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Forum Editorial

Redox Regulation of Growth and Death in Cardiac Myocytes

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THIS AND THE NEXT ISSUE OF THE JOURNAL FOCUS on the regulation of myocardial growth and death by reactive oxygen species (ROS) and reactive nitrogen species (RNS), with a special emphasis on cell-signaling mechanisms. During the past decade, a growing number of studies published in peer-reviewed journals have examined the role of oxidative stress in mediating cell growth and death of cardiac myocytes, and the relevance of such mechanisms in cardiac diseases, such as ischemic heart diseases and congestive heart failure. Although oxidative stress has detrimental effects on the heart, such as apoptosis/necrosis, fibrosis, cardiac arrhythmia, and mitochondrial dysfunction, it also mediates physiologic and/or protective responses, such as (compensatory) hypertrophy, angiogenesis, and preconditioning effects. The better to illustrate the cardiac role of ROS/RNS, this editorial reviews these contrasting cellular functions of ROS/RNS and their effect on cell-signaling mechanisms.

Cell signaling is generally mediated by specific and reversible modifications of signaling molecules. Such modifications must be under very precise control, spatially and temporally, which is attained, for example, by the balancing of protein kinases and phosphatases, and also by protein–protein interactions mediated through scaffold proteins in the signaling complex. Important questions regarding the cell-signaling mechanisms mediated by ROS/RNS include how ROS/RNS achieve high levels of specificity and reversibility, compared with other signaling molecules/second messengers, and whether such signaling mechanisms are really physiologic [See discussion in (17)]. Growing lines of evidence suggest that ROS/RNS manage to attain the specificity and reversibility and work as signaling molecules/second messengers. This forum issue reviews many of the key signaling mechanisms, in the heart, that are mediated through modifications of cysteine (Cys) residues by redox-sensitive mechanisms. Sugden and colleagues (17) summarize the reversible oxidation of Cys-dependent phosphatases, such as PTEN and SHP-2, a mechanism commonly used for activation of phosphatidyl inositol 3 kinase (PI3K) and mitogen-activated protein kinases (MAPKs), respectively, in response to growth-factor stimulation. Colucci and colleagues (8) describe modulation of Ras by *S*-thiolation, such as glutathiolation. Murphy and colleagues (18) describe modulation of Akt and other signaling molecules, by nitrosylation and the role of *S*-nitrosothiols (SNOs), as physiologic second messengers. Haendeler (5) describes regulation of thioredoxin (Trx) function, by ROS/RNS and other antioxidants, through its Cys modifications at noncatalytic sites. These protein modifications affect the function of the target protein through either conformation changes in the catalytic domains or regulation of protein–protein interaction. In addition, nitrosylation could prevent further oxidation and irreversible changes in the target protein (18), whereas glutathiolation could be a modality to preserve the cellular content of glutathione under prooxidant conditions, because the oxidized glutathione (GSSG) is rapidly extruded from cells (8). Korichneva (7) summarizes the involvement of zinc in the redox regulation of signaling molecules. Zinc can bind to Cys residues in proteins, such as protein kinase C (PKC), making Cys thiol sensitive to oxidation. Zinc is released on protein oxidation, which induces conformational changes in the protein, from which zinc is released. Taken together, ROS/RNS modify specific Cys residues on redox-sensitive molecules, which are localized near the site where ROS/RNS are produced. Localized production of ROS/RNS by mitochondria, NAD(P)H oxidase (Nox), xanthine oxidase, or nitric oxide synthase (NOS) would modulate downstream targets in specific subcellular components. Perhaps glutathione (in the presence of glutathione reductase) and Trx (in the presence of Trx reductase) would confer reversibility to such signaling mechanisms.

Although these examples clearly indicate the involvement of ROS/RNS in cardiac signaling, many important questions still remain. For example, do such redox-dependent mechanisms represent just one of many parallel pathways or the essential signaling mechanism mediating growth-factor and G protein–coupled receptor (GPCR) signaling? Alternatively, ROS/RNS-dependent signaling mechanisms may exist to modify the conventional signaling mechanisms.

Some ROS/RNS have dose-dependent effects; protective/anabolic/preconditioning effects are observed at low doses, and detrimental/apoptotic effects are seen at high doses. This

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seems to be a unique characteristic of ROS/RNS as second messengers. Obviously, the cell/organism has to take a risk to use such a mechanism when conducting routine work, because accidental overproduction could have life-threatening effects. Sugden and colleagues (17) propose that whereas local, cellular production of ROS, such as the production of H_2O_2 by membrane-associated Nox, may be physiologic, global production could be detrimental. Colucci and colleagues (8) propose that as the levels of oxidative stress increase, so will the level of thiol modification; irreversible modifications, such as sulfonic acid formation, will occur at the highest level of oxidative stress. Irreversible modifications would result in the loss of protein function and in the accumulation of defective proteins.

