Journal of Molecular and Cellular Cardiology 73 (2014) 103–111

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc

Review article

Redox regulation of cardiac hypertrophy

Can M. Sag, Celio X.C. Santos, Ajay M. Shah *

King's College London British Heart Foundation Centre of Excellence, Cardiovascular Division, London, UK

ARTICLE INFO

Article history:
Received 2 January 2014
Received in revised form 31 January 2014
Accepted 3 February 2014
Available online 11 February 2014

Keywords:
Hypertrophy
Cardiac
Redox
Signaling
NADPH oxidase

ABSTRACT

It is increasingly evident that redox-dependent modifications in cellular proteins and signaling pathways (or redox signaling) play important roles in many aspects of cardiac hypertrophy. Indeed, these redox modifications may be intricately linked with the process of hypertrophy wherein there is not only a significant increase in myocardial O_2 consumption but also important alterations in metabolic processes and in the local generation of O_2-derived reactive species (ROS) that modulate and/or amplify cell signaling pathways. This article reviews our current knowledge of redox signaling pathways and their roles in cardiac hypertrophy. This article is part of a Special Issue entitled "Redox Signalling in the Cardiovascular System".

Contents

Abbreviations: AKAPs, A-kinase anchoring proteins; AKT, protein kinase b; Ang-II, angiotensin II; ASK1, apoptosis signal-regulating kinase 1; BH4, tetrahydrobiopterin; CaMKII, Ca/calmodulin-dependent kinase II; cGMP, cyclic guanosine monophosphate; CHF, chronic heart failure; CTGF, connective tissue growth factor; DCM, dilated cardiomyopathy; ECC, excitation–contraction coupling; ECM, extracellular matrix; ETC, electron transport chain; ER, endoplasmic reticulum; ERK1/2, extracellular signal-regulated kinase 1/2; Ero1, endoplasmic reticulum oxidase 1; GATA4, transcription factor GATA-4; C/EBPβ, transcription factor C/EBPβ; GPCR, G-protein coupled receptor; GSH, reduced glutathione; HADC, histone deacetylase; HIF, hypoxia-inducible factor; HNO, nitroxyl; H_2O_2, hydrogen peroxide; IGF, insulin-like growth factor; MI, myocardial infarction; MMP2, matrix metalloproteinase-2; LOX, lysyl oxidase; LV, left ventricle; MAO, monoamine oxidase; MEF2, myocyte enhancer factor-2; mTOR, mammalian target of rapamycin; NFAT, nuclear factor of activated T cells; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; NO, nitric oxide; NOS, NO synthase; NOX, NAPDH oxidase; Nrf2, nuclear factor erythroid-2 related factor 2; O_2, molecular oxygen; O_2^-, superoxide; $ONOO^-$, peroxynitrite; p38MAPK, mitogen-activated protein kinase; PDI, protein disulfide isomerase; PGC1 , peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PHD, prolyl oxidase; PKA, cAMP-dependent protein kinase A; PKG, cGMP-dependent protein kinase G; PI3Kα, phosphatidylinositol 3 kinase alpha; RAAS, renin–angiotensin–aldosterone system; RNS, reactive nitrogen species; ROS, reactive oxygen species; RyR2, Ca release channels of the SR; SERCA2a, SR Ca2 + ATPase; SR, sarcoplasmic reticulum; SRF, serum response factor; SOD, superoxide dismutase; TGF , transforming growth factor ; TRX, thioredoxin; VEGF, vascular endothelial growth factor; XO, xanthine oxidase.

* Corresponding author at: James Black Centre, King's College London, 125 Coldharbour Lane, London SE5 9NU, UK. Tel.: +44 2078485189; fax: +44 2078485193.
E-mail address: ajay.shah@kcl.ac.uk (A.M. Shah).

http://dx.doi.org/10.1016/j.yjmcc.2014.02.002

1. Introduction

Cardiac hypertrophy represents an increase in cardiac muscle mass in response to a chronic increase in cardiac workload. It may be associated, at least initially, with an enhanced contractile function of the heart but chronic increases in workload due to disease stress generally result in a progressive decline in cardiac performance and ultimately the development of chronic heart failure (CHF). Such pathological hypertrophy usually occurs in response to chronically increased afterload or "pressure overload" (e.g. due to hypertension), increased preload or "volume overload" (e.g. due to valvular regurgitation), or following myocardial infarction (MI). In addition, pathological hypertrophy may also arise in diabetes and with genetic abnormalities of myocardial structure or function [1]. In contrast, reversible physiological hypertrophy with well compensated contractile function is seen in athletes or healthy pregnancy [2].

The development of cardiac hypertrophy involves a complex remodeling of cardiomyocyte structure and function as well as remodeling of the non-myocyte compartment (i.e. the vasculature and the extracellular matrix [ECM]). For instance, the maintenance of an appropriate capillary density and blood supply to match the increase in muscle mass is crucial for an adaptive hypertrophic response, whereas a mismatch promotes decompensation [3]. Maladaptive cardiac hypertrophy is accompanied by disproportionate interstitial fibrosis, energy deficit, cardiomyocyte death, vascular dysfunction and chamber dilatation [4].

It is increasingly evident that redox-dependent modifications in cellular proteins and signaling pathways (or redox signaling) play important roles in many aspects of cardiac hypertrophy [5]. Indeed, these redox modifications may be intricately linked with the process of hypertrophy wherein there is not only a significant increase in myocardial O_2 consumption but also important alterations in metabolic processes and in the local generation of O_2-derived reactive species (ROS) that modulate and/or amplify cell signaling pathways. This article reviews our current knowledge of redox signaling pathways and their roles in cardiac hypertrophy.

2. General considerations with respect to redox signaling in cardiac hypertrophy

At the cellular level, O_2 specifically undergoes one electron reduction to O_2^- through the action of several oxidases, either as their primary function or as a byproduct of some other reaction. These oxidases include NAPDH oxidases (NOXs), xanthine oxidase (XO), monoamine oxidase (MAO), and uncoupled NO synthases (NOS) (Fig. 1 and below). O_2^- is also produced by mitochondrial complexes I and II under certain circumstances. The O_2^- can become further dismutated to H_2O_2 via superoxide dismutases (SOD). Moreover, O_2 is used by NOS to produce nitric oxide (NO), a reactive nitrogen species (RNS) that may be the precursor of other reactive species (e.g. $ONOO^-$). The complex interplay and specific effect of these reactive species is greatly influenced by the amount

