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The unfolded protein response in ischemic heart disease

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Abstract

Ischemic heart disease is a severe stress condition that causes extensive pathological alterations and triggers cardiac cell death. Accumulating evidence suggests that the unfolded protein response (UPR) is strongly induced by myocardial ischemia. The UPR is an evolutionarily conserved cellular response to cope with protein-folding stress, from yeast to mammals. Endoplasmic reticulum (ER) transmembrane sensors detect the accumulation of unfolded proteins and stimulate a signaling network to accommodate unfolded and misfolded proteins. Distinct mechanisms participate in the activation of three major signal pathways, *viz*. protein kinase RNA-like ER kinase, inositol-requiring protein 1, and activating transcription factor 6, to transiently suppress protein translation, enhance protein folding capacity of the ER, and augment ER-associated degradation to refold denatured proteins and restore cellular homeostasis. However, if the stress is severe and persistent, the UPR elicits inflammatory and apoptotic pathways to eliminate terminally affected cells. The ER is therefore recognized as a vitally important organelle that determines cell survival or death. Recent studies indicate the UPR plays critical roles in the pathophysiology of ischemic heart disease. The three signaling branches may elicit distinct but overlapping effects in cardiac response to ischemia. Here, we outline the findings and discuss the mechanisms of action and therapeutic potentials of the UPR in the treatment of ischemic heart disease.

Keywords

UPR; PERK; ATF6; IRE1; XBP1s; Ischemic heart disease; ER stress

1. Introduction

Ischemic heart disease is the leading cause of cardiovascular disease-related disability and death worldwide, which creates huge burden on the healthcare system and economy [1–3]. Despite extensive interests and mounting needs, our knowledge into the pathophysiology is

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still lagging behind. As a result, current care and cure are unable to arrest the progression and prevalence of ischemic heart disease.

Identification of better and more effective therapeutic approaches is not feasible without a deeper and more thorough understanding of the pathological mechanisms. Further, the present clinical situation of inability to treat ischemic heart disease suggests novel, additional pharmacological targets need to be discovered. Myocardial infarction creates sudden blockage of oxygen and nutrient supply to the myocardium. To survive this detrimental insult, cardiac myocytes undergo extensive remodeling, electrophysiologically, metabolically and structurally [4]. Persistent ischemia causes permanent damage in cardiac cells and renders them beyond rescue. On the other hand, timely and effective restoration of coronary blood flow by thrombolysis and/or percutaneous coronary intervention is the best approach to salvage myocardium from ischemia and improve clinical outcomes [5]. This so-called ischemia/reperfusion (I/R) however does not help without a price [6]. Numerous studies have shown that I/R *per se* leads to significant cardiac damage, in additional to ischemia [7,8], which may account for as much as 40% of the final infarction [6].

A growing body of evidence suggests that the unfolded protein response (UPR) is strongly activated in ischemic heart disease in humans and rodent models by various pathological events, such as overproduction of reactive oxygen species (ROS), inflammation, and metabolic derangement (Fig. 1) [9–13]. The UPR is an adaptive cellular process to accommodate protein-folding stress [14,15]. Upon activation, three signaling arms cooperate to restore cellular homeostasis, including protein kinase RNA-like ER kinase (PERK), inositol-requiring protein 1 (IRE1), and activating transcription factor 6 (ATF6) (Fig. 2). However, during prolonged or overwhelming protein folding stress, the adaptation of UPR starts to fail and ER-initiated apoptosis ensues, which in turn, contributes to the development and progression of ischemic heart disease [16,17]. Here, we focus on the role and mechanisms of action of the UPR in myocardial infarction and I/R and explore potential therapeutic targets to tackle this devastating disease.

2. The UPR and ER molecular chaperones in ischemic heart disease

Glucose-regulated protein of 78 kDa (GRP78), also known as immunoglobulin heavy chain binding protein (BiP), is one of the most abundant molecular chaperones residing in the ER. GRP78 was originally discovered as a glucose-regulated target due to its upregulation by glucose deprivation in transformed cells [18]. Besides as a critical chaperone involved in protein folding, GRP78 also serves as a sensor and regulator of the UPR. At resting conditions, GRP78 binds the three signaling arms of the UPR and retains them on the ER membrane. In response to accumulation of misfolded proteins, however, GRP78 preferentially interacts with protein clients on the hydrophobic patches and releases the tethering with the UPR transducers. Liberated PERK undergoes autophosphorylation and activation, while ATF6 is translocated from ER to the Golgi for regulated intramembrane cleavage and maturation. As for IRE1, autophosphorylation leads to stimulation of an endoribonuclease activity, which acts on a downstream target mRNA X-box binding protein 1 (XBP1). After excision of a cryptic exon of 26 bp, the spliced XBP1 (XBP1s) becomes a potent transcriptional factor. The three signaling branches then transiently suppress protein

synthesis, enhance ER protein-folding capacity, and augment ER-associated degradation, which together orchestrate to restore cellular homeostasis. Additionally, GRP78 is a *bono fide* target of the UPR [19]. XBP1s and activated ATF6 may form heterodimers and directly stimulate the transcription and translation of GRP78. In so doing, the UPR may be quenched after acute adaption, and chronic pathological activation of the UPR may be prevented.