The localization of ROS/RNS and antioxidants also seems important. We have previously shown that overexpression of the cytosolic form of dominant negative Trx1 predominantly causes cardiac hypertrophy (23), whereas others have shown that the loss of the mitochondrial form of Trx2 causes cell death (14). Thus, oxidative stress in mitochondria could be more detrimental than that in cytoplasm. Webster and colleagues (22) suggest that increases in mitochondrial oxidative stress, opening of the mitochondrial permeability transition pore (MPTP), and a tight association with mitochondrial membrane under acidosis are required for the cell death–promoting effects of Bnip3, a BH3-only protein (3, 22). Precise determination of the subcellular locality and identity of ROS/RNS would allow us to elucidate their role in cell signaling, and to determine which functions are physiologic and which are unphysiologic.

ROS are produced primarily in the mitochondria during ischemia and heart failure (20). Gottlieb and colleagues (2) discuss mechanisms by which mitochondria amplify the production of ROS, termed ROS-induced ROS release (RIRR). RIRR allows a local perturbation to mitochondria to spread, becoming cell-wide disturbances, and plays an important role in mediating cardiac injury by ischemia–reperfusion, including apoptosis. Tsutsui and colleagues (20) describe the mechanism by which ROS and mitochondrial dysfunction stimulate one another through increases in mitochondrial DNA (mtDNA) damage. Thus, it appears that mitochondria have multiple positive-feedback mechanisms that enhance both ROS and mitochondrial dysfunction under pathologic conditions.

Modulation of the components of the vicious cycles, such as by inhibition of the MPTP or the inner mitochondrial membrane anion channel (IMAC), both key players of RIRR (2), or by stimulation of TFAM, a key regulator of mtDNA transcription/replication and mtDNA copy number (20), could be a novel mechanism of cardioprotection by disrupting the operation of the amplification loops. It is expected that cardiac myocytes may possess endogenous fail-safe mechanisms to prevent false activation of the vicious cycles, as well as unique postmitochondrial antiapoptotic mechanisms (15). Thus, exploring such endogenous mechanisms may provide clues for the development of a novel modality to protect the heart from myocardial injury.

The 26S proteasome is responsible for degradation of majority of proteins in eukaryotic cells, thereby regulating a wide variety of cellular functions, including cell growth, adaptation to stress, and cell death. Dysfunction of the proteasome system is observed in many pathologic conditions in the heart, such as ischemia–reperfusion injury, cardiomyopathy, and heart failure. Ping and colleagues (4) discuss the interaction between ROS and the 26S proteasome system. The 26S proteasome appears to be susceptible for posttranslational modulation during oxidative stress, including oxidative modification by the lipid peroxidation product 4-hydroxy-2-nonenal (HNE). Because the proteasome system is involved in degradation of oxidatively damaged proteins, oxidative stress may trigger another vicious cycle involving the proteasome dysfunction and accumulation of damaged proteins.

Recently, Trxs have attracted the attention of many investigators, perhaps because its diverse functions (*e.g.,* antioxidants, secreted growth factors, and intracellular second messengers). Trx1 is a 12-kDa protein with multiple functions in the heart, including regulation of tissue levels of oxidative stress, regulation of cell growth, and inhibition of apoptosis. Trx1 exists primarily in the cytoplasm but is translocated into the nucleus with stresses such as myocardial ischemia (10). Mice with cardiac-specific overexpression of Trx1 exhibit either protection against stresses or inhibition of cardiac hypertrophy (21, 23). Trx1 also works, extracellularly, as a secreted factor and promotes cell growth, especially in cancer cells [reviewed in (24)], and protects the heart from ischemic injury (19). Trx1 directly associates with intracellular signaling molecules and transcription factors, and modulates their signaling functions (24). In addition, expression and function of Trx1 is regulated by transcriptional mechanisms, posttranslational modification, and changes in subcellular localization (5, 24). Such complex regulatory mechanisms allow Trx1 to mediate diverse functions in various cell types.

