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Endoplasmic reticulum stress and Nrf2 signaling in cardiovascular diseases

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ABSTRACT

Various cellular perturbations implicated in the pathophysiology of human diseases, including cardiovascular and neurodegenerative diseases, diabetes mellitus, obesity, and liver diseases, can alter endoplasmic reticulum (ER) function and lead to the abnormal accumulation of misfolded proteins. This situation configures the so-called ER stress, a form of intracellular stress that occurs whenever the protein-folding capacity of the ER is overwhelmed. Reduction in blood flow as a result of atherosclerotic coronary artery disease causes tissue hypoxia, a condition that induces protein misfolding and ER stress. In addition, ER stress has an important role in cardiac hypertrophy mainly in the transition to heart failure (HF). ER transmembrane sensors detect the accumulation of unfolded proteins and activate transcriptional and translational pathways that deal with unfolded and misfolded proteins, known as the unfolded protein response (UPR). Once the UPR fails to control the level of unfolded and misfolded proteins in the ER, ER-initiated apoptotic signaling is induced. Furthermore, there is considerable evidence that implicates the presence of oxidative stress and subsequent related cellular damage as an initial cause of injury to the myocardium after ischemia/reperfusion (I/R) and in cardiac hypertrophy secondary to pressure overload. Oxidative stress is counterbalanced by complex antioxidant defense systems regulated by a series of multiple pathways, including the UPR, to ensure that the response to oxidants is adequate. Nuclear factor-E2-related factor (Nrf2) is an emerging regulator of cellular resistance to oxidants; Nrf2 is strictly interrelated with the UPR sensor called pancreatic endoplasmic reticulum kinase. A series of studies has shown that interventions against ER stress and Nrf2 activation reduce myocardial infarct size and cardiac hypertrophy in the transition to HF in animals exposed to I/R injury and pressure overload, respectively. Finally, recent data showed that Nrf2/antioxidant-response element pathway activation may be of importance also in ischemic preconditioning, a phenomenon in which the heart is subjected to one or more episodes of nonlethal myocardial I/R before the sustained coronary artery occlusion.

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Introduction

Although from 2000 to 2010 death rates attributable to all cardiovascular diseases (CVD) significantly declined, in 2010 CVD still accounted for 31.9% of all deaths (more than 2 million people) in the United States [1]. In particular coronary artery disease (CAD) still determined about one of every six deaths, with almost 400,000 Americans dead owing to this disease [1].

Atherosclerosis is the leading cause of cerebrovascular and CV diseases, which together account for a third of all deaths in Western countries [2,3]. Multiple risk factors, including hypertension, smoking, diabetes mellitus, dyslipidemia, obesity, and a sedentary lifestyle contribute to the initiation and progression of atherosclerosis [4]. Compromised blood flow and cellular perfusion lead to ischemia that causes deleterious effects on the organs in question: examples of acute ischemia include stroke, myocardial infarction, and organ transplantation. The primary goal in ischemia is therefore to restore blood flow as soon as possible, keeping in mind, however, that a rapid reestablishment of blood flow can cause oxidative stress-related organ damage, i.e., the so-called ischemia/reperfusion (I/R) injury [5]. Animal and human studies [6–9] have revealed that oxygen deprivation in the heart causes endoplasmic reticulum (ER) stress of myocytes, i.e., ER accumulation of unfolded and misfolded proteins, a situation that can lead to cell apoptosis if not counteracted by the unfolded protein response (UPR), a collection of actions designed to mitigate or eliminate ER stress and regain homeostasis. In addition, it has been reported that ischemic stress through various mechanisms can activate nuclear factor-E2-related factor (Nrf2) [10–12], which is involved in increasing the levels of endogenous antioxidants [13], attenuating apoptosis [14], and increasing mitochondrial biogenesis [15]. In addition, the UPR and Nrf2 have also been reported to be activated in cardiac hypertrophy and heart failure (HF) [16,17], a major and increasing public health problem especially in industrialized societies with aging populations.

The purpose of this article is to examine and discuss the current evidence for a cardioprotective role of UPR and Nrf2 in CAD and in left-ventricular hypertrophy (LVH) in the transition to HF.

ER stress and the UPR

It is known that the majority of protein precursors, i.e., unfolded polypeptides, fold in the ER of eukaryotic cells [reviewed in 18]. In general, cells adjust the protein-folding capacity of the ER according to their requirements, and the proteins that have been correctly folded exit the ER to their final destination. It was initially reported that manipulations that artificially increase the load of unfolded ER proteins activate the expression of genes that encode ER-resident chaperones [19]. It was then shown that various cellular perturbations implicated in the pathophysiology of human diseases, including CVD, neurodegenerative diseases, diabetes mellitus, obesity, and liver diseases [20], can alter ER function and lead to the abnormal accumulation of misfolded proteins. This situation configures the so-called ER stress, a form of intracellular stress that occurs whenever the protein-folding capacity of the ER is overwhelmed [18]. This accumulation signals incipient problems and activates transcriptional and translational pathways that deal with unfolded and misfolded proteins, known as the UPR [18]. Only recently have ER stress and UPR been considered potential contributors to CVD [21,22].