Accumulating evidence has shown that GRP78 is upregulated in the heart under multiple cardiac pathological conditions [20] (Table 1). Using human heart samples, Ortega et al. found that GRP78 is induced at protein level in patients with either dilated or ischemic cardiomyopathy [21]. On the other hand, mouse hearts subjected to *in vivo* myocardial infarction exhibit increased GRP78 expression in cardiac myocytes near the infarct region but not in healthy cells in the remote area [10]. Additionally, Hardy and Raiter found that simulated ischemia for 4 h leads to upregulation of GRP78 in cultured cardiac myocytes [22]. Moreover, Shintani-Ishida et al. showed that early ischemic preconditioning increases myocardial GRP78 in a rat coronary artery occlusion model [23]. More importantly, transfection of GRP78 antisense oligonucleotides attenuates the preconditioning-mediated protection to ischemia, suggesting that GRP78 plays a critical role in ischemic preconditioning [23].

The mechanisms of GRP78-mediated cardioprotection remain to be fully clarified. Most studies attribute this beneficial effect to the chaperone function, to ameliorate protein-folding stress and quench chronic pro-apoptotic signaling of the UPR [20,24]. Recently, emerging evidence suggests that upregulation of GRP78 in response to ER stress may trigger its translocation to cell surface and mediate a pro-survival signaling transduction pathway [25]. Studies have shown that GRP78 is localized on the cell surface, such as endothelial cells [26], macrophages [27], immortalized cell lines [28], and tumor cells [29–32]. Jacobsen et al. found that a human monoclonal antibody derived from the phage display library recognizes cell surface localized GRP78 in breast cancer cells [33]. Additionally, Pizzo and colleagues isolated autoantibodies from prostate cancer patients, which bind GRP78 on cell surface. Interestingly, the epitope on GRP78 for these autoantibodies is also recognized by activated α2-macroglobin (α2M*) [34]. Further studies show that the interaction between cell surface GRP78 and antibodies or α2M* elicits intracellular anti-apoptotic signaling [35]. In addition, using a phage display peptide library, Hardy et al. identified a 12-amino acids peptide that specifically binds cell surface GRP78 in endothelial cells [36]. This interaction leads to enhancement of angiogenesis and protection against limb ischemia [37]. Moreover, Hardy and Raiter show that AdoPep1, a GRP78-binding peptide of 12-amino acids derived from the metalloproteinase domain of ADAM15 (a disintegrin and metalloproteinase 15), can protect cardiac myocytes from ischemia-induced cell death at both *in vitro* and *in vivo* levels [22]. In aggregate, these results suggest that GRP78 may confer cardioprotective effects in the heart by both chaperone function and cell surface localization and pro-survival pathway activation.

ER is also the host of other protein folding chaperones and quality control system. Glucose-regulated protein of 94 kDa (GRP94) is the HSP90 (heat shock protein of 90 kDa) counterpart in the ER lumen. Like GRP78, GRP94 plays a critical role in ER protein folding [38]. Targeted disruption of GRP94 leads to embryonic lethality on day 7 of gestation [39].

While GRP94$^{+/-}$ embryonic stem (ES) cells does not affect the ER stress response, homozygous deletion in ES cells severely impairs differentiation to cardiac myocytes, highlighting an essential role in cardiogenesis [40]. Indeed, GRP94 is highly expressed in both atrial and ventricular myocytes in the developing heart [41]. However, the role of GRP94 in the heart under pathological conditions remains to be fully clarified. The other critical component of the protein quality control system in the ER is calnexin/calreticulin, which functions to ensure correct folding and assembly of glycoproteins. After N-glycan is transferred from donor to substrate glycoproteins, glucosidases I and II cooperate to remove the terminal two glucose molecules, and the exposed, third glucose is recognized by calnexin/calreticulin. ER protein of 57 kDa (ERp57), an integral component of the calnexin/calreticulin system and an oxidoreductase in the ER, catalyzes disulfide bond formation between ERp57 and substrates. When protein folding is complete, cleavage of the last glucose in *N*-glycan releases cargo proteins from calnexin/calreticulin. If folding is failed, UDP glucose: glycoprotein glucosyltransferase attaches a new glucose to the *N*-glycan and another round of calnexin/calreticulin cycle starts for further protein folding. Using a fluorescent reporter mouse model, Mesaeli et al. found that calreticulin is highly abundant in the developing heart [42]. Homozygous deletion of calreticulin shows marked decreases in ventricular wall thickness and defects in trabeculation, which may stem from impairments in calcium signaling [42].

2.1. The PERK pathway in ischemic heart disease

PERK is a transmembrane serine/threonine kinase activated by ER stress *via* dimerization and autophosphorylation, which leads to phosphorylation of downstream target eIF2α (eukaryotic initiation factor 2α) and global inhibition of translation [43]. The attenuation of translation causes decreases in protein synthesis and reduction in new ER folding clients, which provides additional time for repair in the ER. However, translation of ATF4 (activating transcription factor 4) is activated under this condition due to the existence of a positive-acting upstream open-reading frame (uORF) in the 5′ untranslated region (UTR) [44]. ATF4 in turn stimulates a downstream target CHOP (C/EBP homologous protein), which is expressed at a very low level under resting conditions. As a transcription factor, CHOP has been shown to regulate multiple apoptosis-related genes, including Bcl-2 (B cell lymphoma −2) and GADD34 (growth arrest and DNA damage inducible 34) [45].

