The superoxide dismutases (SODs), which catalyze the dismutation of 2 superoxide anions to hydrogen peroxide and oxygen, represent major antioxidant mechanisms in single-cell organisms, plants, bacteria and eukaryotes. In mammalian cells, there are 3 forms of SOD that include the mitochondrial manganese SOD (MnSOD or SOD2), the cytoplasmic SOD that is a copper/zinc-containing enzyme (Cu/Zn SOD or SOD1), and an extracellular SOD that is also a copper/zinc-containing enzyme (ecSOD or SOD3). The ecSOD is unique in that it is actively secreted via the trans-Golgi network and binds to glycosaminoglycans in the extracellular matrix. In most tissues, the amount of ecSOD is very small, on the order of 1% to 5% of the total SOD. In contrast, blood vessels, the lung, and to a lesser extent, the heart contain substantial amounts of this enzyme. The ecSOD is therefore of particular interest to investigators interested in cardiovascular and pulmonary biology. Overexpression of ecSOD protects mice against lung damage, and mice lacking ecSOD are predisposed to lung injury caused by hyperoxia. Between 30% and 50% of the total SOD in blood vessels is in this extracellular form, and mice lacking this enzyme have vascular dysfunction and are predisposed to hypertension.

In cardiovascular tissues and, likely the lung, an important role of the SODs is to protect NO against oxidative inactivation by superoxide. Both NO and superoxide are free radicals with unpaired electrons in their outer orbitals and react with one another in a diffusion-limited fashion. Studies in which the Cu/ZnSODs (SOD1 and SOD3) have been pharmacologically inhibited have shown that NO cannot be released from the endothelium without being oxidatively degraded. Thus, these enzymes play a role in promoting vasodilatation and sustaining the protective roles of NO in the vascular wall. In the case of the ecSOD, its extracellular location allows it to act as a shepherd guiding NO on its way from one cell to another (Figure). Of note, and to be discussed later, the product of the reaction of superoxide and NO is the strong oxidant peroxynitrite. As evident from the Western blots in panel A, the protein levels of the intracellular SOD1 in ecSOD−/− mice subjected to TAC as detected by Western analysis. Nitrotyrosines were once thought to be a footprint of peroxynitrite’s reaction with tyrosines in various proteins; however, it is now clear that they can also be formed via a reaction of hydrogen peroxide with peroxidases and nitrite, leading to formation of higher oxides of nitrogen that react with protein tyrosines. In the setting of ecSOD deficiency, however, the most likely reason for nitrotyrosine formation is almost certainly increased peroxynitrite formation in the extracellular space. The half-life of peroxynitrite and its diffusion distance is substantially greater than that of superoxide, and unlike superoxide, it can cross cell membranes. It is therefore very likely that when formed in the extracellular space of hearts in ecSOD−/− mice after TAC, it entered myocytes and vascular cells where it proceeded to create havoc. Thus, the ecSOD, although outside the cell, protects it from intracellular onslaught by peroxynitrite.

A second major hint from Lu et al’s article is shown in their Figure 3. As evident from the Western blots in panel A, protein levels of the intracellular SOD1 in ecSOD−/− mouse hearts were identical to those of wild-type mice both at baseline and after TAC. In contrast to this, SOD1 activity was diminished by almost half in ecSOD−/− hearts after TAC. Why did this occur? It has been well documented that peroxynitrite can react with SOD1 and reduce its activity by almost exactly as much as that observed by Lu et al. This is associated with formation of a histidyl radical, indicative of interplay with the copper center of the enzyme that is transaortic constriction (TAC) to cause left ventricular pressure overload. The responses to this challenge were strikingly different between the wild-type and ecSOD-deficient mice. The degree of hypertrophy, ventricular dilatation, and myocardial fibrosis was markedly increased in mice lacking ecSOD. TAC also caused a striking increase in collagen I and III, atrial natriuretic factor, and matrix metalloproteinases-2 and -9 in ecSOD−/− mice that was either much less or absent in wild-type mice.

These findings demonstrate that the ecSOD is essential for protecting the heart against pressure overload and implicates extracellular superoxide in the genesis of heart failure. The question is how does this occur? Superoxide is short lived and charged, such that it does not cross cell membranes in substantial quantities. How then, does extracellular superoxide increase the expression of proteins like atrial natriuretic factor, matrix metalloproteinases, or collagens? How can it change the ratio of reduced:oxidized glutathione, which are largely intracellular molecules? How can it turn on a hypertrophy program in these hearts?