Three main ER transmembrane stress sensors initiate UPR: inositol-requiring enzyme 1 (IRE-1), pancreatic endoplasmic reticulum kinase (PERK), and activating transcription factor 6 (ATF6) [18,23,24]. All three sensors are maintained in an inactive state through the interaction of their N-terminus with the glucose-regulated protein 78 kDa (GRP78) [18].

IRE-1 is activated by the presence of misfolded proteins in the ER lumen [reviewed in 18 and 25]. The binding of misfolded proteins to IRE-1 causes a conformational change of IRE-1 that activates RNase activity. Depending on the degree of ER stress the sequelae of IRE-1 activation may be different: in the presence of low levels of ER stress it catalyzes the splicing of the mRNA encoding X-box-binding protein 1 (XBP1), removing a 26-nucleotide intron, which changes the reading frame. Active XBP1 enters the nucleus and increases the transcription of chaperones and other UPR-related proteins that enhance the ER-associated degradation (ERAD) of misfolded proteins. IRE-1 also degrades certain mRNAs through regulated IRE-1-dependent decay, so reducing the protein load to the ER. If otherwise ER stress is sustained and prolonged, IRE-1 can also be involved in activation of inflammation and apoptosis [25–30].

PERK inactivates eukaryotic translation initiation factor (eIF) 2α, which reduces protein translation, so allowing the cell to fold the proteins already present in the ER. ATF4 mRNA escapes translational suppression induced by PERK, because it possesses an internal ribosome entry site sequence in its 5′ untranslated region [31]. In the presence of low-grade ER stress, ATF4 induces the expression of C/EBP homologous protein (CHOP), which at low concentrations favors the induction of several genes central in cell survival and the UPR [32]. However, if ER stress is prolonged, PERK greatly increases CHOP, thus blocking the expression of the anti-apoptotic BCL family member protein Bcl2, favoring cell death [33]. In addition, PERK phosphorylates Kelch-like ECH-associated protein 1 (Keap1), thereby freeing Nrf2 from its inhibitor and allowing its nuclear import for the expression of antioxidant and detoxification enzymes [34].

ATF6, including two isoforms, ATF6α (90 kDa) and ATF6β (110 kDa), is another transmembrane sensor protein that becomes a powerful transcription factor upon regulated intramembrane proteolysis-specific cleavage in the Golgi complex [18]. After cleavage, the cytosolic N-domain of ATF6 translocates into the nucleus where it induces the expression of many UPR-related genes, including GRP78, CHOP, and XBP1 [18].

There is a cross talk between ER stress and autophagy, an evolutionary process by which cellular macromolecules and organellar components are sequestered within double-membrane vesicles and delivered to lysosomes for degradation and recycling of bioenergetic substrates [reviewed in 35]. ER stress can induce autophagy, through at least two UPR pathways, PERK–eIF2α and IRE-1 [35,36].

Once the UPR fails to control the level of unfolded and misfolded proteins, ER-initiated apoptotic signaling pathways are induced, and in this setting CHOP has been shown to play a key role [18]. In ER-stressed cells, CHOP has been demonstrated to induce ER oxidase 1α, which activates inositol triphosphate receptor-mediated release of calcium into the cytosol and subsequent activation of calcium/calmodulin-dependent protein kinase kinase II

[37–39], which triggers apoptosis through various mechanisms. In addition, CHOP has also been reported to upregulate the proapoptotic proteins Bim- and p53-upregulated modulator of apoptosis (PUMA), which induce mitochondrial-dependent apoptosis [40,41]. Finally, CHOP, through the induction of its transcriptional target DNA damage-inducible protein 34 (GADD34), blocks the activity of eIF2α and restores protein translation with ER perturbation and cell death [42].

The Keap1–Nrf2 pathway

Nrf2 belongs to a small family of transcription factors containing a unique basic-leucine-zipper (bZIP) motif, the cap-n-collar (CNC) family [43,44]. Although three other CNC–bZIP transcription factors have been reported in mammals, which have been designated NF-E2 p45, Nrf1, and Nrf3 [reviewed in 44 and 45], Nrf2 reportedly is the main mediator of cellular adaptation to redox stress [46]. Nrf2 activates a series of enzymes with antioxidant and detoxifying activity that play a key role in the protection of the cells against various environmental stresses, such as electrophiles, reactive oxygen species, and reactive nitrogen species [47]. Additionally, Nrf2 controls the transcription of several drug-metabolizing enzymes, transporters, cellular reducing equivalents (glutathione (GSH) and NADPH), and proteasomes [48].

