdominal aortic constriction, and **olmesartan** decreased the expression of GRP78, caspase-12 and p-JNK in cardiomyocytes from rats with heart failure (Sukumaran *et al.,* 2011). Additionally, **calcitriol** and **paricalcitol**, agonists of **Vitamin D receptor**s, were protective in MI/R injury by inhibition of caspase-12 and CHOP expression in mice (Yao *et al.,* 2015). This information supports the potential use of inhibitors of ER stress-induced apoptosis, in pressure-overloaded hearts, as a novel therapeutic approach.

Recently, cardiac-specific gene transfer has appeared as a novel therapeutic approach in the treatment of heart disease (Zacchigna *et al.,* 2014). The use of Adeno-associated virus (AAV) has become one of the most promising gene transfer tools for gene therapy, and understanding the molecular basis of myocardial dysfunction has allowed the development of AAV-mediated cardiac gene transfer strategies in animal models. Importantly, the use of these delivery vectors has proven to be safe and effective, as clinical applications of AAV-mediated gene therapy have been tested in an increasing number of Phase I–III clinical trials, with promising results (Collins and Thrasher, 2015). However, this therapy approach is still in its infancy. Administration of an AAV1 vector containing SERCA2 initially resulted in improvement of patients with advanced heart failure (Zsebo *et al.,* 2014). However, no improvement was observed in patients with heart failure in the CUPID-2b trial for the AAV1/SERCA2a vector (Greenberg *et al.,* 2016). Thus, strategies to further improve the efficiency and effectiveness of these delivery systems, along with the development of vectors with higher cardiac tropism, have to be considered. Among the different AAV serotypes, AAV9 appears to be the most efficient in gene transfer studies in rodents, making it the preferred AAV serotype for potential clinical approaches in cardiac disease (Inagaki *et al.,* 2006). In line with this, Hrd1, a key component of ERAD, has been shown to positively act in the adaptive ER stress response in mammalian cardiac myocytes (Doroudgar *et al.,* 2015). AAV9-mediated Hrd1 expression directed to ventricular myocytes contributed to preserving heart function and reduced cardiac hypertrophy in mice with

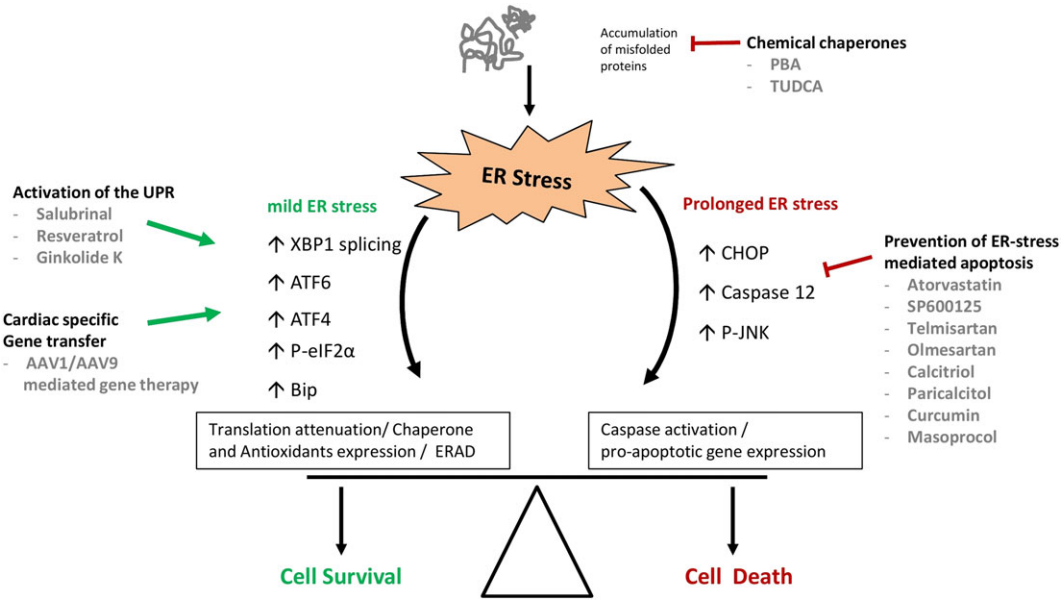

Figure 3

Restoring ER homeostasis as a therapeutic target in cardiac diseases. Different therapeutic approaches aim to restore the balance between the pro-survival and pro-apoptotic ER responses, towards a protective outcome in cardiomyocytes under stress. The main approaches are as follows: (i) the direct alleviation of ER stress by lessening protein misfolding using chemical chaperones; (ii) The use of a compound that is able to directly enhance the protective responses of the UPR; and (iii) the use of a compound that prevents the trigger of ER stress-mediated apoptosis by suppression of CHOP, caspase 12 and JNK activity and expression. In addition, gene therapy based on cardiac-specific expression of protective genes may contribute to the maintenance of cardiomyocyte homeostasis under stress conditions.

pressure overload-induced cardiac pathology (Doroudgar *et al.,* 2015). Similarly, AAV9-mediated ATF6 cardiac overexpression reversed the damage and decreased function observed in ATF6 knockout mice under I/R by ER stress and oxidative stress alleviation in heart (Jin *et al.,* 2016), further highlighting the protective role of the UPR and the potential use of AAV-mediated gene transfer as a therapeutic strategy in ER stress-related cardiac pathology (Figure 3).

In conclusion, ER stress is involved in many pathological processes of cardiovascular disease. The UPR is a defensive mechanism, which can protect cardiomyocytes by maintaining ER homoeostasis. However, prolonged ER stress will cause dysfunction and apoptosis of cardiomyocytes, leading to cardiovascular diseases. Over the recent years, understanding of the pathophysiological role of ER stress in cardiovascular disease has progressed significantly and several potential therapeutic agents have been investigated. However, there are still many unresolved questions that need to be answered. An improved understanding of the molecular mechanisms underlying ER stress in heart diseases will help to identify novel potential targets for new therapeutic interventions and drug discovery.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.,* 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.,* 2015a,b,c,d,e).

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Conflict of interest

The authors declare no conflicts of interest.

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