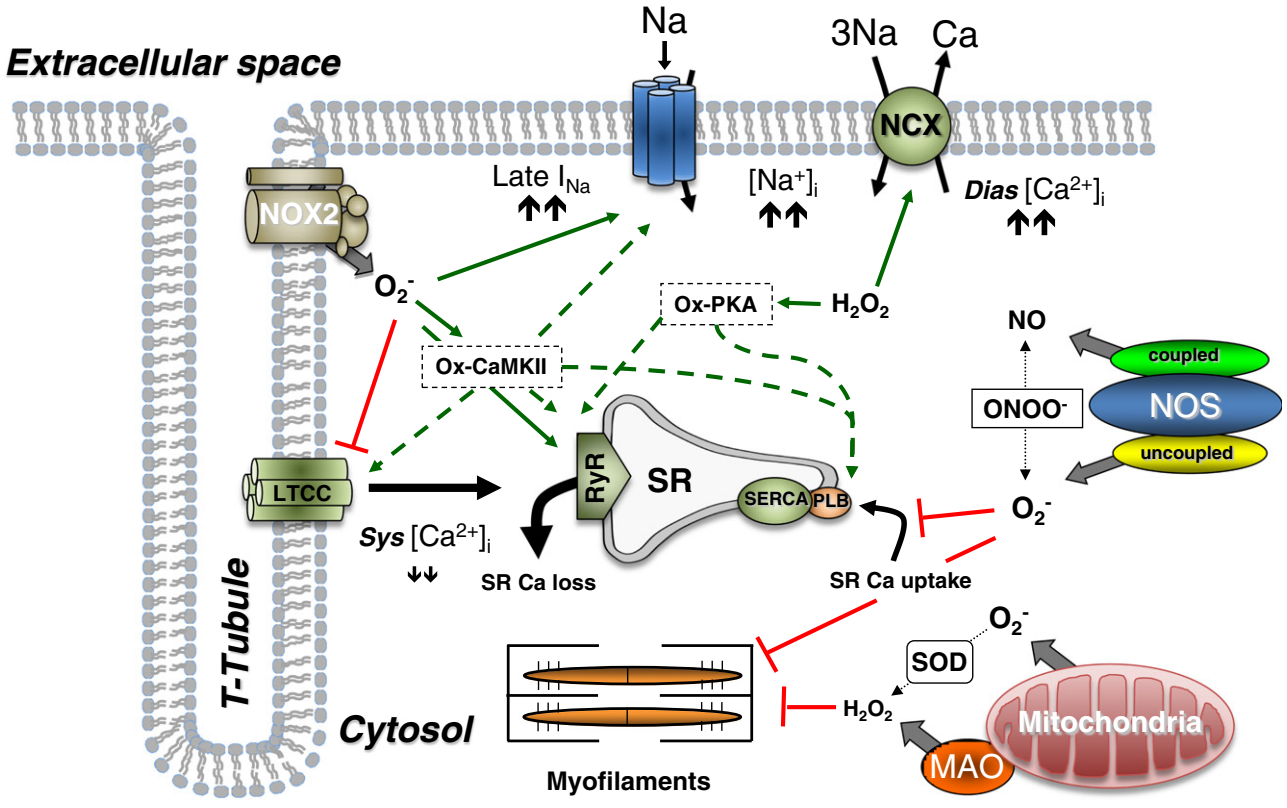

Fig. 1. Important intracellular ROS sources and selected targets involved in excitation-contraction coupling in the hypertrophied cardiac myocyte. ROS may have direct actions or indirect actions through modification of various kinases, resulting in an impairment of excitation–contraction coupling, i.e. a decrease of systolic (sys) Ca fluxes in the face of Na-dependent diastolic (dias) Ca overload. Solid green lines indicate activation, solid red lines inhibition; dashed lines indicate indirect effects via protein kinases. O_2^-, superoxide; H_2O_2, hydrogen peroxide.

generated, half-life of the chemical species, subcellular location, site-specific antioxidant pools, and the local chemical environment (e.g. pH, the buffer pair CO_2/HCO_3^-). For instance, NO reacts extremely rapidly with O_2^- to generate $ONOO^-$ (or $ONOOCO_2^-$ in the presence of CO_2/HCO_3) in a reaction that is favored over the dismutation of O_2^- by SOD [6].

ROS may directly modulate the redox status of macromolecules and signaling pathways or may exert indirect effects, e.g. by reacting with NO and decreasing local NO bioavailability [7]. Among the most susceptible molecular targets of "signaling" ROS in cardiac cells are protein cysteine thiols and methionines. Cysteine oxidation leads to intra- or intermolecular disulfide formation or other thiol modifications such as nitrosylation (NO bound to protein thiol) and glutathiolation (a disulfide bond of a protein thiol with glutathione). Other common post-translational redox modifications include proline and arginine hydroxylation, and the nitration of aromatic amino acids (e.g. tryptophan or tyrosine). Such modifications can affect the conformation, stability and function of proteins. Examples of signaling molecules that are redox modified in cardiac hypertrophy are shown in Figs. 1 and 2, and include protein kinases (e.g. calcium-calmodulin kinase II [CaMKII], protein kinase A [PKA], and protein kinase G [PKG]), the ryanodine receptor Ca release channel (RyR2), sarcoplasmic reticulum Ca ATPase (SERCA2a), GTPases (e.g. RAS), antioxidant proteins such as thioredoxin (TRX), and histone deacetylases (HDAC). It is important to note that local antioxidant pools and specific antioxidant enzymes, notably peroxiredoxin and TRX, may also be critically important in redox signaling. For example, transient spatially confined inactivation of peroxiredoxin is thought to be a crucial event in reversible growth factor-induced redox signaling [8]. Reduced glutathione (GSH) is the major cellular redox buffer and the mechanisms involved in its regeneration (e.g. NADPH-dependent activity of glutathione reductase) therefore also significantly influence redox signaling. An appropriate balance between ROS generation and antioxidant pools is important for normal cellular function and either an over-oxidized or an over-reduced environment ("oxidative stress" and "reductive stress", respectively) may be detrimental [9].

2.1. Cellular sources of ROS

Most of the ROS sources depicted in Fig. 1 have been implicated in some aspect of pressure-overload induced maladaptive cardiac hypertrophy [10–13].

2.1.1. Mitochondria

ROS generation in mitochondria is related to a partial reduction of O_2 to O_2^- by complexes I and III of the electron transport chain (ETC.), through electron "leakage" during mitochondrial respiration [14]. Other mitochondrial enzymes may also contribute to ROS generation

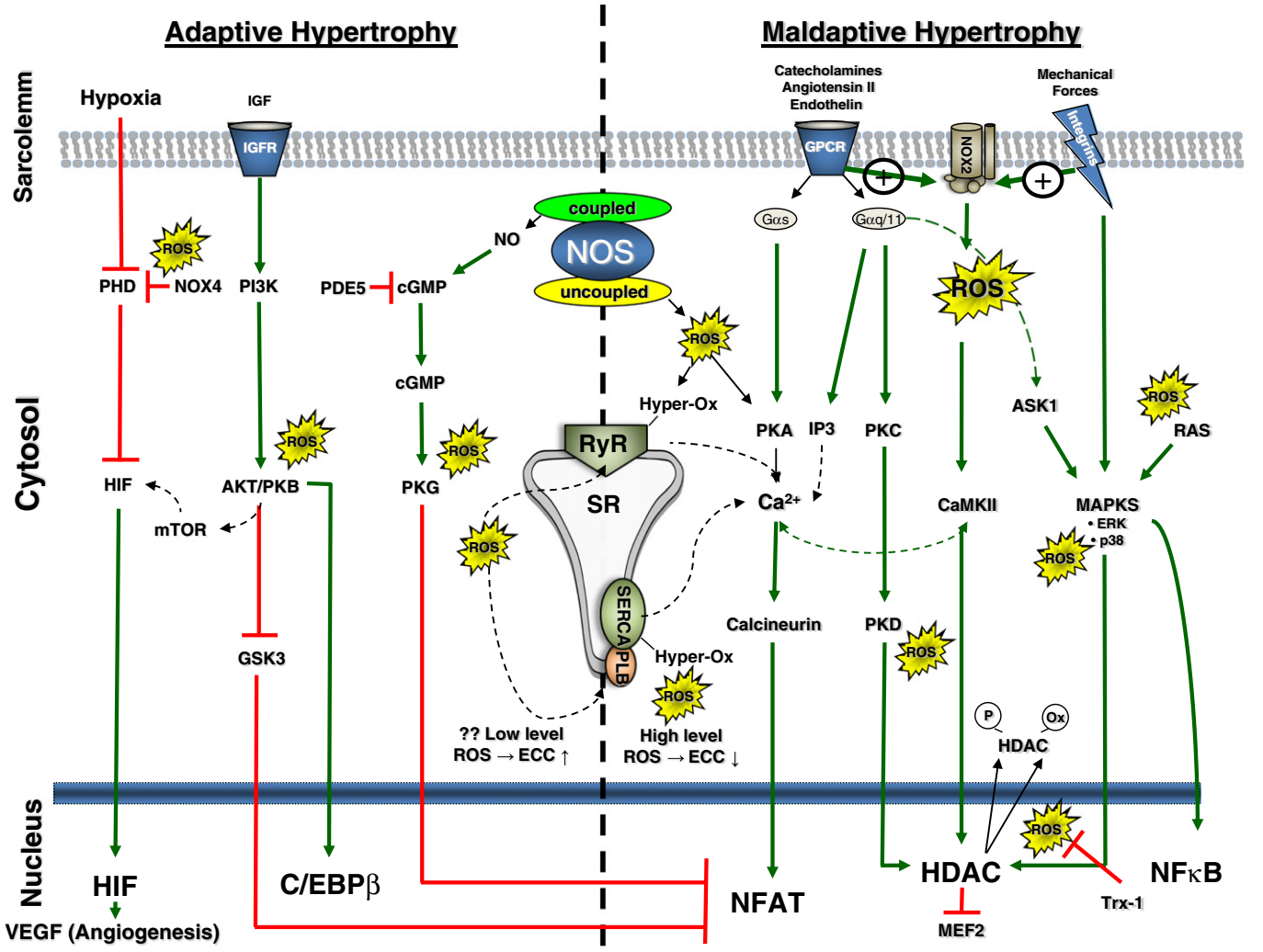

Fig. 2. Key redox-sensitive signaling pathways involved in cardiomyocyte hypertrophy. Redox-regulated signaling pathways involved in adaptive hypertrophy are shown on the left, while pathways involved in maladaptive hypertrophy are illustrated on the right. Schematically shown are sarcolemmal receptors, cytosolic signaling cascades and their main nuclear transcription factor targets. Red lines indicate inhibition whereas green lines indicate activation of downstream targets.