Emerging evidence shows that CHOP exerts strong pro-apoptotic function in multiple ischemic conditions. Nashine et al. found that I/R in the eye leads to upregulation of the UPR in retinal ganglion cells. Consistently, deficiency of CHOP significantly improves cell survival and functional recovery in response to I/R [46]. Miyazaki et al. showed that cardiac I/R activates the phosphorylation of eIF2α and upregulates CHOP gene expression [17]. More importantly, CHOP knockout mice show reduction of myocardial inflammation and improvements in cardiac function against I/R. In addition, Myoishi et al. found CHOP-dependent pathway is activated in unstable plaques [47]. Knockdown of CHOP by siRNA decreases ER stress-dependent death of cultured coronary artery smooth muscle cells and THP-1 cells [47]. Similar findings have been observed in a renal I/R model [48]. On the other hand, pharmacological inhibition of the UPR by 4-PBA (4-phenylbutyric acid) confers strong cardioprotection against myocardial infarction, which is accompanied by significant

reduction of CHOP expression [49]. Collectively, these results suggest that the pro-apoptotic role of CHOP under chronic ER stress is a universal phenomenon in response to various ischemic insults.

Consistently, CHOP expression is elevated in human heart failure of ischemic origin, along with other markers of the UPR [50,51]. Interestingly, CHOP is also stimulated in the heart of dilated cardiomyopathy in human [50–52]. In rodent models, thoracic aortic constriction (TAC) causes significant cardiac hypertrophy, pathological remodeling and heart failure, which are accompanied by augmentations of CHOP and other UPR markers [50,52]. Although the hypertensive cardiomyopathy is different compared with ischemic heart disease, hypoxia has been identified in pressure overload-induced heart failure [53]. It is therefore possible that the hypoxic response by TAC triggers the UPR and CHOP expression in the heart. In addition, cardiac myocyte hypertrophic growth involves new protein production, metabolic reprogramming, phospholipid biosynthesis and membrane expansion, most of which are potent inducers of the UPR [54–56]. Importantly, germline deletion of CHOP shows strong protection against heart failure progression in response to pressure overload [50]. Mechanistically, Caspase 12 expression is significantly diminished and pro-surviving proteins, including Bcl-2, are restored [50]. Moreover, expression of GADD34 is reduced in CHOP deficiency hearts after TAC. Since GADD34 is involved in translation stimulation and ER client expression [57], decrease of GADD34 may lead to a drop in ER load and improvements in cellular homeostasis. Collectively, these results indicate that chronic activation of the UPR under pathological conditions elicits cell death pathways and promotes progression of heart failure.

2.2. The IRE1/XBP1s pathway in ischemic heart disease

IRE1 is the most evolutionarily conserved ER stress transducer from yeast to mammals [14]. Accumulation of misfolded proteins in the ER stimulates dimerization and autophosphorylation of IRE1. The active IRE1 manifests an endoribonuclease activity toward multiple downstream targets. In mammalian cells, IRE1 recognizes and cleaves an atypical exon from XBP1, which creates frame-shift in the XBP1 mRNA. The resultant spliced XBP1 (XBP1s) is translated as a fully functional, larger basic leucine zipper transcriptional factor. Studies have shown that XBP1s stimulates genes involved in chaperone production, protein folding, ER-associated degradation and metabolic regulation.

Using a mouse I/R model, we have shown that reperfusion of as early as 5 minutes post coronary artery ligation stimulates XBP1s expression, which rises to approximately 6-fold by 4 h [58]. More importantly, XBP1s downstream target genes, such as GRP78, GRP94, start to increase 4 h after reperfusion. We have also examined the level of XBP1s in myocardial samples from patients with end-stage heart failure, and found that XBP1s mRNA level is reduced in human hearts following left ventricular assistant device mechanical support compared to patients prior to device implantation [58]. These results indicate that XBP1s is an acute, early response to I/R stress in the heart.

To further investigate the role of the IRE1/XBP1s pathway in the heart during I/R, we took advantage of the cardiac-specific XBP1 knockout (cKO) animal model [58]. We found that a significant increase in myocyte death and more profound pathological remodeling in cKO

mice compared with either $XBP1^{fl/fl}$ or αMHC-Cre controls, suggesting that XBP1s induction is necessary to protect the heart from I/R injury *in vivo*. In addition, using a tetracycline inducible transgenic mouse model, we observed dramatic protection against reperfusion injury by XBP1s overexpression, with the infarct area of the transgenic group reduced by nearly 50%, suggesting that XBP1s expression is sufficient to protect the heart from I/R injury. Consistently, in a cerebral I/R injury model, Ibuki et al. found that overexpression of XBP1s in the brain suppresses cell death. Moreover, inhibition of XBP1s activation accelerates neuronal cell death in response to I/R [59]. Although the gain- and loss-of-function studies of XBP1s show consistent results in I/R, caution needs to be exercised when interpreting these results. In the transgenic mouse model, we only triggered short-term overexpression for 2 weeks [58]. However, prolonged induction of XBP1s in cardiac myocytes may cause persistent ER stress and detrimental consequences.