In this issue, Das and colleagues (10) show that shRNA inhibition of Trx1 eliminates its cardioprotective effects during preconditioning in the mouse heart, suggesting that Trx1 is an important mediator of preconditioning. During myocardial ischemia, Trx1 is upregulated and translocated into the nucleus, stimulating NF-κB–mediated gene expression. Although many signaling mechanisms have been shown to mediate preconditioning, this is the first study to show that Trx1 works as an intracellular second messenger that mediates the effects of preconditioning. Thus, Trx1 is able to work as both an intracellular (10) and extracellular (19) messenger. In this regard, Trx1 would be an ideal mechanism for cardioprotection, because preconditioning in even a portion of the heart would alert the entire myocardium to coming disasters, producing a diffusible factor that travels through extracellular spaces and stimulates nuclear events of cell protection in remote areas.

Using microarray analysis, Sadoshima and colleagues (1) report a new function of Trx1: the upregulation of genes encoding the TCA cycle and mitochondrial oxidative phosphorylation. Mitochondrial function is generally impaired during heart failure and myocardial ischemia [See (20)]. Augmenting mitochondrial function, by antioxidants, would be beneficial because it would effectively extinguish the vicious cycle driven by both mitochondrial dysfunction and increases in ROS (20).

Among the many antioxidants, perhaps the protein–protein interaction has been best characterized in Trx1. Although

many Trx1-interacting proteins are reduced by Trx1, some of them are not, indicating that Trx1 may possess antioxidant independent functions. Lee and colleagues (24) reviewed the function of thioredoxin-interacting protein (Txnip), which inhibits the function of Trx1 by interacting with the catalytic center of Trx1. Downregulation of Txnip, by oxidative or mechanical stress, enhances the activity of Trx1. It should be noted, however, Txnip has diverse cellular functions. For example, Txnip is transported into the nucleus and inhibits cell growth, possibly by interacting with transcriptional co-repressors (13). Thus, Txnip may also function as either a transporter protein or an adapter protein, thereby assisting nuclear actions (perhaps redox-independent functions) of Trx1. Otsu and Nishida (12) reviewed the function of apoptosis signal-regulating kinase-1 (Ask1), a serine/threonine kinase, which plays an important role in mediating both hypertrophy and apoptosis in cardiac myocytes. In this case, binding of Trx1 inhibits the activation of Ask1 through conformational changes, rather than through regulation of the redox status of Ask1. In both cases, the redox status of Trx1 affects the binding of Trx1 to partner proteins.

During the past few years, the molecular mechanisms regulating the life span of animals have been studied extensively. In many cases, the longevity mechanism is activated by a low grade of stresses, and an upregulation of stress-resistance mechanisms confers longevity to the organism (9). Interestingly, among the many antioxidants, thus far, only the systemic overexpression of Trx1 (11) and the mitochondrial overexpression of catalase (16) have been shown to induce substantial life span extension in mice. Thus, we speculate that Trxs may have unique mechanisms that make cells stress resistant. Tsutsui and colleagues (20) also presented the beneficial effects of glutathione peroxidase in preventing cardiac injury over other antioxidants (20). Kajstura and colleagues (6) proposed a novel concept that aging of the heart is controlled by damage of cardiac stem cells, forced activation of cellular senescence, and marked decrease in the number of functionally competent primitive cells. Because the increased level of oxidative stress is a prime suspect for the induction of DNA damage, telomere erosion, and senescence, it will be interesting to test whether Trx1 protects cardiac stem cells from the aging effect. As pointed out by Webster and colleagues (22), results from clinical studies testing the delivery of exogenous enzymatic and nonenzymatic antioxidants are mostly negative. Perhaps we may need antioxidants with targeted functions for cardioprotection; attacking the mitochondrial vicious cycles while preserving the housekeeping functions of ROS. Clearly, additional studies are needed to understand better the signaling mechanisms mediated by ROS, and the molecular actions of antioxidants, to translate our knowledge of ROS into bedside treatment for cardiovascular patients.

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ABBREVIATIONS

Ask1, apoptosis signal-regulating kinase-1; Cys, cysteine; GPCR, G protein–coupled receptor; GSSG, oxidized glutathione; HNE, 4-hydroxy-2-nonenal; IMAC, inner mitochondrial membrane anion channel; MAPKs, mitogen-activated protein kinases; MPTP, mitochondrial permeability transition pore; mtDNA, mitochondrial DNA; NOS, nitric oxide synthase; Nox, NAD(P)H oxidase; PI3K, phosphatidyl inositol 3 kinase; PKC, protein kinase C; RNS, reactive nitrogen species; RIRR, ROS-induced ROS release; ROS, reactive oxygen species; SNO, *S*-nitrosothiols; Trx, thioredoxin; Txnip, thioredoxin-interacting protein.

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