as described later. The maintenance of the mitochondrial antioxidant status has been recognized as an important determinant of mitochondrial ROS levels [15]. Elevation in ROS generation by mitochondria has been detected in different cardiovascular settings whereby mitochondrial ROS generation can have beneficial or detrimental effects [16]. The deletion of mitochondrial antioxidants such as TRX reductase 2 is embryonically lethal and related to impaired cardiac function [17]. Moreover, in isolated mitochondria, pharmacological inhibition of TRX reductase 2 resulted in unremitted H_2O_2-emission from these organelles, while the mitochondrial energetic status is reflected in the levels of reduced TRX [18]. In another animal model, deletion of mitochondrial manganese SOD led to severe fatal dilated cardiomyopathy. On the other hand, mice overexpressing mitochondrial-targeted catalase were protected from cardiac disease and showed a prolonged life span [19].

2.1.2. The endoplasmic reticulum (ER)

The ER lumen has a strongly oxidizing milieu where O_2 drives protein oxidation and folding. O_2 is used in the oxidation of lysine and proline by lysyl oxidase (LOX) and prolyl oxidase (PHD), respectively. Similar reactions mediated by other PHD isoforms occur in the cell cytosol and regulate the stability of the transcriptional factor hypoxia-inducible factor (HIF). Another important reaction in the ER is the introduction of disulfide bonds into nascent proteins. This enzyme ER oxidase 1 (Ero1) is first oxidized by molecular O_2 to form oxidized Ero1, generating H_2O_2 in this process. Subsequently, protein disulfide isomerase (PDI) mediates the transfer of disulfides from Ero1 to nascent proteins, as part of normal protein folding. Thus, H_2O_2 is produced during normal cell metabolism in the ER. Higher levels of H_2O_2 may be generated in the ER during cellular stresses, and other oxidases such as NOXs may contribute to this. Recently, it was shown that Ero1 regulates excitation–contraction coupling in cardiomyocytes during increased loading [20].

2.1.3. NADPH oxidases

NOX proteins were initially discovered through their critical role in phagocytic cells [21]. 7 distinct NOX isoforms (NOX1–NOX5, and DUOX1 and 2) have since been identified. NOX1, 2, 4, and 5 have been identified in the cardiovascular system where they generate low levels of ROS (within the nanomolar range) and have been implicated in the regulation of many redox-sensitive signaling pathways, playing multifarious roles in cell differentiation, proliferation and migration [21,22]. The main cardiac isoforms are NOX2 and NOX4 both of which occur as heterodimers with a 22 kD subunit termed $p22^{phox}$. NOX2 is a sarcolemmal enzyme that is activated by common hypertrophic stimuli such as G-protein coupled receptor (GPCR) agonists (e.g., angiotensin II (Ang-II) or endothelin-1), growth factors, cytokines and mechanical forces in a process that involves the binding of 4 regulatory subunits ($p67^{phox}$, $p47^{phox}$, $p40^{phox}$ and Rac1). In contrast, NOX4 is found in intracellular membranes, notably the ER [23], and is constitutively active. It has no requirement for regulatory subunits and is thought to be regulated mainly by its abundance [22]. NOX2 and NOX4 are both upregulated during the response to hypertrophic stimuli but appear to have distinct effects in the heart (see Section on redox-sensitive signaling pathways).

2.1.4. Uncoupled NO synthases (NOS)

Endothelial and neuronal NO synthases are expressed in cardiac myocytes. Under pathological conditions such as inflammation, inducible NOS is also expressed. NOS-derived NO is reported to have beneficial antihypertrophic effects through cGMP-related signaling, and the inhibition of cGMP hydrolysis with a phosphodiesterase 5 inhibitor is beneficial in an animal model of pressure overload [24] (Fig. 2). However, NOS enzymes can become uncoupled during oxidative stress situations due to the oxidation and depletion of the cofactor tetrahydrobiopterin (BH_4) [25] or due to S-glutathionylation [26]. The enzymes then switch from NO to O_2^- generation and may act to further amplify detrimental ROS-signaling induced by other ROS sources (e.g. NOXs). NOS uncoupling may therefore represent an important step in the initiation of maladaptive hypertrophy to common pro-hypertrophic triggers (Fig. 2).

2.1.5. Monoamine oxidases (MAO)

MAO are located in the mitochondria and act in the oxidative deamination of catecholamines. This reaction generates H_2O_2 and the enzymes have been reported to be important sources of ROS in mice subjected to pressure overload [27] and to contribute to cardiac dysfunction in this setting [28]. The mitochondrial location of MAO also raises the possibility of cross-talk with other mitochondrial ROS sources.

2.1.6. Cytochrome P450 oxidase

Cytochrome P450 oxidase can function as an important source of ROS under certain circumstances. Cytochrome P450 oxidase is reported to be upregulated in heart disease and interestingly cytochrome P450 knockout mice were found to ameliorate heart dysfunction when crossed with a mouse model of dilated cardiomyopathy [29].

2.1.7. Xanthine/xanthine oxidase

During hypoxia or ischemia, xanthine dehydrogenase (XD) is converted to xanthine oxidase (XO) which can convert O_2 to ROS [30]. XO appears to be an important source of ROS in ischemia but its role in the development of cardiac hypertrophy remains elusive [31]. It should be noted that in human hearts, the expression levels of XO may be very low, limiting this enzyme's relevance with respect to human pathology [32].

3. Redox regulation of cardiac hypertrophy

3.1. Redox-sensitive signaling pathways involved in cardiomyocyte hypertrophy

During cardiac hypertrophy the normal pattern of cardiac gene expression is reprogrammed, which can impact on different cellular processes (e.g., Ca homeostasis, contractile function, metabolism, oxygen sensing and cell viability). A number of transcriptional factors (e.g. NFAT, GATA4, MEF2, SRF, NF-κB and PGC1α) are involved in driving this hypertrophic program, and these factors in turn are under the control of a multitude of interacting signaling pathways (see Fig. 2) [5,33,34]. An increasing body of data indicates that these hypertrophy-related signaling pathways are subject to redox regulation as a consequence of spatially and temporally confined ROS production from distinct ROS sources as outlined above [5,35]. In addition, ROS may also directly influence transcription factor binding, e.g. through regulation of histone deacetylases (HDACs). For example, it has been demonstrated that HDAC4 (which inhibits the expression of MEF2-dependent genes) undergoes specific oxidation in response to α-adrenergic stimulation to form an intra-disulfide bond at Cys 274/276, resulting in its nuclear export and dis-inhibition of gene transcription [36]. A similar oxidation has also been recently reported in response to α-adrenergic stimulation [37].

Overlapping signaling pathways that drive maladaptive hypertrophy are also redox sensitive. For example, G-protein-coupled receptor (GPCR) agonists such as angiotensin II, endothelin-1 and α-adrenoceptor agonists, cytokines (through gp130 protein) and biomechanical stress all cause increased NOX-derived ROS production which modulates specific signaling pathways during the development of cardiac hypertrophy. NOX2 expression and activation are increased in response to chronic pressure overload [10] and are involved in angiotensin II-induced cardiac hypertrophy. Mice deficient in NOX2 [38] or Rac1 [39] have a significantly blunted hypertrophic response to angiotensin II, and similar results are reported in cellular models of hypertrophy [40,41]. NOX2-dependent enhancement of ERK signaling, ASK1/p38MAPK signaling, and NF-κB

activation have been implicated in these effects [41–44]. With respect to ERK signaling, a specific redox modification of Cys118 in RAS, upstream of Raf-MEK-ERK activation, was shown to mediate cardiac hypertrophy in response to α-adrenoceptor stimulation [45] and mechanical stress-induced hypertrophy [46]. The hypertrophic response to pressure overload is more complex because NOX2 null mice develop a similar extent of hypertrophy to wild-type controls although contractile function is better [47] suggesting that other pathways are also involved.