It has long been appreciated that chronic activation of the UPR leads to cell death, of which the IRE1 branch plays an essential role [60]. Pharmacological induction of the UPR by tunicamycin or thapsigargin stimulates the canonical UPR signaling, and also c-Jun N-terminal kinase (JNK) activation [61]. Importantly, the UPR-JNK phenotype is absent in $IRE1\alpha^{-/-}$ mouse embryonic fibroblasts (MEFs), highlighting a critical role of IRE1 in this process. At the molecular level, the cytoplasmic domain of IRE1 recruits and interacts with tumor necrotic factor receptor associated factor 2 (TRAF2) and stimulates JNK phosphorylation and activation [61].

Further, emerging studies show that sustained IRE1 signaling under ER stress promotes cell death *via* other mechanisms [62]. Chronic activation of IRE1 triggers the regulated IRE1-dependent decay (RIDD) [63]. While early phase of RIDD may exert beneficial actions by reducing ER protein load, persistent RIDD targets ER chaperones for degradation, which contributes to impairment in ER folding capacity and cellular homeostasis [63]. In addition, prolonged activation of IRE1 may cause rapid decay of several microRNAs for Caspase 2 [64]. Therefore, Caspase 2 protein level is elevated and cell death ensues. To achieve these divergent actions, the cytoplasmic region of IRE1 may form distinct scaffold complexes with different cellular components, which are referred as UPRosome [60].

Along these lines, IRE1 has been implicated in pro-apoptotic and proinflammatory pathways in atherosclerosis models. A recent study by Tufanli et al. suggests that IRE1 regulates the expression of many proatherogenic genes, including several important cytokines and chemokines. This study reveals that at the *in vivo* level, IRE1 inhibitors lead to a significant decrease in hyperlipidemia-induced IL-1 (interleukin -1) and IL-18 (interleukin -18) production, lower T-helper type-1 immune responses, and reduced atherosclerotic plaque size without altering the plasma lipid profiles in $ApoE^{-/-}$ mice [65].

2.3. The ATF6 pathway in ischemic heart disease

ATF6 is a 670 amino acids single pass type 2 transmembrane protein. At basal conditions, ATF6 is localized on the ER membrane *via* interaction with GRP78. Upon induction of the UPR, dissociation of GRP78 leads to liberation of ATF6 and consequent translocation from ER to the Golgi, which requires a conserved region of amino acids 468–500 in the ER luminal domain [66]. In the Golgi, ATF6 is subjected to regulated intramembrane

proteolysis by site-1 and site-2 proteases (S1P and S2P), which resembles the posttranslational processing of sterol regulatory element binding proteins (SREBPs) [67]. The other conserved domain of amino acids 550–640 is indispensible for S1P recognition and initiation of intramembrane cleavage. The soluble cytoplasmic region of 400 amino acids is then translocated to the nucleus. This nuclear ATF6 (ATF6n) possesses both DNA-binding and transactivation domains, which contributes to the upregulation of a host of ER chaperones to enhance the folding capacity of the ER and restore cellular homeostasis.

Ischemia in the heart leads to potent activation of the UPR at both *in vitro* [10,68] and *in vivo* levels [69,70]. Doroudgar et al. show that simulated ischemia for 20 h using cultured myocytes stimulates the ER-resident chaperone GRP78 expression and this effect is largely diminished when ATF6 is reduced by siRNA [68]. At the functional level, ATF6 knockdown causes more severe cell death upon ischemia in neonatal rat ventricular myocytes (NRVMs). These *in vitro* findings are further confirmed in an *in vivo* setting. Martindale et al. took a transgenic approach to overexpress ATF6n in an inducible manner in cardiac myocytes [69]. Upon induction by tamoxifen injection, the transgenic hearts show significant protection against global I/R as assessed by increases in recovery of ventricular developed pressure, decreases in cardiomyocyte apoptosis, and reduction in necrotic release of lactate dehydrogenase. Consistently, ATF6 inhibition by a specific inhibitor 4-(2-aminoethyl) benzenesulfonyl fluoride leads to deterioration of cardiac function after myocardial infarction, which is similar to a transgenic mouse model expressing the dominant negative mutant of ATF6 [71]. In aggregate, these results suggest that the ATF6 branch of the UPR confers strong cardioprotection against ischemic heart disease.

Multiple mechanisms have been proposed to explain the pro-surviving effects of ATF6. The active ATF6n may form a heterodimer with XBP1s and bind the ER stress responsive element (ERSE) in promoters of ER chaperones, including GRP78, GRP84, ERp72, *etc*. In addition, ATF6n may directly stimulate sarco/endoplasmic reticulum calcium ATPase 2 (SERCA2) and promote the restoration of calcium homeostasis [72]. Indeed, dominant negative mutation of ATF6 strongly diminishes calcium depletion-mediated upregulation of SERCA2, suggesting that ATF6 is required for maximal, optimal induction of SERCA2. Not surprisingly, a conserved ERSE site has been discovered in the SERCA2 promoter, indicating that SERCA2 is a direct transcriptional target of ATF6. Moreover, a recent study highlights a novel role of ATF6 in protecting the heart against I/R. Overproduction of ROS is at the central stage of reperfusion injury. Studies by Jin et al. show that overexpression of ATF6n is sufficient to protect NRVMs from H_2O_2-induced cell damage [70]. Knockdown of ATF6 leads to exacerbation of ROS production by simulated I/R and more profound cell death. Moreover, a comprehensive survey shows that ATF6 directly stimulates a group of antioxidant genes, including catalase and peroxiredoxin 5. Further analysis discovers 2 conserved ERSE sites in the catalase promoter, suggesting that catalase is a direct transcriptional target of ATF6. In addition, catalase induction by I/R is significantly diminished by ATF6 knockout. *Ex vivo* studies show that deficiency of ATF6 exacerbates recovery from I/R, and overexpression of either ATF6 or catalase leads to a strong rescue. Collectively, these findings suggest that ATF6 confers strong cardioprotection against I/R, which is mediated by multiple pro-surviving mechanisms.