Domain analysis of Nrf2 has shown that Nrf2 is composed of seven conserved Nrf2-erythroid cell-derived protein with CNC homology (Neh) domains [49,50]. The Neh1 domain contains the bZIP motif, which allows Nrf2 to bind to the antioxidant-response element (ARE) sequence. In addition, this domain can interact with E2 ubiquitin-conjugating enzyme to regulate the Nrf2 protein stability [51]. The Neh2 domain, located in the most N-terminal region, acts as a negative regulatory domain by binding to the Nrf2 inhibitor Keap1 [45]. Keap1 possesses four functional domains: Broad complex, Tramtrack, and Bric-a-Brac (BTB); intervening region; double glycine repeat or Kelch repeat (DGR); and C-terminal region [52]. Under basal or unstressed conditions, Nrf2 remains in an inactive cytoplasmic form through association with the BTB domain-containing protein Keap1 [53]. Although it was initially thought that Nrf2 activation was strictly regulated through the inhibition of nuclear import, increasing evidence has shown that Nrf2 protein levels are kept at low levels through proteasome-mediated degradation [54–56]. In general, proteins are targeted to the 26 S proteasome through the covalent attachment of polyubiquitin chains. Ubiquitin conjugation is mediated by the sequential activities of an E1 enzyme, which mediates the ATP-dependent activation of ubiquitin; an E2 ubiquitin-conjugating enzyme; and an E3 ubiquitin ligase; E2 and E3 coordinate the transfer of ubiquitin to the substrate protein [57]. Keap1 functions as a substrate adaptor protein for a Cullin 3 (Cul3)/ring box 1 (Rbx1)-dependent ubiquitin–protein ligase complex that forms the E3 ligase complex [58]. Via its N-terminal BTB domain, Keap1 binds to Cul3/Rbx1 and, via its C-terminal DGR domain, binds to Nrf2, leading to the ubiquitination and subsequent degradation of Nrf2 through the 26 S proteasome [59,60]. Under basal conditions, therefore, Nrf2 is rapidly degraded by the cytosolic Keap1/Cul3/Rbx1 complex. In contrast, under stressed conditions, the ability of the Cul3–Keap1–E3 ligase to ubiquitinate Nrf2 is inhibited, allowing Nrf2 to translocate to the nucleus and initiate the antioxidant response [44,45]. The mechanisms by which Nrf2 is released from Keap1 have been actively investigated. One mechanism proposed is that the cysteine thiol groups of Keap1 function as sensors for oxidative stress, causing formation of disulfide bonds between cysteines of two Keap1 peptides and leading to conformational changes that render Keap1 unable to bind Nrf2 [61]. Alternatively, dissociation of Nrf2 from Keap1 has been reported to be caused by protein kinase C-induced phosphorylation at Nrf2 Ser-40 [62]. It has been reported that these two mechanisms can work in concert [63]. As for antioxidant defense genes, Nrf2 induces a series of enzymes and molecules involved in (1) catabolism of superoxide and peroxides, such as superoxide dismutase, catalase, peroxiredoxin, and glutathione peroxidase; (2) regeneration of oxidized cofactors and proteins, whereby oxidized GSH is reduced by glutathione reductase, oxidized thioredoxin by thioredoxin reductase, and oxidized peroxiredoxin by sulfiredoxin; and (3) synthesis of reducing factors such as GSH and NADPH [48]. Finally, Nrf2 induces the expression of several oxidant-signaling proteins that affect particular cellular functions such as autophagy [64], inflammation [65], inflammasome signaling [66], ER stress and the UPR [67], apoptosis [14], and mitochondrial biogenesis [15].

There is evidence that persistent accumulation of Nrf2 in the nucleus is harmful; to avoid accumulation of Nrf2 in the nucleus, cells contain mechanisms that autoregulate the cellular abundance of Nrf2 [45,68,69]. As recently reviewed by Niture et al. [45], the complex Keap1/Cul3/Rbx1 is present not only in the cytosol, but also in the nucleus, where it degrades Nrf2 and therefore controls the “switching off” of Nrf2-activated gene expression.

A feedback autoregulatory loop between Keap1 and Nrf2 controls the cellular abundance of Keap1 and Nrf2 [70]: Nrf2 regulates Keap1 by regulating its transcription, and Keap1 controls Nrf2 by facilitating its degradation. More recently, it was shown that another autoregulatory loop exists between Nrf2 and Cul3/Rbx1 [71]. Nrf2 controls the transcription of Cul3 and Rbx1 and the Cul3/Rbx1 complex ubiquitinates and degrades Nrf2. In other words, there exists cellular homeostasis between Nrf2 and the Keap1/Cul3/Rbx1 complex. In addition, in the nucleus there are other Nrf2 negative regulators. In particular it has been reported that all four Src subfamily members, including Fyn, Src, Yes, and Fgr, follow Nrf2 in the nucleus and phosphorylate Nrf2 Tyr-568, which leads to nuclear export and ubiquitination/degradation of Nrf2 [68]. Furthermore, there is evidence that the transcription factor Bach1 is a further negative regulator of Nrf2 because it has been reported to compete with Nrf2 for binding to the ARE [69]. So in response to stress, the kinetics of the Nrf2 cycle has two phases: in the first Nrf2 is activated in response to stress and imported in the nucleus, where it forms heterodimers with its partners and binds to the ARE; subsequently, the nuclear Keap1/Cul3/Rbx1complex, Src subfamily members, and Bach1 switch Nrf2 activation off. As recently reported it is likely that the first phase runs in parallel to a preinduction phase in which the Nrf2 negative regulators are exported out of the nucleus [45].

In addition to mediating stress-stimulated induction of antioxidant and detoxification genes, Nrf2 contributes to adaptation by modulating intermediary metabolism. In particular, Nrf2 inhibits lipogenesis, supports β-oxidation of fatty acids, facilitates flux through the pentose phosphate pathway, and increases NADPH regeneration and purine biosynthesis [72].