NOX4 levels also rise in response to chronic pressure overload and other stress stimuli but its effects appear to be distinct from those of NOX2. Notably, NOX4 is capable of driving several distinct pro-survival processes and an increase in autophagy [48]. During chronic pressure overload, NOX4 was found to exert a protective role through the enhancement of cardiomyocyte HIF-VEGF signaling, which through paracrine angiogenic effects resulted in a higher myocardial capillary density [49]. Interestingly, similar NOX4-dependent enhancement of HIF signaling has been reported in the kidney [50]. NOX4 may also enhance the expression of cytoprotective genes through the activation of Nrf2 [51,52], and promote autophagy in the setting of starvation [48], although the extent to which these pathways contribute to the response to hypertrophic stimuli remains to be fully defined. These findings exemplify the importance of ROS source-specific signaling with respect to the resulting remodeling phenotype [53]. Such signaling is likely to be highly dependent upon temporally and spatially confined ROS production and the specificity derived from such compartmentation. On the other hand, the loss of such compartmentation (e.g. due to very high level ROS production, mis-localization of ROS sources or aberrant biochemical function) may also lead to the loss of specific protective signaling and be replaced by non-specific detrimental such as mitochondrial dysfunction. Interestingly, detrimental effects of NOX4-derived ROS in a model of severe pressure overload have been reported [54] and it will be of interest to define the reasons why protective mechanisms such as those discussed above are overwhelmed in some situations.

Other signaling pathways that drive adaptive hypertrophy include the insulin-like growth factor (IGF)/phosphatidylinositol 3 kinase alpha (PI3Kα)/protein kinase B (AKT) pathway. AKT activation is an important convergence point with cross-talk to other adaptive signals such as HIF via the activation of mTOR. The redox regulation of AKT and of HIF is well established [55] although the details of ROS sources that influence these pathways remain to be fully worked out. Another example of a redox-regulated signaling pathway with anti-hypertrophic effects is the cGMP/PKG pathway [24]. Oxidative activation of PKG has been discovered to be an important regulatory mechanism [56] and elucidation of the relevance of this to cardiac hypertrophy is awaited.

3.2. Redox-regulation of excitation–contraction coupling (ECC) and Ca handling

A gradual worsening of contractile function is a cardinal feature of the disease progression of maladaptive cardiac hypertrophy to heart failure. Abnormalities of excitation–contraction coupling (ECC), including cardiac myofilament dysfunction, play a key role in this contractile dysfunction and growing evidence suggests an important role for redox modifications of key signaling components of ECC in this process (Fig. 1). Although this is not the primary focus of the current article, in view of its importance we provide a brief overview of the main redox abnormalities. The interested reader is referred to excellent recent reviews for more detail [57].

Important effects of ROS on ECC include indirect effects through the redox modification of protein kinase activity and direct effects on channels and ion transporters. PKA activation in response to β-adrenergic stimulation is of key importance in regulating the phosphorylation status of L-type Ca channels, the SR Ca release channels (ryanodine receptors, RyR2), troponin I, and myosin binding protein C. PKA can be redox activated through the formation of an inter-disulfide bond between its catalytic subunits [58], an event that also promotes the translocation and association of PKA with specific A-kinase anchoring proteins (AKAPs), thereby targeting active PKA to specific subcellular sites. An analogous mechanism has been identified for PKG activation and subcellular targeting through the formation of an intra-disulfide bond between Cys42 residues on PKG monomers, leading to its dimerisation [56]. The functional consequences of oxidative PKA or PKG activation in the setting of cardiac hypertrophy and failure remain to be elucidated. Another kinase involved in ECC, CaMKII, can undergo oxidation on methionine residues (Met-281/282) in its regulatory domain, resulting in a preservation of CaMKII activity even after the removal of Ca/calmodulin [59]. CaMKII oxidation has been associated with the development of cell death and ventricular rupture after myocardial infarction [60], atrial fibrillation [61] and diabetes-related bradycardia after myocardial infarction [62], and either NOX2-derived ROS or mitochondrial ROS were implicated in these effects. A specific role for oxidized CaMKII in the development of cardiac hypertrophy and failure remains to be investigated but seems likely in view of the data implicating CaMKII-related regulation of ECC in this setting. For example, CaMKII-dependent misregulation of RyR2 phosphorylation was implicated in altered calcium cycling in a mouse model of hypertrophy [63]. Such kinase-mediated redox regulation is particularly interesting because it may represent tractable potential therapeutic targets in cardiac hypertrophy and failure.

RyR2 may be direct redox targets by virtue of their very large number of cysteine residues [64]. Superoxidation of the RyR2 receptor is associated with irreversible activation and increased Ca leakage [65] but acute low-level exposure to ROS may have positive inotropic effects due to an enhancement of SR Ca release [64]. In line with this, the reactive nitrogen species nitroxyl (HNO) was shown to induce Ca release from the SR via RyR2-oxidation [66], a process that might result in an enhancement of myocyte contractility [67]. Similar observations have been made with respect to common cancer therapeutics such as thoracic radiation or anthracycline chemotherapy that are both capable of inducing high levels of ROS in isolated cardiac myocytes. In this setting, elevated ROS may oxidatively activate CaMKII, which results in increased CaMKII-dependent systolic SR Ca release, as well as persistent diastolic SR Ca leakage, which ultimately leads to contractile dysfunction [68,69]. On the other hand, a physiological role of NOX2-derived ROS in ECC through modulation of SR Ca release has also recently been reported [70]. These authors showed that NOX2-derived ROS release within T-tubules enhances length-dependent contractile activation of isolated cardiomyocytes by enhancing SR Ca release upon physiological stretch. Hyperactivation of this mechanism in Duchenne muscular dystrophy or after angiotensin II stimulation led to dysfunctional Ca release and arrhythmia. A previous study suggested that NOX2-dependent S-glutathionylation of RyR2 channels may be involved in tachycardia-related increase in contractility in normal myocardium [71]. ROS may also enhance SERCA2a function, e.g. through phospholamban oligomerization and consecutive dissociation from SERCA2a [72] whereas high dose ROS can disrupt SERCA2a function [73]. Other Ca handling proteins whose function may be disrupted by redox modifications include the sarcolemmal Na/Ca exchanger (NCX) [74], voltage gated L-type Ca channels [75] and sarcolemmal Na channels [76] (Fig. 1).

Redox modifications of the contractile machinery also contribute to contractile dysfunction although the role of specific modifications needs to be further investigated [77]. One example of a specific redox modification is the formation of a disulfide bridge in the N2-B segment of the giant myofilament, titin, which is implicated in increased muscle stiffness and diastolic dysfunction [78]. Also, HNO was shown to promote disulfide bond formation between critical cysteine residues in cardiac myofilaments thereby increasing Ca sensitivity of the myofilaments and mediating a redox-dependent positive inotropic effect [79].

3.3. Myocardial vascularization during hypertrophy

Myocardial vascularization is a critical factor during cardiac compensation in response to chronic pressure overload [3]. In the heart, a mismatch in the number of capillaries relative to the increase in cardiomyocyte size during hypertrophy is related to pathological remodeling resulting in an increase in the levels of hypertrophy, fibrosis, cardiac dilatation, and contractile failure. The transcriptional factor HIF1 is central to this process [80]. Although persistent HIF1 activation is reported to be detrimental during cardiac hypertrophy [81], several studies show that regulated HIF activation is cardioprotective (Fig. 2). As mentioned earlier, NOX4-derived ROS enhance cardiomyocyte HIF activation in response to chronic pressure overload to mediate protective effects at least in part through enhanced capillarization [49]. In the vasculature, NOX4-dependent pro-angiogenic effects have been shown to involve eNOS activation [51,82], and it is therefore of interest to assess whether a similar mechanism may apply in the heart. NO-dependent signaling also appears crucial for a proportionate vessel to myocytes growth in the hypertrophying heart [83].

