Oxidative stress, resulting from increased production of reactive oxygen species (ROS) or reduced antioxidant defenses, has been implicated in cardiovascular disease pathophysiology for >2 decades. Based on the concept that this drives both the genesis and progression of conditions such as heart failure, numerous clinical trials of antioxidant therapies were undertaken but were unsuccessful. Nevertheless, experimental data linking oxidative stress and heart disease remain compelling and support continued efforts to develop more effective therapies than antioxidant vitamins.1 In the current issue of Circulation, Zhao et al2 report that cardiomyocyte-specific high-level overexpression of the ROS-generating enzyme NADPH oxidase-4 (Nox4) aggravated angiotensin II–induced cardiac remodeling and was mitigated by a small molecule Nox inhibitor. The authors propose that Nox4 inhibition may have a therapeutic potential to treat cardiac remodeling. Is this proposal reasonable, and how should such studies be interpreted within a pathophysiological framework for the roles of ROS in heart failure?

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ROS have complex physiological and pathological effects depending on the ROS source, species and local concentration, subcellular location, local antioxidant environment, and the disease stage.1 Major cardiac ROS sources include the mitochondria, metabolic enzymes, uncoupled nitric oxide synthases, and Nox proteins. ROS species such as hydroxyl and lipid radicals are almost always detrimental, especially in large amounts. However, superoxide anions (O₂⁻) and the non-radical hydrogen peroxide (H₂O₂, formed by superoxide dmutation) may be involved in redox signaling, ie, the specific, usually reversible, oxidation-reduction (redox) modification of cellular signaling pathway components.

Physiological redox signaling involves low-level, regulated ROS production in subcellular microdomains where specific targets of oxidative modification may include ion channels, receptors, kinases, phosphatases, and transcription factors. Oxidative modification of the target typically converts the ROS signal into a functional output. In the heart, redox signaling regulates physiological processes such as cardiomyocyte differentiation and excitation-contraction coupling.3 Pathophysiological redox signaling implies an increase in a specific redox signal or enhanced coupling to specific targets. Such signaling is involved in many aspects of cardiac remodeling, eg, cardiomyocyte hypertrophy related to the activation of ERK1/2 and NF-kB, or contractile dysfunction related to the activation of calcium-calmodulin kinase II.1 Importantly, some redox-sensitive pathways activated in pathological settings may promote beneficial responses, eg, adaptation to hypoxia and cytoprotection. Nox proteins are especially suited for redox signaling in that they generate ROS as their primary function, exhibit distinct subcellular locations and spatial compartmentation with signaling targets, and often have specialized activation mechanisms.3

In contrast to such specific redox signaling, unregulated ROS production may occur during reperfusion after myocardial ischemia or in heart failure. Here, mitochondria are central players, both as sources and targets of ROS. Excessive unconfined ROS production not only causes nonspecific damage throughout the cell, but also is particularly detrimental for mitochondrial function.4 Mitochondrial DNA, lipid, Krebs cycle enzymes, and the F1 ATPase are all highly susceptible to oxidative damage, leading to impaired energetic function and cell viability. Mitochondrial dysfunction promotes further oxidative stress through electron leakage, promoting a vicious cycle between oxidative stress and mitochondrial dysfunction. Spatially confined pathophysiological redox signaling can progress to mitochondrial dysfunction and more global oxidative stress by triggering ROS-induced ROS release by mitochondria. On the other hand, high mitochondrial ROS production may also affect diverse cytosolic signaling pathways even though the primary defect lies at the mitochondrial level.

From these considerations, it is clear that distinct therapeutic approaches might be needed for different disease stages. Targeting mitochondrial ROS could disrupt the vicious cycle between oxidative stress and mitochondrial dysfunction in chronic heart failure, and potential agents include various mitochondrial-targeted antioxidants.4 On the other hand, targeting specific redox-signaling pathways or specific ROS sources that drive such pathways might be beneficial in defined settings where the pathway in question is critical. Nox proteins are of particular interest in this regard.1

Of the 7 Nox family isoforms, good evidence supports the presence of Nox2 and Nox4 in cardiomyocytes and other cardiac cells such as endothelial cells and fibroblasts.3 Nox2 is acutely activated by angiotensin II, endothelin-1, tumor necrosis factor-α, growth factors, or mechanical forces, predominantly at the plasma membrane and T tubules. Previous work indicates roles for increased Nox2 activation in driving several...
components of the cardiac-remodeling phenotype, including angiotensin II–induced hypertrophy (via ERK1/2, Akt, and NF-κB signaling); fibrosis induced by increased renin-angiotensin activity; calcium dysregulation attributable to increased sarcoplasmic reticulum calcium leak; arrhythmia, involving calcium-calmodulin kinase II and protein kinase A activation; and postmyocardial infarction remodeling and rupture (involving matrix metalloproteinase activation). As such, targeting Nox2 might be a promising approach in these settings.