Cross talk between UPR and Nrf2

Among the various transcription factors that are induced or repressed by the UPR, Nrf2 has been proposed to play a major role in regulating the non-antioxidant and antioxidant response triggered by the UPR [reviewed in 73].

Although it is well established that PERK participates in the regulation of Nrf2 phosphorylation and dissociation from Keap1 [34], the induction of ASK1 is also likely to play a role in this route, through the TRAF2-mediated kinase action of IRE-1 [74]. Furthermore it has been assessed that there is an interaction between Nrf2 and the ubiquitin/proteasome system, because Nrf2 has been shown to directly activate ubiquitin/proteasome genes [73]. In

particular, recent results indicate that the Nrf2 pathway contributes to the ER stress response by enhancing proteasome-mediated ERAD [75]. Although Nrf1 is an ER-membrane-settled transcription factor that possesses many similarities with Nrf2 [73], there is now evidence suggesting that Nrf1 strongly enhances, with an affinity even greater than Nrf2, the expression of proteasomal subunits [76]. Furthermore it has been reported that Nrf1, but not Nrf2, is likely to induce de novo expression of proteasomal subunits after application of proteasomal inhibitors [77]. So, it has been hypothesized that after ERAD failure to remove unfolded protein accumulation, Nrf1 may stimulate an alternative ERAD pathway [78].

Finally it has been recently reported that Hrd1, a downstream effector of the IRE-1 branch of the UPR, mediates the suppression of the Nrf2 pathway in liver cirrhosis [79]. In response to ER stress XBP1 mRNA is spliced by IRE-1 to produce active XBP1, which in turn induces transcriptional upregulation of Hrd1 [79]. In Wu et al.'s paper [79], it is shown that activation of the XBP1–Hrd1 arm of ER stress causes an enhanced Nrf2 ubiquitylation and degradation of the Nrf2 signaling pathway.

Role of ER stress and Nrf2 in evolving vulnerable atherosclerotic plaques

Previous studies on sudden coronary death found that plaque rupture with subsequent thrombotic occlusion is a central mechanism of acute myocardial infarction and sudden coronary death [80,81]. Rupture-prone or vulnerable plaques are generally characterized by the presence of a high content of inflammatory cells and a large necrotic core covered by a thin fibrous cap, with decreased smooth muscle cell (SMC) and extracellular matrix content [82], the so-called thin-cap fibroatheromata (TCFA). Other common features of the TCFA include expansive remodeling, large plaque size, plaque hemorrhage, neovascularization, adventitial inflammation, and “spotty” calcifications [83].

Many studies have shown that apoptotic macrophages accumulate in advanced atherosclerotic plaques [84]. When phagocytes cannot adequately remove the apoptotic macrophages (defective efferocytosis), these apoptotic cells become secondarily necrotic and coalesce over time into a key feature of vulnerable plaque, the necrotic core [85,86]. The consequence of accelerated macrophage apoptosis coupled with defective efferocytosis is the expansion of the necrotic core [85,86], a major feature responsible for plaque disruption [87].

It has been shown that ER stress may play a crucial role in the evolvement of atherosclerotic plaque. Initial works by Feng et al. [88] and then by Zhou and collaborators [89] have shown that plaque macrophages of chow-fed or Western diet-fed $ApoE^{-/-}$ mice were characterized by UPR and high expression of CHOP. Furthermore, Myoishi et al. [90] found a higher expression of GRP78, GRP94, and CHOP in the macrophages and SMCs of TCFA and ruptured plaques than in thick-cap human atheroma. In agreement with these results, we have recently reported [91] that in the tissue around the necrotic core of carotid plaques there was an abnormal amount of macrophage-derived apoptotic cells. This phenomenon was associated with a sustained ER stress with abundance of CHOP and apoptosis-related gene expression, suggesting that ER stress may promote macrophage apoptosis and favor the expansion of the necrotic core. In contrast to the increased CHOP and apoptosis-related gene expression, Nrf2 was very poorly expressed in the macrophages of the tissue around the necrotic core, whereas it was strongly represented in the cells at the periphery of the plaque [91].

Evidence also indicates that Nrf2 may play a central role in stimulating the non-antioxidant response through interrelation with UPR-gene regulation [73]. In this context, Nrf2 signaling has been shown to upregulate the expression of the proteasome catalytic subunits in several cell types and to contribute to the ER stress response by enhancing proteasome-mediated ERAD [75,92]. So far, however, there are no data showing that this mechanism operates also in macrophages of vulnerable plaques.

Cardioprotective role of the UPR

Evidence indicates that the ER plays a key role in cardiomyocytes for multifunctional organelle support: in fact proper synthesis and correct folding of proteins in the ER are extremely important for the normal function of the heart [93]. Recent studies indicate that various cellular stresses including hypoxia, I/R, pressure overload, hypertrophy, and drug-induced insults may activate ER stress [94]. However, although ER stress and UPR have been extensively studied in nonmuscle ER, relatively few studies of ER stress and UPR have been performed in the cardiovascular context [21].