3.4. Redox regulation of interstitial fibrosis

Excessive interstitial fibrosis is another central pathogenic feature of maladaptive cardiac hypertrophy, and contributes to impaired diastolic and systolic function [84] and re-entrant arrhythmia [85]. Several different cell types are involved in the development of pathological cardiac fibrosis. Fibroblasts play a key role in pathological fibrosis following their transformation to myofibroblasts in response to stimuli such as cytokines, transforming growth factor β (TGFβ) and angiotensin II. Cardiomyocytes release pro-fibrotic factors such as TGFβ and connective tissue growth factor (CTGF) upon stimulation by hypertrophic triggers [85,86]. Inflammatory cells are also involved through the release of cytokines and may be attracted into the stressed heart in response to diverse stimuli including damage-associated molecular patterns (DAMPs). Activated endothelial cells promote inflammatory cell influx and are also reported to contribute directly to pathological cardiac fibrosis through the process of endothelial-to-mesenchymal transition (EndoMT), in which TGFβ plays a key role [87]. Increased ROS production may drive or enhance the above processes. Redox signaling has been implicated in fibroblast proliferation, endothelial and inflammatory cell activation, and in EndoMT. Consistent with an important role for ROS signaling in fibrosis, many experimental studies have shown that various antioxidant approaches can reduce fibrosis.

In vivo studies in mouse models suggest an important role for NOX2-derived ROS in driving fibrosis. As such, NOX2-deficient mice subjected to chronic angiotensin II or aldosterone infusion were significantly protected against interstitial cardiac fibrosis [88], interestingly even when the extent of hypertrophy was unaltered. Similar results were found in mice with cardiomyocyte-specific Rac1-deletion that were subjected to angiotensin II infusion [39], NOX2-deficient mice subjected to aortic banding [89] or myocardial infarction [90,91], or in apoptosis signal-regulating kinase 1 (ASK-1)-deficient mice in a model of aldosterone/salt-induced fibrosis [92]. NOX2-dependent production of CTGF and the activation of NF-κB and matrix metalloproteinases (MMPs) appear to be involved in these effects [88]. Recently, we have also found an important pro-fibrotic role of endothelial cell NOX2 in a model of chronic angiotensin II infusion, where NOX2 enhanced inflammatory cell infiltration and EndoMT (Murdoch et al., JACC in press) [93]. Other ROS sources are also implicated in pro-fibrotic effects. The Rabinovitch lab recently provided evidence that angiotensin II-dependent mitochondrial ROS formation in cardiomyocytes contributed to fibrosis [12] while other studies found a role for ROS derived from uncoupled NOS in the setting of TAC-induced hypertrophy [11]. Finally, increased XO expression and activity were found in the infarcted heart and associated with increased myocardial ROS formation while inhibition of XO using allopurinol attenuated myocardial fibrosis suggesting that XO-derived ROS may also participate [94].

3.5. Maladaptive hypertrophy after myocardial infarction

Maladaptive remodeling of the left ventricle (LV) following myocardial infarction is one of the most common causes of heart failure in the western world. It involves a substantial remodeling of the extracellular matrix that results in dilatation of the LV as well as major changes in the non-infarcted myocardium, e.g. cardiomyocyte hypertrophy, contractile dysfunction, fibrosis, cell death and arrhythmia. A growing body of evidence suggests ROS to be centrally involved in these processes [95]. At a cellular level, there are many similarities to the redox signaling events discussed earlier in this article but also differences with respect to stimuli that evoke ROS production, the time-course of development of remodeling, and regional heterogeneity, which remain to be fully defined. Similar to other stresses such as chronic pressure overload, myocardial infarction induces the compensatory activation of the renin–angiotensin system but may also have pro-remodeling effects via direct mechanical forces, local inflammation and cytokine activation. In human tissue samples from infarcted hearts, cardiac myocyte NOX2 expression was increased [96] suggesting that NOX2 is regulated in post-MI remodeling. In fact, NOX2-deficient mice (either induced by a deletion of $p47^{phox}$ [91] or $gp91^{phox}$ [90]) subjected to left coronary ligation had better preserved contractile function, less LV remodeling and reduced mortality associated with less cardiomyocyte hypertrophy, apoptosis and interstitial fibrosis despite a similar initial infarct size to wild-type controls. Antioxidants such as probucol have been reported to be reducing post-MI remodeling in experimental models [97]. XO-derived ROS has also been implicated because the XO inhibitor allopurinol reduced ROS generation following MI and attenuated LV remodeling and dysfunction [94]. In infarcted rat hearts, uncoupled NOS and associated ROS generation augmented detrimental LV remodeling after MI [98]. On the other hand, NOS-derived NO can limit detrimental LV remodeling after MI, possibly via the cGMP/PKG pathway and inhibition of the mitochondrial permeability transition pore [99]. At a molecular level, increased mitochondrial ROS formation after MI was associated with RyR2 S-nitrosylation and depletion of calstabin2 from the RyR2 complex, resulting in diastolic SR Ca leakage and arrhythmias as well as aggravated LV remodeling [100]. Interestingly, NOX2-mediated oxidative CaMKII activation contributed to aldosterone-induced cardiac rupture after MI [60], which was reversible by methionine sulfoxide reductase A. Similarly, oxidized CaMKII was suggested to mediate diabetes-attributable mortality after MI as a consequence of increased mitochondrial ROS formation (which could be reduced using the mitochondrial targeted antioxidant MitoTEMPO) and severe bradycardia [62].

4. Potential clinical implications

The data discussed in this article indicate that disrupted redox signaling plays important roles in the initiation of stress-induced cardiac hypertrophy as well as in its disease progression towards heart failure. It appears to have an impact on all the key features of cardiac remodeling including the cardiomyocyte hypertrophic response, contractile dysfunction, arrhythmia, capillary density, fibrosis and ECM remodeling. Importantly, redox signaling contributes both to protective and detrimental pathways, likely depending upon the ROS source and disease stage, and is often highly specific. As such, it is perhaps not surprising that non-specific antioxidant approaches (e.g. vitamin C or E supplementation) have generally proven unsuccessful in clinical trials despite positive results from focused short-term experimental animal studies [101]. A much more targeted approach is likely to be required, e.g. targeting specific ROS sources (perhaps even in a tissue-specific or cell compartment-specific manner) or specific downstream molecular pathways. The inhibition of pathologically activated NOX2 may be a

promising approach although it may be necessary to avoid concurrent inhibition of NOX4. The theoretical problem of increased infection susceptibility from inhibiting NOX2 in phagocytes may in practice not be a major problem in light of data that phagocyte function is only impaired after near complete inhibition of NOX2 [102]. The "re-coupling" of uncoupled NOS by oral supplementation of tetrahydrobiopterin (BH4) was considered a promising approach [103] but in the clinical setting appears disappointing since orally administered BH_4 becomes oxidized to BH_2 which is not effective with respect to NOS re-coupling [104]. An alternative approach might be to target mitochondrial ROS, for example with specific antioxidant peptides (such as SS-31) that localize to this compartment [105] or with mitochondria-targeted antioxidants such as MitoQ, both of which appear promising in animal studies [106]. An elevation of endogenous antioxidant capacity by non-vitamin approaches might be another approach. For example, thioredoxin-1 gene therapy has been shown to reduce ventricular remodeling in the infarcted myocardium of diabetic rats [107]. However, such approaches may need to be carefully titrated because an excessively reduced cellular environment may be detrimental by generating reductive stress, as in mutant protein aggregation cardiomyopathy [108]. Redox-regulated signaling molecules that might be interesting therapeutic targets include oxidatively activated CaMKII [109] and leaky RyR2 channels.

5. Conclusions

Advances in redox signaling research over the last two decades have enhanced our understanding of how free radicals and ROS regulate various pathophysiological processes involved in the development of heart diseases. Many hubs in the signaling networks related to cardiac hypertrophy are redox sensitive, and play a role in hermetic cell metabolism to regulate cell growth and survival. Disruption of these redox signaling circuits can be detrimental and lead to cardiac dysfunction. This improved understanding of the roles of redox signaling likely explains why non-specific antioxidant therapies have failed with respect to heart diseases [101], but also suggests the possibility that more targeted approaches would be effective. We still need a more detailed understanding of cell compartment-specific and localized redox signaling, as well as the interplay and cross-talk among different redox-regulated pathways and different ROS sources. Better tools (such as real-time ROS detection probes), newer approaches (such as systems biological approaches), and more human data are likely to be extremely valuable in this endeavor.

Disclosures and conflicts of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by the British Heart Foundation; a Foundation Leducq Transatlantic Network of Excellence Award; the Department of Health via a National Institute for Health Research (NIHR) Biomedical Research Centre Award to Guy's and St. Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust; and a German Cardiac Society Fellowship Award to CMS. We gratefully acknowledge the contributions of all current and past members of the Shah laboratory.