What about Nox4? In contrast to Nox2, this isoform has continuous low-level activity and is regulated mainly by its abundance. Nox4 has an intracellular location, reportedly in the endoplasmic reticulum, nucleus, and possibly mitochondria. Nox4 levels are low in the healthy heart but increase in response to diverse stress stimuli, eg, hypoxia, ischemia, hemodynamic overload, and neurohumoral activation. Whereas Nox2 produces superoxide, Nox4 generates predominantly H$_2$O$_2$ under physiological conditions; this can have opposite effects on nitric oxide signaling because superoxide inactivates nitric oxide, whereas H$_2$O$_2$ may enhance nitric oxide production. To investigate the potential pathophysiological role of Nox4 in the heart, some years ago we used complementary loss-of-function and gain-of-function approaches in mouse models. We found with both approaches that Nox4 upregulation during chronic pressure overload was protective. Cardiomyocyte Nox4 increased the activation of the transcription factor Hif1 and enhanced Hif1–vascular endothelial growth factor paracrine signaling to preserve myocardial capillarization, which is recognized as an important determinant of functional cardiac compensation during hemodynamic overload. We have subsequently identified another Nox4–triggered protective redox-signaling pathway, namely the activation of the transcription factor Nrf2, which regulates a potent cytoprotective gene program. Nrf2 activation during hemodynamic overload was shown to be specifically regulated by Nox4, and the deletion of Nrf2 in cardiomyocyte-specific Nox4-overexpressing mice reduced the protective effects of Nox4 during overload.

In contrast to our work, the Sadoshima laboratory, also using gain-of-function and loss-of-function approaches, reported that cardiomyocyte Nox4 is detrimental during hemodynamic overload as a result of increased mitochondrial ROS production and damage. The precise reasons for this disparity remain to be clarified, but methodological differences between the studies include the type and severity of overload studied (severe transverse aortic constriction in their study versus abdominal aortic banding in ours) and the approaches used to disrupt or overexpress the Nox4 gene. Although our work highlights the importance of Nox4-mediated protective redox signaling, Kuroda et al’s results pointed to a primary impact on mitochondrial function. It is interesting to note that several other laboratories have independently reported the protective effects of Nox4 in the kidney and vasculature, similar to those we found in the heart and also involving Hif1 or Nrf2 signaling. Furthermore, the Sadoshima laboratory also subsequently reported cardioprotective effects of Nox4 mediated by specific redox signaling in other pathological settings, such as ischemia-reperfusion. Taking the totality of the evidence, there seems little doubt that Nox4-activated redox-signaling pathways have significant potential to exert protective effects in the heart and other organs, depending on the pathological context. Clearly, the inhibition of such effects, either by global nonspecific ROS scavenging or targeted Nox inhibition, would be undesirable. The questions therefore are: (1) under what conditions might Nox4 mediate detrimental effects and (2) are there pathological situations where Nox4-mediated detrimental effects are sufficiently severe to outweigh beneficial signaling and, therefore, to merit therapeutic targeting?

Protective redox-signaling pathways, in general, depend on spatially confined, regulated ROS production. In the heart, any situation where ROS production is no longer spatially confined and levels rise throughout the cell is likely both to promote mitochondrial dysfunction (with further ROS production) and to nonspecifically activate diverse redox-sensitive pathways, many of which may be detrimental. It can therefore be envisaged that Nox4 (or indeed any ROS source) might in principle exert detrimental effects if the level of upregulation was very high or if it was mislocalized and thus no longer compartmentalized with specific redox-sensitive targets. To date, limited evidence supports such a scenario for endogenous myocardial Nox4 in disease settings. Failing human myocardium shows an up to 2-fold increase in total NADPH oxidase activity, similar to the levels in our study where we found a protective role of Nox4 against hemodynamic overload. Whether Nox4 levels and ROS production are elevated to a substantially greater extent after severe transverse aortic constriction merits careful assessment. The study by Zhao et al in this issue reports a 10-fold increase in Nox4 abundance and 8-fold increase in ROS levels in their transgenic mouse, making the pathophysiological relevance of their findings questionable. Zhao et al also report changes in the activity of the redox-sensitive Akt/mTOR– and NF-κB–signaling pathways but other redox-sensitive pathways were not analyzed, leaving open the question whether the effects observed were specific or merely the result of suprapathological ROS levels. In fact, similar changes in Akt and NF-κB activation are observed with other ROS sources such as Nox2 and are mitigated by knockout of Nox2, again raising the question whether the effects observed by these authors are specific to Nox4.

These issues are particularly important when considering the therapeutic potential of targeting a specific ROS source. The potential efficacy of such a strategy may be tested by genetic knockout/knockdown of the ROS source or by its pharmacological inhibition. Zhao et al did not study genetic knockout of Nox4 but reported that a small molecule Nox1/Nox4 inhibitor, GKT137831, mitigated the effects of angiotensin II infusion in their Nox4-overexpressing mouse, which is to be expected. The more relevant question concerning whether this inhibitor affects cardiac remodeling in wild-type animals subjected to angiotensin II infusion or hemodynamic overload was surprisingly not addressed. Given these significant limitations and other methodological issues, the case made by these authors for Nox4 as a suitable therapeutic target is weak.

Where does this leave the field? Nox2 appears to be a promising target to tackle arrhythmia and calcium dysregulation related to enhanced angiotensin II signaling. It seems clear that, in many settings, Nox4 mediates protective effects through specific redox signaling. Elucidation of the
mechanisms underlying such signaling may identify novel approaches to boost adaptive cellular stress responses. Further research is required to identify whether there are disease situations where Nox4-mediated ROS production becomes detrimental and, if so, how best this might be addressed. Current evidence suggests that in settings where there is widespread elevation of ROS levels and associated mitochondrial dysfunction, the targeting of ROS in the mitochondrial compartment may be a better approach.

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