Ischemic heart disease

There is now a great interest in understanding the mechanisms that link ER stress signaling to human pathology and in particular to CAD. Reduction in blood flow as a result of arterial atherosclerotic occlusion causes tissue hypoxia and hypoglycemia, two conditions that have been proposed to induce protein misfolding and ER stress [93]. In support of this finding, there are previous studies showing that cerebral ischemia activates several pathways of the UPR [95,96]. On the basis of these results in the brain, several following studies have addressed the role of ER stress in the myocardium exposed to ischemia or I/R. In this context it has been reported that many ER stress genes were expressed in mouse hearts with ischemia-induced myocardial infarction [97]. Thuerauf et al. [6] reported that IRE-1, by encoding XBP1, increased the transcription of chaperones and other UPR-related proteins and enhanced ERAD in infarcted mouse heart and hypoxic-cultured cardiac myocytes. Furthermore IRE-1 has been implicated in the activation of autophagy, a prosurvival important defense mechanism in cardiac injuries such as hypertrophy and I/R [98]. Vitadello et al. [8] showed that the XBP1- and ATF6-regulated overexpression of GRP94 reduced the necrosis due to calcium overload or simulated ischemia in cardiac myocytes. Similarly, Martindale et al. [99] and Doroudgar et al. [100] demonstrated that ATF6/XBP1 and therefore GRP94 overexpression protected mouse hearts and cultured cardiac myocytes from ischemia. Belmont et al. [101] demonstrated that the ATF6-induced overexpression of Derlin-3, which encodes an important component of the ERAD machinery, attenuated long-term ER stress in myocytes and in an in vivo mouse model of myocardial infarction, suggesting that enhancing elements of the ERAD can protect heart from ischemic injury. Severino et al. [9] focused on the molecular mechanisms activated during postinfarction remodeling in human hearts and identified protein disulfide isomerase (PDI), a member of the UPR, as a key factor of the survival pathway. In fact, they found that the PDI gene was upregulated in the viable peri-infarct myocardial region within the first days after infarction, and in a postmortem model, the expression of PDI was inversely correlated with apoptotic rate and presence of HF. Taken together these results suggest that the protective role of PDI in human infarcted hearts is mediated by a reduction in apoptotic rate and by prevention of cardiac remodeling [9]. These findings support the involvement of the UPR in ischemia and its protective role in preventing ischemia injury in CAD. On the other hand, Terai et al. [102] demonstrated that simulated hypoxia in neonatal rat

cardiomyocytes also induced CHOP expression and cleavage of caspase 12, indicating that ER-initiated apoptotic signaling is involved in cell death after a hypoxic insult. Furthermore Nickson et al. [103] reported that in primary cultures of rat and mouse neonatal cardiomyocytes, ER stress increased the expression of PUMA, a proapoptotic member of the Bcl2 family, and that PUMA expression inhibition protected ischemic cardiomyocytes from apoptosis. The results of all these studies are schematically shown in Fig. 1.

Very recently [104] we set out to determine the expression of the UPR in peripheral mononuclear cells derived from patients with stable angina and found that GRP78 and CHOP expression was significantly higher in stable angina patients than in healthy controls. This augmented expression was correlated with the abnormal cellular content of superoxide and hydrogen peroxide generated by NADPH oxidase (Nox). Although it has been shown that Nox4-mediated hydrogen peroxide production may be cardioprotective through the induction of autophagy [105], it is likely that it is an excess of oxidative stress that switches from protection to ER-initiated apoptotic signaling [104].

The results of all these studies show that ischemia-associated ER stress can cause both cell survival and apoptosis and suggest that the degree and the length of ischemia may be the discriminating factors between cell survival or death.

LVH and HF

LVH is generally believed to be a compensatory mechanism in response to stress from neurohumoral activation, hypertension, or other myocardial injury. The heart initially compensates with an adaptive enlargement of the myocardium that is characterized by an increase in the size of cardiac myocytes and whole-organ mass. However, prolonged LVH is harmful and leads to HF [106,107].

In the past decade, several studies have demonstrated the involvement of ER stress in the transition from compensated LVH to HF. In cardiac tissue from patients with dilated cardiomyopathy Okada et al. first demonstrated an increased expression of GRP78, indicating that UPR activation is associated with HF in humans [108]. In the same paper, the authors also found that mice subjected to transverse aortic constriction (TAC) developed LVH after 1 week and HF after 4 weeks. They also focused on the changes in UPR activation and ER-initiated apoptosis signaling after TAC: intriguingly, although UPR activation was present both in LVH and in HF, activation of CHOP occurred only in HF [108]. Then to clarify the mechanism of ER induction by TAC, cardiomyocytes were treated with angiotensin II (Ang II): the results indicated that Ang II acts as an ER stress inducer in rat cardiomyocytes; however, long-term induction of ER stress by Ang II was shown to induce apoptosis, through CHOP/GADD153 expression [108]. As confirmation of the role of ER stress-mediated apoptosis in HF, an increased expression of CHOP in myocardial samples from patients with HF has been demonstrated [109]. The authors also examined the effect of CHOP gene deletion on ER-mediated apoptosis and HF dysfunction induced by pressure overload: CHOP-deficient mice showed less LVH, fibrosis, and cardiac dysfunction compared with wild-type mice at 4 weeks after TAC, suggesting that CHOP may also contribute to the development of LVH in the transition to HF [109]. In this context, it has been reported that CHOP regulates the expression of GADD34, which inhibits the phosphorylation of eIF2α [110]; therefore, under CHOP-deficient conditions decreased expression of GADD34 in pressure-overloaded hearts may lead to enhanced phosphorylation of eIF2α and decreased protein synthesis, thus contributing to the prevention of LVH.