2.4. The UPR and inflammation

Inflammation is a collection of inflammatory responses to tissue injury or infection, which plays critical roles in maintaining homeostasis at both cellular and organism levels. Numerous epidemiological, clinical, and experimental evidence has firmly established a causal effect of inflammation in disease initiation and progression, including cardiovascular disease [73]. Like the UPR, acute inflammatory response aims to repair and protect physiological function, whereas chronic stimulation of inflammation is implicated in pathogenesis under various conditions. Importantly, multiple stimuli of the UPR are potent inducers of inflammation, such as overproduction of ROS, calcium derangements, and metabolic dysregulation [74]. Indeed, previous studies have shown that the UPR may directly elicit inflammatory response [75]. ER is an oxidative environment for disulfide bond formation. The oxidative folding machinery, consisting of protein disulfide isomerase and ER oxidoreductase, catalyzes disulfide bond formation in client proteins and transmits electrons to oxygen, which is a major source of ER-derived ROS. Moreover, PERK phosphorylation and activation leads to global translational attenuation. As a consequence, the short-lived IκB kinase may not be efficiently regenerated, the inhibition of NFκB pathway is therefore diminished, and inflammatory response ensues [76]. Further, chronic activation of the UPR leads to formation of IRE1-TRAF2 complex in cytosol that induces JNK phosphorylation and inflammation [61]. On the other hand, multiple cytokines from the inflammatory response may directly stimulate the UPR [77,78]. Taken these findings together, the UPR and inflammation are intimately intercalated with prominent crosstalk, which may play critical roles in the pathogenesis of ischemic heart disease.

2.5. Temporal dynamics of the UPR

Activation of the UPR may activate both cytoprotective and pro-apoptotic signaling pathways, which together determine the final fate of the cell (Fig. 3). Studies have shown that the three branches are stimulated with different temporal dynamics. Administration of tunicamycin or thapsigargin in HEK293 cells leads to activation of all three signaling branches, albeit at different time course [79]. The IRE1 pathway is quickly stimulated, peaks at 4 h and then diminishes after 12 h of tunicamycin treatment. ATF6 follows a similar pattern. PERK, however, manifests persistent activation throughout the treatment time. Lin et al. propose that the temporal regulation may determine cell fate. Forced activation of IRE1 to prevent the quenching improves cell survival, which is associated with induction of XBP1s, not JNK. In contrast, chemical-genetic induction of PERK in cultured cells, without activating either IRE1 or ATF6, leads to exacerbation of cell death even in the absence of UPR stimuli [80]. Using a mouse I/R model, we have shown that the induction of XBP1s is acute, potent and transient, which is consistent with a temporal cytoprotective role of XBP1s in I/R injury [58]. Whereas most studies with acute induction of the UPR show beneficial effects in cell survival, sustained stimulation of all three branches may disrupt the feedback regulation, and lead to impairments in cellular homeostasis.

Emerging evidence suggests that the IRE1 branch acts as a unique cell fate executor [62]. Whereas acute UPR induction stimulates the pro-surviving XBP1s signaling *via* IRE1 autophosphorylation and oligomerization, chronic protein-folding stress leads to the pro-apoptotic action of IRE1. Activation of IRE1 stimulates RIDD toward multiple RNA

substrates, including ER folding cargos [63]. This action, together with PERK-mediated translation attenuation, contributes to reduction of ER folding load and helps restore cell homeostasis. Sustained activation of RIDD however degrades transcripts of ER chaperones, which may impair the ER folding capacity [63]. Moreover, recent studies indicate that RIDD targets multiple microRNAs for pro-apoptotic Caspase 2 [64]. As a consequence, sustained activation of IRE1 leads to upregulation of Caspase 2 and following cell death. Indeed, allosteric inhibition of the RIDD activity of IRE1 preserves ER stress-induced retinal degeneration and pancreatic β cell loss [81]. Taken together, these results suggest that IRE1 may function as a cell fate executor, controlled by dynamic cytosolic scaffolds with different components [60].

3. Conclusions and future perspectives

Despite extensive interests and mounting needs, ischemic heart disease remains a leading cause of morbidity and mortality {Benjamin, 2018 #1331}. Current therapies are insufficient to arrest disease initiation and progression. Discovering new treatment approaches requires a better and further understanding of the underlying pathophysiology. Molecular alterations occurring in cardiac ischemia, such as ROS overproduction, metabolic derangements, inflammation, *etc.*, may lead to potent, acute induction of the UPR in the heart. Indeed, accumulating evidence points to a critical role of the UPR in the etiology of ischemic heart disease. Whereas the pro-surviving effects of the UPR may dominate in the early phase of ischemia, persistent activation of the UPR could cause adverse consequences. While we continue to gain insights about the versatile roles of the UPR in ischemic heart disease over the past decades, future work may be focused on dissecting the temporal dynamics of the UPR and fine-tuning individual signaling branches for therapeutic gain.

