Mitigation of myocardial ischemia-reperfusion injury via HIF-1α-frataxin signaling

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MYOCARDIAL INFARCTION (MI) is a leading cause of death and disability globally that typically results from the abrupt occlusion of an epicardial coronary artery (9). As a result, cardiomyocytes distal to the occlusion are subject to oxygen and nutrient deprivation, which triggers mitochondrial electron transport chain (ETC) inhibition, reactive oxygen species (ROS) generation, and necrotic death of the ischemic core (6). While prompt reopening of the infarct-related artery is critical for salvaging viable myocardium, reperfusion itself is posited to inflict further damage via its ability to transiently hyperpolarize mitochondrial membranes, leading to massive bursts of ROS that open permeability transition pores to initiate apoptosis (4). Reperfusion injury can account for up to 50% of the final infarct size, but despite extensive research, no effective treatment has emerged. Greater understanding of the endogenous cardioprotective pathways recruited during ischemia-reperfusion injury (IRI) might foster novel therapeutic strategies to improve outcomes after an MI.

Hypoxia-inducible factor (HIF)-1 orchestrates the bodies adaptive response to oxygen deprivation and is the critical mediator of ischemic pre- and postconditioning (14, 15, 17, 18). It is a heterodimeric protein consisting of an oxygen-sensitive α-subunit and a constitutive β-subunit. Under normoxic conditions, HIF-1α undergoes rapid degradation, which is initiated by prolylhydroxylase domain proteins (PHDs) that are dependent on oxygen and iron-sulfur clusters (Fe-S) (5). At the onset of myocardial ischemia, cellular hypoxia directly inhibits PHDs to stabilize HIF-1α and, by inhibiting ETC activity, increases ROS release from complex I and III (7). In turn, ROS further augment HIF-1α levels partly by destabilizing Fe-S. HIF-1α mRNA levels are elevated in the hearts of patients with acute myocardial ischemia, and enhancement or inhibition of HIF-1α attenuates and worsens experimental IRI, respectively (8, 14). HIF-1α acts by binding onto hypoxia-responsive elements in over 200 target genes and transcriptional coactivators (17). By augmenting angiogenic proteins, HIF-1α improves oxygen supply to ischemic territories. By suppressing mitochondrial function and switching metabolism from oxidative to glycolytic pathways, HIF-1α attenuates cardiac contractility to reduce oxygen demand. Inhibition of ETC activity during the ischemic phase is important as it reduces the magnitude of ROS production, permeability transition, and apoptosis at the onset of reperfusion. In this issue of the American Journal of Physiology-Heart and Circulatory Physiology, Nanayakkara et al. (13) provide evidence that the upregulation of frataxin is another mechanism via which HIF-1α mediates cardioprotection.

Frataxin is an evolutionary-conserved mitochondrial matrix protein whose putative functions are thought to limit ROS production and oxidative stress (1). It is heavily implicated in the biogenesis of Fe-S, which are critical cofactors involved in ATP generation, intracellular oxygen, iron, and ROS sensing, and the replication and maintenance of the nuclear genome (10). During de novo Fe-S biogenesis, frataxin is hypothesized to chaperone iron onto mitochondrial protein complexes that assemble the clusters and transfers them onto apoproteins. Frataxin, which can sequester over 3,000 iron atoms, also donates iron to IX protoporphyrin during heme biosynthesis. Primary frataxin deficiency, as seen in the neurodegenerative disease Friedreich’s ataxia, is characterized by Fe-S depletion, mitochondrial iron accumulation, oxidative stress, and cardiomyopathy (1). This observation, coupled with other experimental data (16), inform the view that frataxin, irrespective of its role in Fe-S and heme biogenesis, functions also to maintain mitochondrial redox balance by sequestering free labile iron that can potentially generate dangerous hydroxyl radicals.