References

[1] Cohn JN, Ferrari R, Sharpe N. Cardiac remodeling-concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. Behalf of an international forum on cardiac remodeling. J Am Coll Cardiol 2000;35:569–82.
[2] Catalucci D, Latronico MV, Ellingsen O, Condorelli G. Physiological myocardial hypertrophy: how and why? Front Biosci 2008;13:312–24.
[3] Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R, et al. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. J Clin Invest 2005;115:2108–18.
[4] Opie LH, Commerford PJ, Gersh BJ, Pfeffer MA. Controversies in ventricular remodelling. Lancet 2006;367:356–67.
[5] Burgoyne JR, Mongue-Din H, Eaton P, Shah AM. Redox signaling in cardiac physiology and pathology. Circ Res 2012;111:1091–106.
[6] Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev 2007;87:315–424.
[7] Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. J Clin Invest 2005;115:500–8.
[8] Woo HA, Yim SH, Shin DH, Kang D, Yu DY, Rhee SG. Inactivation of peroxiredoxin I by phosphorylation allows localized H_2O_2 accumulation for cell signaling. Cell 2010;140:517–28.
[9] Brewer AC, Mustafi SB, Murray TV, Rajasekaran NS, Benjamin IJ. Reductive stress linked to small HSPs, G6PD, and Nrf2 pathways in heart disease. Antioxid Redox Signal 2013;18:1114–27.
[10] Li JM, Gall NP, Grieve DJ, Chen M, Shah AM. Activation of NADPH oxidase during progression of cardiac hypertrophy to failure. Hypertension 2002;40:477–84.
[11] Takimoto E, Champion HC, Li M, Ren S, Rodriguez ER, Tavazzi B, et al. Oxidant stress from nitric oxide synthase-3 uncoupling stimulates cardiac pathologic remodeling from chronic pressure load. J Clin Invest 2005;115:1221–31.
[12] Dai DF, Johnson SC, Villarin JJ, Chin MT, Nieves-Cintron M, Chen T, et al. Mitochondrial oxidative stress mediates angiotensin II-induced cardiac hypertrophy and Galphaq overexpression-induced heart failure. Circ Res 2011;108:837–46.
[13] Xu X, Hu X, Lu Z, Zhang P, Zhao L, Wessale JL, et al. Xanthine oxidase inhibition with febuxostat attenuates systolic overload-induced left ventricular hypertrophy and dysfunction in mice. J Card Fail 2008;14:746–53.
[14] Kowaltowski AJ, de Souza-Pinto NC, Castilho RF, Vercesi AE. Mitochondria and reactive oxygen species. Free Radic Biol Med 2009;47:333–43.
[15] Bayeva M, Gheorghiade M, Ardehali H. Mitochondria as a therapeutic target in heart failure. J Am Coll Cardiol 2013;61:599–610.
[16] Ide T, Tsutsui H, Kinugawa S, Utsumi H, Kang D, Hattori N, et al. Mitochondrial electron transport complex I is a potential source of oxygen free radicals in the failing myocardium. Circ Res 1999;85:357–63.
[17] Conrad M, Jakupoglu C, Moreno SG, Lippl S, Banjac A, Schneider M, et al. Essential role for mitochondrial thioredoxin reductase in hematopoiesis, heart development, and heart function. Mol Cell Biol 2004;24:9414–23.
[18] Stanley BA, Sivakumaran V, Shi S, McDonald I, Lloyd D, Watson WH, et al. Thioredoxin reductase-2 is essential for keeping low levels of H(2)O(2) emission from isolated heart mitochondria. J Biol Chem 2011;286:33669–77.
[19] Dai DF, Santana LF, Vermulst M, Tomazela DM, Emond MJ, MacCoss MJ, et al. Overexpression of catalase targeted to mitochondria attenuates murine cardiac aging. Circulation 2009;119:2789–97.
[20] Chin KT, Kang G, Qu J, Gardner LB, Coetzee WA, Zito E, et al. The sarcoplasmic reticulum luminal thiol oxidase ERO1 regulates cardiomyocyte excitation-coupled calcium release and response to hemodynamic load. FASEB J 2011;25:2583–91.
[21] Brandes RP, Weissmann N, Schroder K. NADPH oxidases in cardiovascular disease. Free Radic Biol Med 2010;49:687–706.
[22] Brown DI, Griendling KK. Nox proteins in signal transduction. Free Radic Biol Med 2009;47:1239–53.
[23] Santos CX, Nabeebaccus AA, Shah AM, Camargo LL, Filho SV, Lopes LR. Endoplasmic reticulum stress and nox-mediated reactive oxygen species signaling in the peripheral vasculature: potential role in hypertension. Antioxid Redox Signal 2014;20:121–34.
[24] Zhang M, Takimoto E, Lee DI, Santos CX, Nakamura T, Hsu S, et al. Pathological cardiac hypertrophy alters intracellular targeting of phosphodiesterase type 5 from nitric oxide synthase-3 to natriuretic peptide signaling. Circulation 2012;126:942–51.
[25] Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, et al. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest 2003;111:1201–9.
[26] Chen CA, Wang TY, Varadharaj S, Reyes LA, Hemann C, Talukder MA, et al. S-glutathionylation uncouples eNOS and regulates its cellular and vascular function. Nature 2010;468:1115–8.
[27] Kaludercic N, Carpi A, Menabo R, Di Lisa F, Paolocci N. Monoamine oxidases (MAO) in the pathogenesis of heart failure and ischemia/reperfusion injury. Biochim Biophys Acta 1813;2011:1323–32.
[28] Kaludercic N, Carpi A, Nagayama T, Sivakumaran V, Zhu G, Lai EW, et al. Monoamine oxidase B prompts mitochondrial and cardiac dysfunction in pressure overloaded hearts. Antioxid Redox Signal 2014;20:267–80.
[29] Lu D, Ma Y, Zhang W, Bao D, Dong W, Lian H, et al. Knockdown of cytochrome P450 2E1 inhibits oxidative stress and apoptosis in the cTnT(R141W) dilated cardiomyopathy transgenic mice. Hypertension 2012;60:81–9.
[30] Nishino T, Okamoto K, Eger BT, Pai EF, Nishino T. Mammalian xanthine oxidoreductase — mechanism of transition from xanthine dehydrogenase to xanthine oxidase. FEBS J 2008;275:3278–89.
[31] Minhas KM, Saraiva RM, Schuleri KH, Lehrke S, Zheng M, Saliaris AP, et al. Xanthine oxidoreductase inhibition causes reverse remodeling in rats with dilated cardiomyopathy. Circ Res 2006;98:271–9.
[32] Halliwell BG, Gutteridge JMC. Free radicals in biology and medicine. 4th ed. Oxford, United Kingdom: Oxford University Press; 2007.
[33] Heineke J, Molkentin JD. Regulation of cardiac hypertrophy by intracellular signalling pathways. Nat Rev Mol Cell Biol 2006;7:589–600.
[34] Shah AM, Mann DL. In search of new therapeutic targets and strategies for heart failure: recent advances in basic science. Lancet 2011;378:704–12.
[35] Santos CX, Anilkumar N, Zhang M, Brewer AC, Shah AM. Redox signaling in cardiac myocytes. Free Radic Biol Med 2011;50:777–93.