PERK has recently been demonstrated to protect the heart from pressure-overload-induced congestive HF: in response to chronic TAC, PERK-knockout mice exhibited decreased ejection fraction, increased left-ventricular fibrosis, and enhanced cardiomyocyte apoptosis [111]. The increased CHOP expression also found in PERK-knockout hearts after TAC confirms that CHOP may contribute to the left-ventricular dysfunction by increasing apoptosis. In the same paper [111] the authors also observed a significant decline in sarcoplasmic reticulum Ca^{2+} ATPase (SERCA2a) expression. SERCA2a is a well-defined regulator of calcium homeostasis in cardiomyocytes [112], and ER calcium depletion resulting from reduced SERCA2a activity has been shown to induce ER stress [113]. Because SERCA2a activity is critical for preserving cardiac function, the dramatic SERCA2a fall in PERK-knockout mice after TAC is probably an important mechanism for pressure-overload-induced congestive HF.

Prostatic androgen-repressed message 1 (PARM-1) protein has been recently related to LVH and HF in Dahl salt-sensitive rats [114]. Furthermore, downregulation of PARM-1 expression by siRNA in cultured cardiomyocytes markedly attenuated the expression of PERK and ATF6 under ER stress; in contrast, CHOP induction was markedly augmented by PARM-1 silencing. These findings indicate that PARM-1 plays a crucial role in maintaining PERK and ATF6 expression in the setting of ER stress conditions

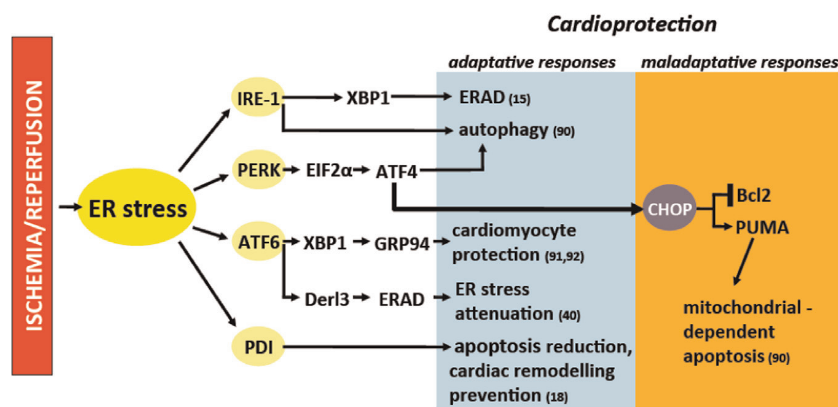

Fig. 1. Endoplasmic reticulum stress-dependent responses to ischemia/reperfusion. ER, endoplasmic reticulum; IRE-1, inositol-requiring enzyme 1; PERK, pancreatic endoplasmic reticulum kinase; ATF6, activating transcription factor 6; PDI, protein disulfide isomerase; XBP1, X-box-binding protein 1; ERAD, endoplasmic reticulum-associated degradation; EIF2α, eukaryotic translation initiation factor 2α; ATF4, activating transcription factor 4; GRP94, glucose-regulated protein 94 kDa; Derl3, Derlin-3; CHOP, C/EBP homologous protein; Bcl2, B-cell lymphoma 2; PUMA, proapoptotic proteins Bim- and p53-upregulated modulator of apoptosis. References are in parentheses.

and controls cardiomyocyte survival by suppressing CHOP-mediated apoptotic pathways [114].

In conclusion, the animal and human studies performed so far [115,116] have revealed that UPR and ER-initiated apoptosis are implicated in the pathophysiology of LVH leading to HF. The initial effects of ER stress would favor the prosurvival phase of the pathway and therefore might provide cardiovascular protection. However, during prolonged stress, cardiomyocytes may exhibit a relatively compressed time of transition from prosurvival to proapoptotic phases of ER stress, thus contributing to HF.

Cardioprotective role of Nrf2

Ischemic heart disease

There is considerable evidence that implicates the production of oxidative stress and subsequent related cellular damage as an initial cause of injury to the myocardium after ischemia and I/R injury [117]. During myocardial reperfusion, in fact, the myocardium already damaged by the ischemic insult is further subjected to several abrupt biochemical and metabolic changes, which compound the injuries generated during the period of myocardial ischemia [118]. These changes include mitochondrial reenergization, oxidative stress, intracellular Ca^{2+} overload, the restoration of physiologic pH, and inflammation; all these factors interact with one another to mediate cardiomyocyte death [96]. Oxidative stress is counterbalanced by complex antioxidant defense systems regulated by a series of multiple pathways to ensure that the response to oxidants is adequate. It has been shown that Nrf2 is an important factor in controlling both constitutive and inducible expression of a wide spectrum of antioxidants and phase 2 enzymes in cardiomyocytes and is responsible for protecting these cells against oxidative and electrophilic stress. These findings also implicate Nrf2 as an important signaling molecule for myocardial cytoprotection [119]. In this context, a variety of interventions against Nrf2 induction have been shown to offer myocardial protection in animals exposed to ischemia and I/R injury [10,120–127].