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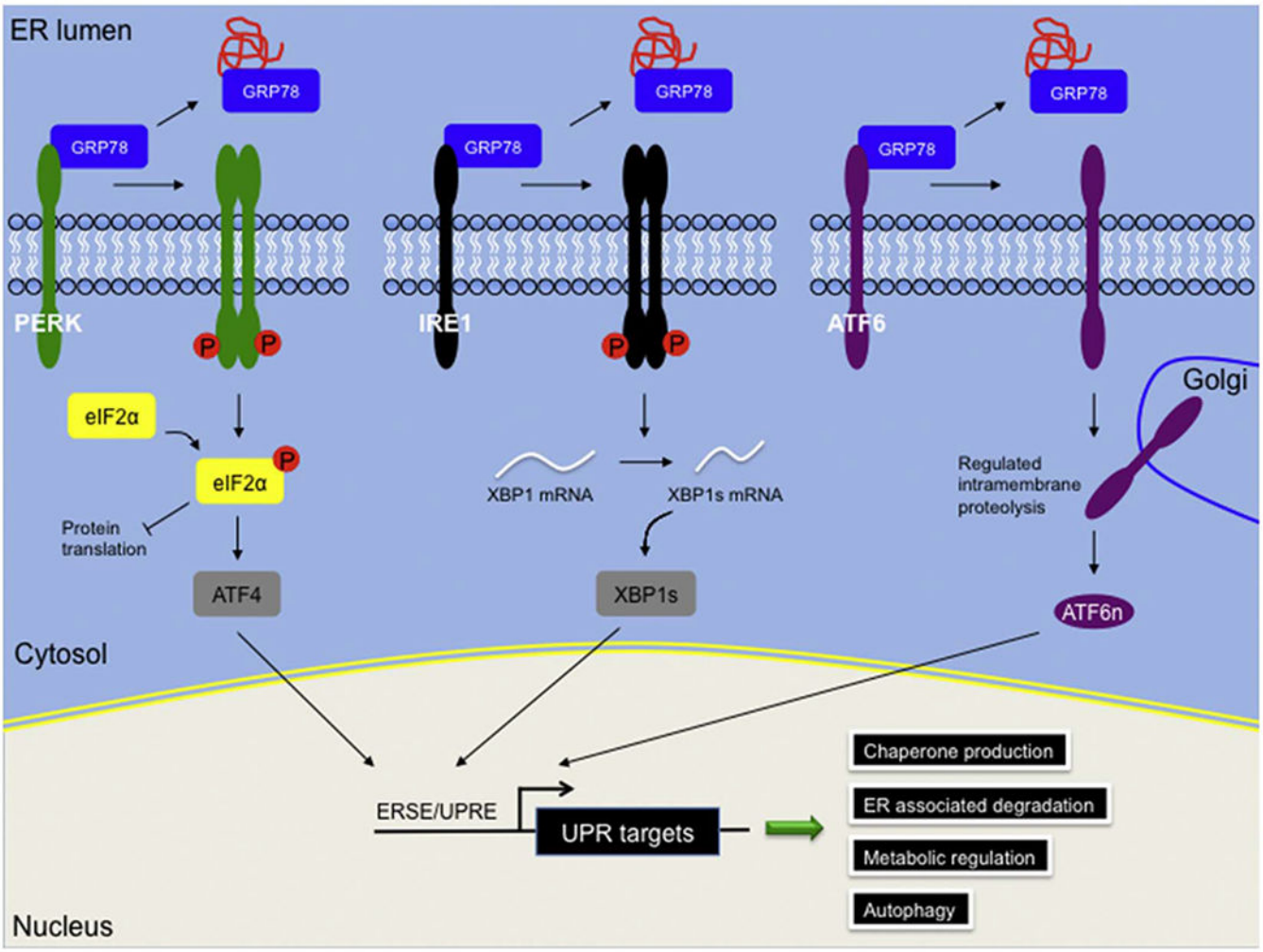

Fig. 1.

The mammalian unfolded protein response (UPR).

The mammalian UPR consists of three distinct but overlapping signaling branches. Under resting conditions, PERK, IRE1 and ATF6 are sequestered on the ER membrane by interacting with ER-resident chaperone GRP78. Accumulation of unfolded proteins leads to dissociation of GRP78 and activates the three downstream pathways *via* different mechanisms. Dimmerization and autophosphorylation of PERK stimulates phosphorylation of eIF2α, which on one hand transiently attenuates protein translation to create a "window-of-repair" for protein folding, and on the other hand, increases ATF4 translation. Dimerization and autophosphorylation of IRE1 enhances an endoribonuclease activity that cleaves a cryptic intron from downstream target XBP1. The spliced XBP1 (XBP1s) is a potent transcriptional factor targeting the ER stress responsive element (ERSE) / unfolded protein response element (UPRE) sites of numerous UPR genes. ATF6 is single transmembrane protein, which translocated to the Golgi from ER upon protein folding stress. ATF6 undergoes regulated intramembrane proteolysis on the Golgi membrane and the cytosolic N-terminus of nuclear ATF6 (ATF6n) acts as a strong transcriptional factor. Stimulation of the UPR leads to activation of various downstream signaling, such as

chaperone production, ER-associated degradation, metabolic regulation and autophagy, which together aim to restore ER homeostasis.