[36] Ago T, Liu T, Zhai P, Chen W, Li H, Molkentin JD, et al. A redox-dependent pathway for regulating class II HDACs and cardiac hypertrophy. Cell 2008;133:978–93.
[37] Haworth RS, Stathopoulou K, Candasamy AJ, Avkiran M. Neurohormonal regulation of cardiac histone deacetylase 5 nuclear localization by phosphorylation-dependent and phosphorylation-independent mechanisms. Circ Res 2012;110:1585–95.
[38] Bendall JK, Cave AC, Heymes C, Gall N, Shah AM. Pivotal role of a gp91(phox)-containing NADPH oxidase in angiotensin II-induced cardiac hypertrophy in mice. Circulation 2002;105:293–6.
[39] Satoh M, Ogita H, Takeshita K, Mukai Y, Kwiatkowski DJ, Liao JK. Requirement of Rac1 in the development of cardiac hypertrophy. Proc Natl Acad Sci U S A 2006;103:7432–7.
[40] Nakagami H, Takemoto M, Liao JK. NADPH oxidase-derived superoxide anion mediates angiotensin II-induced cardiac hypertrophy. J Mol Cell Cardiol 2003;35:851–9.
[41] Hingtgen SD, Tian X, Yang J, Dunlay SM, Peek AS, Wu Y, et al. Nox2-containing NADPH oxidase and Akt activation play a key role in angiotensin II-induced cardiomyocyte hypertrophy. Physiol Genomics 2006;26:180–91.
[42] Izumiya Y, Kim S, Izumi Y, Yoshida K, Yoshiyama M, Matsuzawa A, et al. Apoptosis signal-regulating kinase 1 plays a pivotal role in angiotensin II-induced cardiac hypertrophy and remodeling. Circ Res 2003;93:874–83.
[43] Hirotani S, Otsu K, Nishida K, Higuchi Y, Morita T, Nakayama H, et al. Involvement of nuclear factor-kappaB and apoptosis signal-regulating kinase 1 in G-protein-coupled receptor agonist-induced cardiomyocyte hypertrophy. Circulation 2002;105:509–15.
[44] Aikawa R, Nagai T, Tanaka M, Zou Y, Ishihara T, Takano H, et al. Reactive oxygen species in mechanical stress-induced cardiac hypertrophy. Biochem Biophys Res Commun 2001;289:901–7.
[45] Kuster GM, Pimentel DR, Adachi T, Ido Y, Brenner DA, Cohen RA, et al. Alpha-adrenergic receptor-stimulated hypertrophy in adult rat ventricular myocytes is mediated via thioredoxin-1-sensitive oxidative modification of thiols on Ras. Circulation 2005;111:1192–8.
[46] Pimentel DR, Adachi T, Ido Y, Heibeck T, Jiang B, Lee Y, et al. Strain-stimulated hypertrophy in cardiac myocytes is mediated by reactive oxygen species-dependent Ras S-glutathiolation. J Mol Cell Cardiol 2006;41:613–22.
[47] Byrne JA, Grieve DJ, Bendall JK, Li JM, Gove C, Lambeth JD, et al. Contrasting roles of NADPH oxidase isoforms in pressure-overload versus angiotensin II-induced cardiac hypertrophy. Circ Res 2003;93:802–5.
[48] Sciarretta S, Zhai P, Shao D, Zablocki D, Nagarajan N, Terada LS, et al. Activation of NADPH oxidase 4 in the endoplasmic reticulum promotes cardiomyocyte autophagy and survival during energy stress through the protein kinase RNA-activated-like endoplasmic reticulum kinase/eukaryotic initiation factor 2alpha/activating transcription factor 4 pathway. Circ Res 2013;113:1253–64.
[49] Zhang M, Brewer AC, Schroder K, Santos CX, Grieve DJ, Wang M, et al. NADPH oxidase-4 mediates protection against chronic load-induced stress in mouse hearts by enhancing angiogenesis. Proc Natl Acad Sci U S A 2010;107:18121–6.
[50] Nlandu Khodo S, Dizin E, Sossauer G, Szanto I, Martin PY, Feraille E, et al. NADPH-oxidase 4 protects against kidney fibrosis during chronic renal injury. J Am Soc Nephrol 2012;23:1967–76.
[51] Schroder K, Zhang M, Benkhoff S, Mieth A, Pliquett R, Kosowski J, et al. Nox4 is a protective reactive oxygen species generating vascular NADPH oxidase. Circ Res 2012;110:1217–25.
[52] Brewer AC, Murray TV, Arno M, Zhang M, Anilkumar NP, Mann GE, et al. Nox4 regulates Nrf2 and glutathione redox in cardiomyocytes in vivo. Free Radic Biol Med 2011;51:205–15.
[53] Zhang M, Perino A, Ghigo A, Hirsch E, Shah AM. NADPH oxidases in heart failure: poachers or gamekeepers? Antioxid Redox Signal 2013;18:1024–41.
[54] Kuroda J, Ago T, Matsushima S, Zhai P, Schneider MD, Sadoshima J. NADPH oxidase 4 (Nox4) is a major source of oxidative stress in the failing heart. Proc Natl Acad Sci U S A 2010;107:15565–70.
[55] Pouyssegur J, Mechta-Grigoriou F. Redox regulation of the hypoxia-inducible factor. Biol Chem 2006;387:1337–46.
[56] Burgoyne JR, Madhani M, Cuello F, Charles RL, Brennan JP, Schroder E, et al. Cysteine redox sensor in PKGIa enables oxidant-induced activation. Science 2007;317:1393–7.
[57] Wagner S, Rokita AG, Anderson ME, Maier LS. Redox regulation of sodium and calcium handling. Antioxid Redox Signal 2013;18:1063–77.
[58] Brennan JP, Bardswell SC, Burgoyne JR, Fuller W, Schroder E, Wait R, et al. Oxidant-induced activation of type I protein kinase A is mediated by RI subunit interprotein disulfide bond formation. J Biol Chem 2006;281:21827–36.
[59] Erickson JR, Joiner ML, Guan X, Kutschke W, Yang J, Oddis CV, et al. A dynamic pathway for calcium-independent activation of CaMKII by methionine oxidation. Cell 2008;133:462–74.
[60] He BJ, Joiner ML, Singh MV, Luczak ED, Swaminathan PD, Koval OM, et al. Oxidation of CaMKII determines the cardiotoxic effects of aldosterone. Nat Med 2011;17:1610–8.
[61] Purohit A, Rokita AG, Guan X, Chen B, Koval OM, Voigt N, et al. Oxidized CaMKII triggers atrial fibrillation. Circulation 2013;128:1748–57.
[62] Luo M, Guan X, Luczak ED, Lang D, Kutschke W, Gao Z, et al. Diabetes increases mortality after myocardial infarction by oxidizing CaMKII. J Clin Invest 2013;123:1262–74.
[63] Toischer K, Rokita AG, Unsold B, Zhu W, Kararigas G, Sossalla S, et al. Differential cardiac remodeling in preload versus afterload. Circulation 2010;122:993–1003.
[64] Zima AV, Blatter LA. Redox regulation of cardiac calcium channels and transporters. Cardiovasc Res 2006;71:310–21.
[65] Terentyev D, Gyorke I, Belevych AE, Terentyeva R, Sridhar A, Nishijima Y, et al. Redox modification of ryanodine receptors contributes to sarcoplasmic reticulum Ca2+ leak in chronic heart failure. Circ Res 2008;103:1466–72.
[66] Cheong E, Tumbev V, Abramson J, Salama G, Stoyanovsky DA. Nitroxyl triggers Ca2+ release from skeletal and cardiac sarcoplasmic reticulum by oxidizing ryanodine receptors. Cell Calcium 2005;37:87–96.
[67] Tocchetti CG, Wang W, Froehlich JP, Huke S, Aon MA, Wilson GM, et al. Nitroxyl improves cellular heart function by directly enhancing cardiac sarcoplasmic reticulum Ca2+ cycling. Circ Res 2007;100:96–104.
[68] Sag CM, Wolff HA, Neumann K, Opiela MK, Zhang J, Steuer F, et al. Ionizing radiation regulates cardiac Ca handling via increased ROS and activated CaMKII. Basic Res Cardiol 2013;108:385.
[69] Sag CM, Kohler AC, Anderson ME, Backs J, Maier LS. CaMKII-dependent SR Ca leak contributes to doxorubicin-induced impaired Ca handling in isolated cardiac myocytes. J Mol Cell Cardiol 2011;51:749–59.
[70] Prosser BL, Ward CW, Lederer WJ. X-ROS signaling: rapid mechano-chemo transduction in heart. Science 2011;333:1440–5.
[71] Sanchez G, Pedrozo Z, Domenech RJ, Hidalgo C, Donoso P. Tachycardia increases NADPH oxidase activity and RyR2 S-glutathionylation in ventricular muscle. J Mol Cell Cardiol 2005;39:982–91.
[72] Sivakumaran V, Stanley BA, Tocchetti CG, Ballin JD, Caceres V, Zhou L, et al. HNO enhances SERCA2a activity and cardiomyocyte function by promoting redox-dependent phospholamban oligomerization. Antioxid Redox Signal 2013;19:1185–97.
[73] Xu KY, Zweier JL, Becker LC. Hydroxyl radical inhibits sarcoplasmic reticulum Ca(2+)-ATPase function by direct attack on the ATP binding site. Circ Res 1997;80:76–81.
[74] Goldhaber JI. Free radicals enhance Na+/Ca2+ exchange in ventricular myocytes. Am J Physiol 1996;271:H823–33.
[75] Gill JS, McKenna WJ, Camm AJ. Free radicals irreversibly decrease Ca2+ currents in isolated guinea-pig ventricular myocytes. Eur J Pharmacol 1995;292:337–40.
[76] Kassmann M, Hansel A, Leipold E, Birkenbeil J, Lu SQ, Hoshi T, et al. Oxidation of multiple methionine residues impairs rapid sodium channel inactivation. Pflugers Arch 2008;456:1085–95.
[77] Sumandea MP, Steinberg SF. Redox signaling and cardiac sarcomeres. J Biol Chem 2011;286:9921–7.
[78] Grutzner A, Garcia-Manyes S, Kotter S, Badilla CL, Fernandez JM, Linke WA. Modulation of titin-based stiffness by disulfide bonding in the cardiac titin N2-B unique sequence. Biophys J 2009;97:825–34.
[79] Gao WD, Murray CI, Tian Y, Zhong X, DuMond JF, Shen X. et al. Nitroxyl-mediated disulfide bond formation between cardiac myofilament cysteines enhances contractile function. Circ Res 2012;111:1002–11.
[80] Sano M, Minamino T, Toko H, Miyauchi H, Orimo M, Qin Y, et al. p53-Induced inhibition of Hif-1 causes cardiac dysfunction during pressure overload. Nature 2007;446:444–8.
[81] Krishnan J, Suter M, Windak R, Krebs T, Felley A, Montessuit C, et al. Activation of a HIF1alpha-PPARgamma axis underlies the integration of glycolytic and lipid anabolic pathways in pathologic cardiac hypertrophy. Cell Metab 2009;9:512–24.
[82] Craige SM, Chen K, Pei Y, Li C, Huang X, Chen C, et al. NADPH oxidase 4 promotes endothelial angiogenesis through endothelial nitric oxide synthase activation. Circulation 2011;124:731–40.
[83] Jaba IM, Zhuang ZW, Li N, Jiang Y, Martin KA, Sinusas AJ, et al. NO triggers RGS4 degradation to coordinate angiogenesis and cardiomyocyte growth. J Clin Invest 2013;123:1718–31.
[84] Creemers EE, Pinto YM. Molecular mechanisms that control interstitial fibrosis in the pressure-overloaded heart. Cardiovasc Res 2011;89:265–72.
[85] Weber KT, Sun Y, Bhattacharya SK, Ahokas RA, Gerling IC. Myofibroblast-mediated mechanisms of pathological remodelling of the heart. Nat Rev Cardiol 2013;10:15–26.
[86] Koitabashi N, Danner T, Zaiman AL, Pinto YM, Rowell J, Mankowski J, et al. Pivotal role of cardiomyocyte TGF-beta signaling in the murine pathological response to sustained pressure overload. J Clin Invest 2011;121:2301–12.
[87] Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nat Med 2007;13:952–61.
[88] Johar S, Cave AC, Narayanapanicker A, Grieve DJ, Shah AM. Aldosterone mediates angiotensin II-induced interstitial cardiac fibrosis via a Nox2-containing NADPH oxidase. FASEB J 2006;20:1546–8.
[89] Grieve DJ, Byrne JA, Siva A, Layland J, Johar S, Cave AC, et al. Involvement of the nicotinamide adenosine dinucleotide phosphate oxidase isoform Nox2 in cardiac contractile dysfunction occurring in response to pressure overload. J Am Coll Cardiol 2006;47:817–26.
[90] Looi YH, Grieve DJ, Siva A, Walker SJ, Anilkumar N, Cave AC, et al. Involvement of Nox2 NADPH oxidase in adverse cardiac remodeling after myocardial infarction. Hypertension 2008;51:319–25.
[91] Doerries C, Grote K, Hilfiker-Kleiner D, Luchtefeld M, Schaefer A, Holland SM, et al. Critical role of the NAD(P)H oxidase subunit p47phox for left ventricular remodeling/dysfunction and survival after myocardial infarction. Circ Res 2007;100:894–903.
[92] Nakamura T, Kataoka K, Fukuda M, Nako H, Tokutomi Y, Dong YF, et al. Critical role of apoptosis signal-regulating kinase 1 in aldosterone/salt-induced cardiac inflammation and fibrosis. Hypertension 2009;54:544–51.
[93] Murdoch CE CA, Zeng L, Yu B, Ivetic A, Walker SJ, Vanhoutte D, et al. Endothelial NADPH oxidase-2 promotes interstitial cardiac fibrosis and diastolic dysfunction through pro-inflammatory effects and endothelial–mesenchymal transition. J Am Coll Cardiol 2014 [in press].
[94] Engberding N, Spiekermann S, Schaefer A, Heineke A, Wiencke A, Muller M, et al. Allopurinol attenuates left ventricular remodeling and dysfunction after experimental myocardial infarction: a new action for an old drug? Circulation 2004;110:2175–9.