Nanayakkara et al. (13) performed a series of studies to test the notion that a HIF-1α-frataxin-iron axis operates during myocardial IRI. After induction of IRI, the authors observed frataxin upregulation in the hearts of wild-type mice but not in those of cardiomyocyte-specific HIF-1α knockout (KO) animals. Infarct size was significantly greater in HIF-1α KO mice, confirming the cardioprotective effect of HIF-1α. Knockdown of HIF-1α in H9C2 cardiomyocytes diminished frataxin levels, and a functional hypoxia-responsive element was found in the mouse frataxin promoter, implying a direct regulation of frataxin expression by HIF-1α. To explore downstream mechanisms, mitochondrial iron levels were quantified and found to be markedly elevated in the ischemic region of HIF-1α KO hearts compared with their nonischemic regions and wild-type animals. Mitochondrial iron levels were also increased in normoxic frataxin knockout cells and become more so after the induction of hypoxia and/or HIF-1α inhibition. Furthermore, hypoxic H9C2 cells treated with a HIF-1α inhibitor exhibited the highest levels of ROS and mitochondrial membrane depolarization (a preapoptotic event), whereas cells overexpressing frataxin and those treated with an iron chelator and a HIF-1α inhibitor failed to show augmented ROS expression or mitochondrial membrane depolarization. Consequently, the authors concluded that HIF-1α partly cardioprotecst by directly upregulating frataxin and that this upregulation restrains ROS production and mitochondrial membrane depolarization by preventing iron accumulation.

While the study provides robust evidence that HIF-1α is cardioprotective partly via frataxin upregulation, the in vivo...
relevance of frataxin-mediated iron buffering as the downstream mechanism is unclear. First, as the authors admit, the reduction in ROS and mitochondrial membrane depolarization with frataxin overexpression might have been due to glutathione peroxidise and Nrf2 activation by frataxin (19, 20). Nrf2 is the master regulator of antioxidant defenses, and its upregulation would be expected to have a quantitatively greater impact on redox down regulation than iron sequestration. Second, it remains fundamentally unresolved whether iron accumulation during acute cellular stress is truly harmful or whether the bulk of this accumulated iron is even redox active. Because chronic and substantial iron overload, as seen in hemochromatosis and thalassemia, causes tissue oxidative damage, the prevailing wisdom has therefore held that the iron accrued during acute inflammation is also redox active and detrimental. Yet, the body responds to inflammatory oxidative stress by abruptly liberating iron from ferritin, heme and Fe-S proteins into the cytosol and mitochondrial matrix. This is physiologically incongruous if expansion of the intracellular iron pool is detrimental in this scenario. Moreover, the proportion of this “inflammatory” iron that is redox active might serve a beneficial purpose by enhancing Nrf2 antioxidant defenses or triggering ROS-induced stabilization of ambient HIF-1α proteins (3, 12). Thus the inhibition of Fe-S biogenesis by HIF-1α that leads to mitochondrial iron accumulation during IRI might be a mechanism in which HIF-1α amplifies its own levels to maximize cardioprotection. This might explain the lack of benefit of iron chelation in patients with nonspecific inflammation such as those with an MI (2). While chelation beneficially activates HIF-1α by inducing sustained iron deficiency (which inactivates PHDs), it could also cause harm by preventing maximal HIF-1α expression and inhibiting multiple heme and Fe-S-dependent proteins. In a recent report, Martelli et al. (11) provide compelling data suggesting that mitochondrial iron accumulation after frataxin deficiency is an adaptation to diminished heme levels which acts to maintain some degree of residual heme and Fe-S biosynthesis. Interestingly, despite mitochondrial iron loading, no signs of protein or lipid oxidation were observed, indicating that the increase in iron content did not trigger oxidative damage.

Clinically, the work by Nanayakkara et al. (13) validates currently evolving cardioprotective paradigms targeted at augmenting HIF-1α such as ischemic pre- and postconditioning and the use of PHDs inhibitors. More importantly, the study suggests that bypassing HIF-1α and directly augmenting frataxin expression might in itself limit IRI. An obvious experimental step before this, however, would be to show that cardiac-specific frataxin KO animals with intact HIF-1α function sustain exaggerated myocardial damage after IRI. It is also crucial that in vivo data is provided, confirming that iron sequestration is a fundamental mechanism underlying HIF-1α-frataxin cardioprotection and not just a bystanding effect. Moreover, the existence of a HIF-1α-frataxin axis in human hearts after an MI needs to be shown. More general questions that are of fundamental biological importance include what the definitive role of frataxin in health and disease is, and whether the moderate accumulation of iron and ROS seen during acute cellular stress is helpful or harmful. Resolution of these issues might ultimately lead to novel therapeutics that yield greater myocardial salvage after an MI.

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