A recently classified small-molecule effector produced in the body by the enzyme cystathionine lyase, hydrogen sulfide (H_2S), has been reported to provide cardioprotection in various models of cardiac injury [128]. Calvert et al. [10] found that transgenic mice with cardiac-restricted overexpression of the H_2S-generating enzyme cystathione lyase displayed a clear protection against left-ventricular structural and functional impairment in response to ischemia-induced heart failure. The cardioprotective effect of H_2S was mostly related to the induction of endogenous antioxidants, through Nrf2 pathway activation [10].

Ashrafian et al. [12] generated fumarate hydratase cardiac-knockout mice. These fumarate-replete hearts were strongly protected from ischemia and I/R injury because fumarate potently increased the expression of ARE-regulated genes, through activation of Nrf2. In addition they found that exogenous oral fumaric acid derivatives gave the same cardioprotective effects in non-transgenic animals experiencing ischemia and I/R [12]. Similar results were reported by Deng et al. [125], who demonstrated that α-lipoic acid reduces infarct size and preserves cardiac function in rat myocardial I/R injury through activation of the Nrf2 pathway. Although in all these animal studies the activation of the Nrf2/ARE pathway has shown to reduce myocardial infarction size, so far there are no studies showing that activation of Nrf2 has a protective effect on myocardial infarction size in humans.

Nrf2/ARE pathway activation may be of importance also in ischemic preconditioning, an endogenous cardioprotective phenomenon; in this experimental setting, myocardial infarct size can be dramatically reduced by subjecting the heart to one or more episodes of nonlethal myocardial I/R before the sustained coronary artery occlusion [129]. Although proof-of-principle studies using different conditioning strategies in CAD patients undergoing elective or emergency coronary revascularization have been performed to clarify if ischemic conditioning can improve clinical outcomes, the results are still contradictory, there being both positive and negative data [130]. The reasons for the negative findings have been mainly attributed to the varying presence of CAD risk factors, comorbidities, and medications in patient cohorts [130]. Other limitations of these studies are the limited number of patients and the use of surrogate rather than clinical outcome endpoints [108]. Ongoing clinical trials (e.g., CIRCUS, ERICCA, RIPHeart) will clarify if ischemic conditioning can improve clinical outcomes [130].

Very recently Xu et al. [127] found that Nrf2-knockout mice, in addition to having an increased infarction size in response to I/R, had a reduced degree of cardiac protection by means of ischemic preconditioning. In contrast, an elevated Nrf2 protein expression was observed in heart wild-type mice exposed to ischemic preconditioning [127]. Of course the activation of the Nrf2 pathway may be only one of the elements that contributes to the intricate mechanisms involved in cardioprotective preconditioning [reviewed in 130] and further studies are needed to clarify if Nrf2 is definitely central in explaining the favorable outcomes of preconditioning. Fig. 2 schematically shows some interventions that through Nrf2/ARE pathway activation induce cardioprotection in ischemia and I/R.

LVH and HF

It is firmly established that oxidative stress plays a causative role in the pathogenesis of CVD, including pathological LVH and HF [131–135]. Oxidative stress derives from the activation of some intracellular enzymes such as NADPH oxidase or xanthine oxidase, uncoupling of nitric oxide synthase, and electron transport and "leakage" during oxidative phosphorylation in the mitochondria [136–138].

Oxidative stress has been identified as a key player in the development of cardiac hypertrophy [139], through the activation of specific pathways leading to adaptive or maladaptive cardiac remodeling processes [140]. In this context, on the other hand, recent studies indicate that the H_2O_2-generating enzyme Nox4 exerts beneficial cardiac and vascular effects during chronic hemodynamic stress [141,142] through Nrf2 activation [143].

Initially, in in vitro experiments Nrf2/ARE signaling was shown to play an important role in preventing oxidative cardiac cell injury [144,145]. Then, it was demonstrated that heme oxygenase (HO)-1 attenuated Ang II-induced cardiac hypertrophy both in vitro and in vivo [146]. Furthermore, Ndisang and Jadhav [147] reported that upregulation of HO-1 reduced LVH and extracellular matrix remodeling in spontaneously hypertensive rats. Similarly, upregulation of cytoprotective HO-1 through Bach1 deletion inhibited TAC-induced LVH [148]. In contrast, Chen et al. [149] demonstrated that in transgenic mice overexpressing HO-1, TAC increased the levels of calcineurin compared to wild-type mice, so worsening the pressure-overload-induced LVH. These results indicate that the role of HO-1 in pressure-overload-induced cardiac remodeling is still controversial and needs further investigation. At variance with this negative finding in transgenic mice overexpressing HO-1, it was shown that after TAC Nrf2-knockout mice developed pathological LVH, significant myocardial fibrosis and apoptosis, and overt HF. Overexpression of Nrf2, instead, drastically inhibited LVH and cardiac fibrosis, suggesting that Nrf2 is a critical regulator for maintaining the structural and functional integrity of the heart that is abnormally stressed [150].