[95] Sun Y. Oxidative stress and cardiac repair/remodeling following infarction. Am J Med Sci 2007;334:197–205.
[96] Krijnen PA, Meischl C, Hack CE, Meijer CJ, Visser CA, Roos D, et al. Increased Nox2 expression in human cardiomyocytes after acute myocardial infarction. J Clin Pathol 2003;56:194–9.
[97] Sia YT, Lapointe N, Parker TG, Tsoporis JN, Deschepper CF, Calderone A, et al. Beneficial effects of long-term use of the antioxidant probucol in heart failure in the rat. Circulation 2002;105:2549–55.
[98] Masano T, Kawashima S, Toh R, Satomi-Kobayashi S, Shinohara M, Takaya T, et al. Beneficial effects of exogenous tetrahydrobiopterin on left ventricular remodeling after myocardial infarction in rats: the possible role of oxidative stress caused by uncoupled endothelial nitric oxide synthase. Circ J 2008;72:1512–9.
[99] Rastaldo R, Pagliaro P, Cappello S, Penna C, Mancardi D, Westerhof N, et al. Nitric oxide and cardiac function. Life Sci 2007;81:779–93.
[100] Fauconnier J, Meli AC, Thireau J, Roberge S, Shan J, Sassi Y, et al. Ryanodine receptor leak mediated by caspase-8 activation leads to left ventricular injury after myocardial ischemia–reperfusion. Proc Natl Acad Sci U S A 2011;108:13258–63.
[101] Lonn E, Bosch J, Yusuf S, Sheridan P, Pogue J, Arnold JM, et al. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. JAMA 2005;293:1338–47.
[102] Kuhns DB, Alvord WG, Heller T, Feld JJ, Pike KM, Marciano BE, et al. Residual NADPH oxidase and survival in chronic granulomatous disease. N Engl J Med 2010;363:2600–10.
[103] Moens AL, Takimoto E, Tocchetti CG, Chakir K, Bedja D, Cormaci G, et al. Reversal of cardiac hypertrophy and fibrosis from pressure overload by tetrahydrobiopterin: efficacy of recoupling nitric oxide synthase as a therapeutic strategy. Circulation 2008;117:2626–36.
[104] Cunnington C, Van Assche T, Shirodaria C, Kylintireas I, Lindsay AC, Lee JM, et al. Systemic and vascular oxidation limits the efficacy of oral tetrahydrobiopterin treatment in patients with coronary artery disease. Circulation 2012;125:1356–66.
[105] Dai DF, Chen T, Szeto H, Nieves-Cintron M, Kutyavin V, Santana LF, et al. Mitochondrial targeted antioxidant peptide ameliorates hypertensive cardiomyopathy. J Am Coll Cardiol 2011;58:73–82.
[106] Graham D, Huynh NN, Hamilton CA, Beattie E, Smith RA, Cocheme HM, et al. Mitochondria-targeted antioxidant MitoQ10 improves endothelial function and attenuates cardiac hypertrophy. Hypertension 2009;54:322–8.
[107] Samuel SM, Thirunavukkarasu M, Penumathsa SV, Koneru S, Zhan L, Maulik G, et al. Thioredoxin-1 gene therapy enhances angiogenic signaling and reduces ventricular remodeling in infarcted myocardium of diabetic rats. Circulation 2010;121:1244–55.
[108] Kannan S, Muthusamy VR, Whitehead KJ, Wang L, Gomes AV, Litwin SE, et al. Nrf2 deficiency prevents reductive stress-induced hypertrophic cardiomyopathy. Cardiovasc Res 2013;100:63–73.
[109] Erickson JR, He BJ, Grumbach IM, Anderson ME. CaMKII in the cardiovascular system: sensing redox states. Physiol Rev 2011;91:889–915.