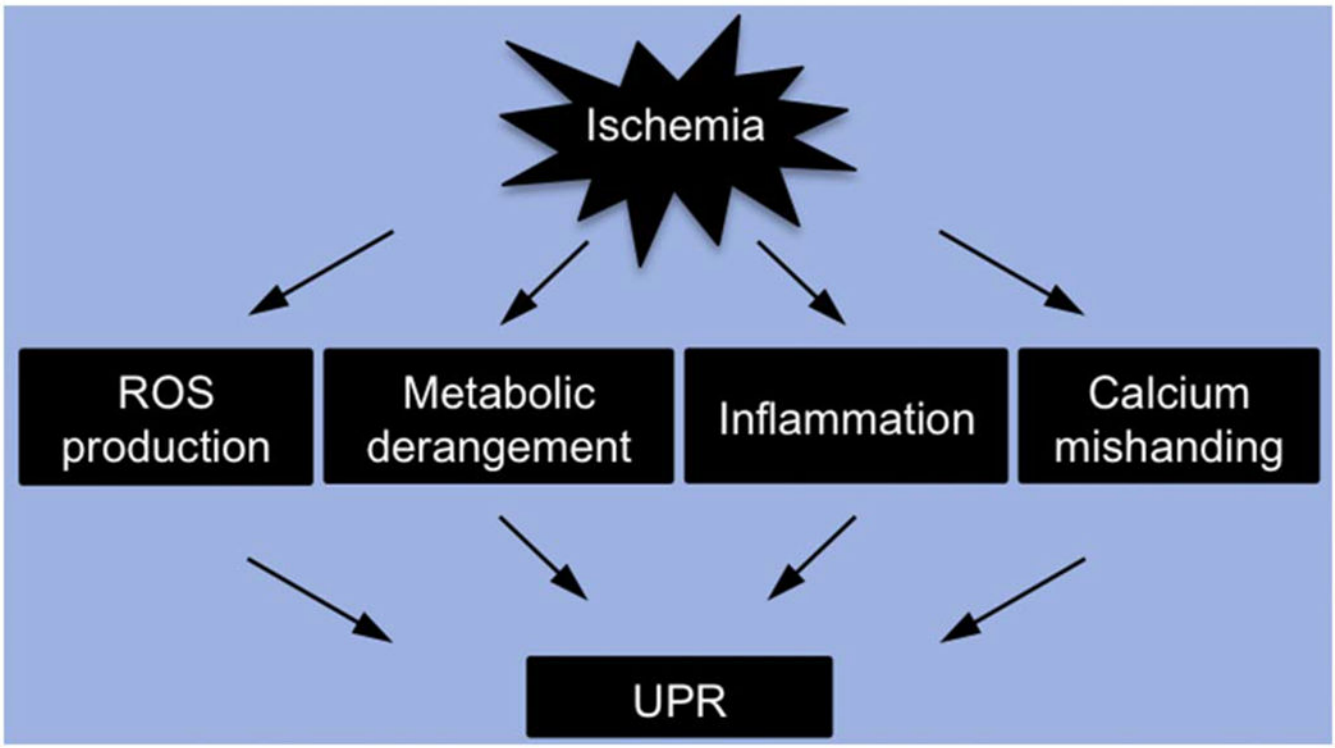

Fig. 2.

Activation of the UPR in ischemic heart disease.

Multiple events participate in the pathogenesis of ischemic heart disease, including overproduction of reactive oxygen species (ROS), metabolic derangement, inflammation, and calcium mishandling. Most, if not all, of these signaling pathways lead to perturbation of the ER homeostasis and induction of the UPR.