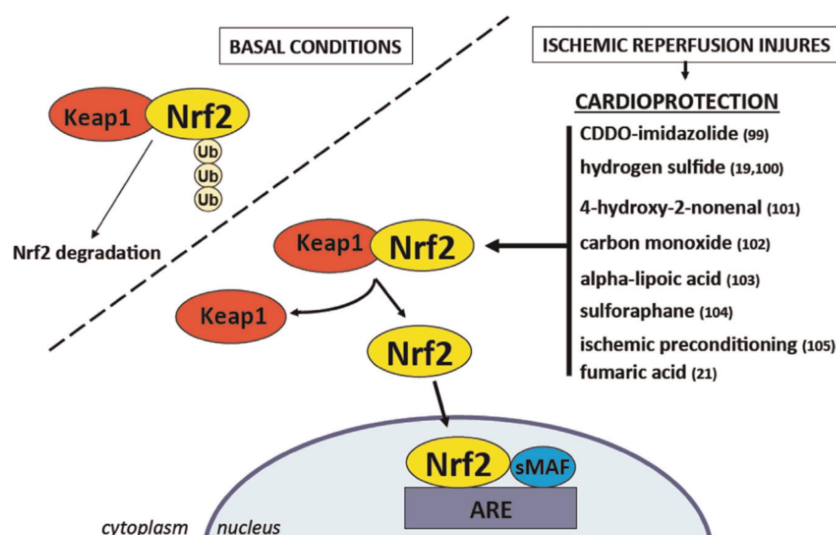

Fig. 2. Cardioprotection induced by Nrf2/ARE pathway activation in ischemia/reperfusion. Keap1, Kelch-like ECH-associated protein 1; Nrf2, nuclear factor-E2-related factor 2; Ub, ubiquitin; ARE, antioxidant-response element; CDDO-imidazolide, 1-[2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oyl] imidazole; sMAF, small musculoaponeurotic fibrosarcoma proteins. References are in parentheses.

Taken together the results of these studies suggest that activation of Nrf2 and its correlated ARE genes, by inhibiting oxidative stress, protects the heart from pressure-overload-induced pathological cardiac hypertrophy, fibrosis, apoptosis, and HF.

The understanding of the mechanisms that upregulate Nrf2 expression has allowed the development of compounds affecting Nrf2 activation and with the potential to treat CVD. Many Nrf2 activators are natural products and plant-derived phytochemicals such as sulforaphane, curcumin, resveratrol, allicin, and garlic organosulfur compounds [151]. Curcumin, for instance, has been shown to reduce acute doxorubicin-induced cardiomyopathy in rats [152]. In an Ang II-induced LVH and HF rat model allicin prevented cardiac remodeling and its progression to HF [153]. In addition, dietary phytochemical intake was shown to reduce oxidative damage and HF in hypertensive rats [154].

Various synthetic Nrf2 activators have also been synthesized, such as carbobenzoxy-Leu-Leu-leucinal (MG132), 4-hydroxynonenal, α-lipoic acid, hydrogen sulfate, and 17β-estradiol [122,123,125]. Both sulforaphane and MG132 have been shown to prevent cardiac LVH, fibrosis, and HF in high blood pressure diabetic mice [155,156]. In addition, in a pressure-overload-induced rodent HF model, treatment with MG132 was reported to attenuate LVH and cardiac fibrosis as well as improving cardiac function [157,158].

Conclusions

In the past decade several studies have addressed the mechanisms involved in heart injury ischemia, I/R, and LVH in the transition to HF. Although some studies suggest that UPR activation during heart ischemia, I/R, and LVH in the transition to HF may contribute to protecting myocytes, there are some others that support the possibility that UPR may even worsen the myocyte damage. The reasons underlying these conflicting results are poorly understood; it is possible that similar to other signaling pathways, the UPR can mediate both protective and damaging effects in the heart, depending upon the context. With the understanding that some functions of ER stress are adaptive, whereas others are maladaptive, it is likely that it is the degree and length of the insult that may discriminate between cell survival or death. In accord with Sozen et al. [159], there seems still to be a gap between the role of the UPR in CVD in humans and the therapeutic implications.

As for Nrf2 and CVD, a variety of interventions against Nrf2 induction have been shown to reduce myocardial infarct size in animals exposed to heart ischemia or I/R injury. Interestingly, H_2S and fumarate have been reported to be cardioprotective also through the activation of the Nrf2/ARE pathway. Nrf2 may also be implicated in the cardioprotection offered by ischemic conditioning.

Finally, the finding that overexpression of Nrf2 significantly inhibits LVH and cardiac fibrosis is very stimulating, suggesting a critical role for Nrf2 in maintaining the structural and functional integrity of the abnormally stressed heart.

Taken together the results of these studies show that Nrf2 activation may play a key role in cardioprotection; in this context the so-called thiol-reactive “indirect antioxidants” [160], a series of compounds shown to increase Nrf2 activity, have the potential to open a new scenario in combatting heart ischemia, I/R injuries, and LVH in the transition to HF.

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