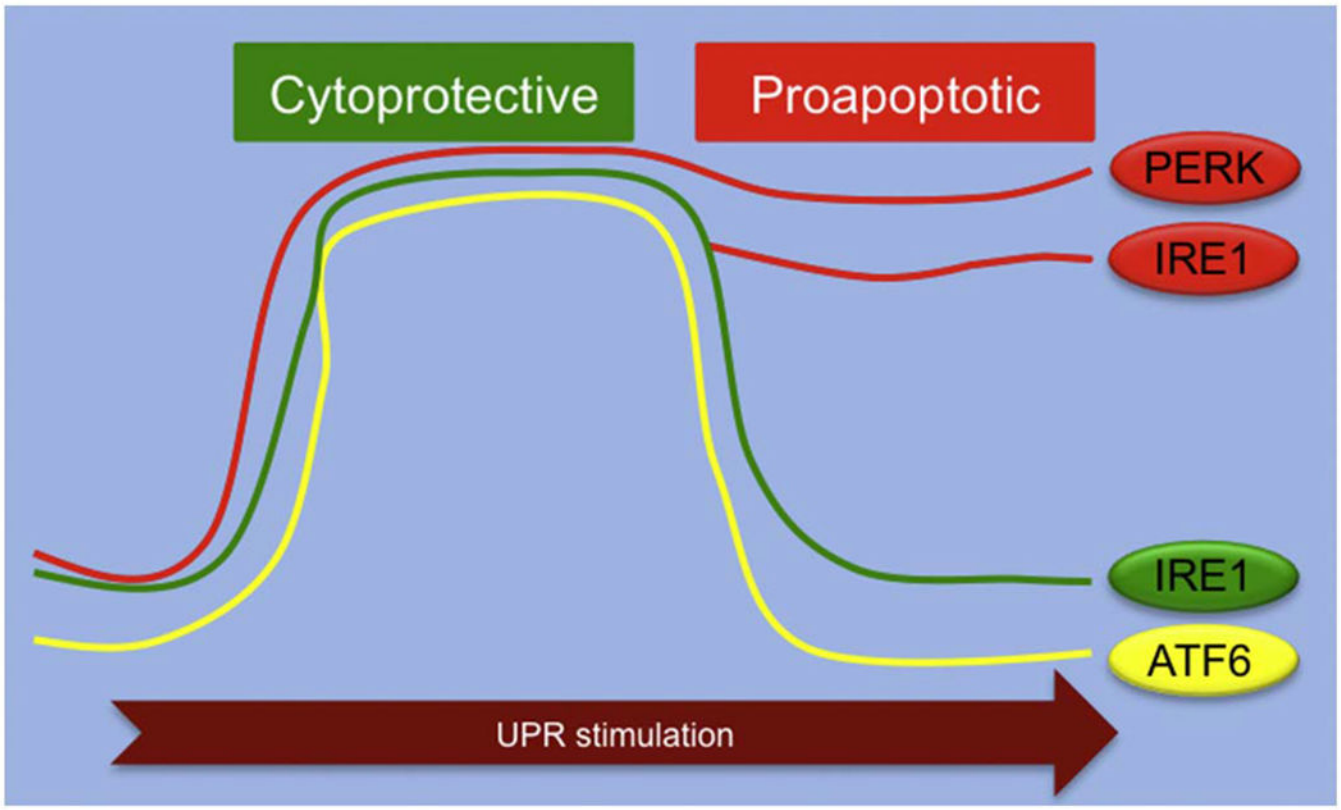

Fig. 3.

The temporal dynamics of the UPR.

The UPR stimuli typically activate all three branches. Both IRE1 and ATF6 are stimulated in an acute, transient manner, which is largely cytoprotective. The diminishment of IRE1 and ATF6 when facing long-lasting stress, combined with persistent activation of PERK, leads to augmentation of the pro-apoptotic signaling and cell death. Note that the pro-apoptotic property of the IRE1 signaling may be induced by irremediable protein folding stress, which contributes to cellular demise under conditions of pathological chronic activation of the UPR. The temporal dynamics of the UPR therefore plays a pivotal role in determining cell fate in response to various stresses.

Table 1

Diseases and defective genes.

Pathway	Gene	Model/disease	Phenotype	Ref.
Protein folding	GRP78	Cardiomyopathy, human	Upregulated	[21]
	GRP78	Myocardial infarction, mouse	Upregulated	[10]
	GRP78	Simulated ischemia, NRVM	Upregulated	[22]
	GRP78	Preconditioning, rat	Upregulated. Knockdown in NRVM dampens protection by preconditioning	[23]
	GRP94	Knockout, mouse	Embryonic lethality; deficient ES cells do not different into cardiac cells	[39–40]
	Calreticulin	Knockout, mouse	Embryonic lethality; ventricular well thinning	[42]
PERK	CHOP	Knockout, mouse	Reduced retinal ganglion cell death after retinal I/R	[46]
	CHOP	Knockout, mouse	Reduced infarction in cardiac I/R	[17]
	CHOP	Atherosclerosis, human	Upregulated in unstable thin-cap atheroma	[47]
	CHOP	Knockout, mouse	Reduced proximal tubule damage in renal I/R	[48]
	CHOP	Cardiomyopathy, human	Upregulated	[50–52]
	CHOP	Knockout, mouse	Reduced pathological remodeling in pressure overload	[50]
IRE	XBP1s	Cardiomyopathy, human	Reduced after implantation of left ventricular assist device	[58]
	XBP1s	Cardiac-specific knockout, mouse	Elevated infarction in cardiac I/R	[58]
	XBP1s	Cardiac-specific overexpression, mouse	Reduced infarction in cardiac I/R	[58]
	XBP1s	Cardiac-specific overexpression, mouse	Reduced infarction in cerebral I/R	[59]
	IRE1	Atherosclerosis, mouse	Inhibitor reduces plaque size	[65]
ATF6	ATF6	Simulated ischemia, NRVM	Upregulated	[68]
	ATF6	Cardiac-specific overexpression, mouse	Reduced infarction in *ex vivo* cardiac I/R	[69]
	ATF6	Cardiac-specific knockout, mouse	Elevated infarction in cardiac I/R	[70]
	ATF6	Cardiac-specific overexpression, dominant negative mutant	Exacerbated response in mouse myocardial infarction	[71]
	ATF6	ATF6 inhibitor, mouse	Exacerbated response in myocardial infarction	[71]

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