The answers to these questions are speculative; however, there are a couple of important hints from the study by Lu et al. First, as shown in Figure 4 of their article, the authors found a striking increase of nitrotyrosine in ecSOD−/− mouse hearts subjected to TAC as detected by Western analysis. Nitrotyrosines were once thought to be a footprint of peroxynitrite’s reaction with tyrosines in various proteins; however, it is now clear that they can also be formed via a reaction of hydrogen peroxide with peroxidases and nitrite, leading to formation of higher oxides of nitrogen that react with protein tyrosines. In the setting of ecSOD deficiency, however, the most likely reason for nitrotyrosine formation is almost certainly increased peroxynitrite formation in the extracellular space. The half-life of peroxynitrite and its diffusion distance is substantially greater than that of superoxide, and unlike superoxide, it can cross cell membranes. It is therefore very likely that when formed in the extracellular space of hearts in ecSOD−/− mice after TAC, it entered myocytes and vascular cells where it proceeded to create havoc. Thus, the ecSOD, although outside the cell, protects it from intracellular onslaught by peroxynitrite.

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coordinated by histidines. Peroxynitrite can also inactivate MnSOD (SOD2), and in this case, it forms nitrotyrosine by reacting with tyrosine-34 of the enzyme. Reductions of SOD activity by this amount would substantially increase the levels of intracellular superoxide, which in turn could affect many intracellular events, including signaling pathways, transcription factor activation, and alteration of the cytoskeleton. These interactions are illustrated in the figure.

Interestingly, recent reports have shown that TAC causes uncoupling of both the endothelial and inducible NO synthases. This is a phenomenon in which these enzymes cannot properly transfer electrons to L-arginine and instead reduce oxygen to form superoxide. The most common cause of this is a lack of the NO synthase cofactor tetrahydrobiopterin. Relevant to the discussion above, of the biologically relevant oxidants, peroxynitrite is by far the most reactive with tetrahydrobiopterin and potently oxidizes it first to the BH$_3$ radical and ultimately to dihydrobiopterin. Dihydrobiopterin can occupy the binding site of NO synthase but does not allow NO catalysis, leading to uncoupling of the enzyme. Generally, not all NO synthase enzymes become uncoupled or the uncoupling is transient such that both NO and superoxide can be made simultaneously, leading to a situation in which the NO synthases become peroxynitrite synthases. A paradigm has arisen over the past several years whereby small amounts of radicals (so-called “kindling radicals”) can lead to formation of peroxynitrite, which oxidizes tetrahydrobiopterin, leading to endothelial NO synthase uncoupling and the formation of large amounts of “bonfire” radicals. In the myocardium, uncoupled endothelial NO synthase can lead to myocardial hypertrophy and dysfunction, and treatment with tetrahydrobiopterin can prevent these events in animals exposed to TAC.

Does the use of knockout mice lacking ecSOD have any implications for the clinical arena? Are there conditions that would lead to a loss or inactivation of ecSOD that would set up a condition such as that observed in this animal? Although ecSOD might never be completely missing, there are situations in which it might be reduced in levels or activity. For example, there is an R213G polymorphism that affects the

Figure. Role of the ecSOD in protection against intracellular oxidative damage. Shown in the top left corner is a normal-functioning capillary endothelial cell in the heart interacting with a cardiac myocyte (bottom left corner). The ecSOD resides in the interstitial space between these cells, where it converts superoxide (O$_2^-$) to hydrogen peroxide (H$_2$O$_2$). This protects NO from reaction with superoxide (O$_2^-$), leading to formation of peroxynitrite (ONOO$^-$). NO freely diffuses from the capillary to the cardiac myocyte. Cardiac myocytes also contain NO synthase. Also shown are the cytoplasmic and mitochondrial SODs (SOD1 and SOD2), which reduce intracellular levels of superoxide. Shown in the bottom right corner is a situation in which abnormal or absent ecSOD is present between a capillary endothelial cell and a cardiac myocyte. NO released from the endothelial cell is oxidized by O$_2^-$ to ONOO$^-$, which enters the myocyte and leads to NO synthase uncoupling and inactivation of SOD1, greatly increasing intracellular O$_2^-$ and promoting myocyte dysfunction.
heparin-binding region of the enzyme such that the enzyme is produced but does not adhere to the extracellular matrix. Affected individuals have very high levels of circulating ecSOD but low levels in the tissue.\textsuperscript{10} This mutation has been linked with various diseases, but larger studies are needed to definitively prove its role in human pathology. Recent studies have shown that injection of this mutant enzyme is less effective than the wild-type ecSOD in ameliorating hypertension and preventing heart failure.\textsuperscript{11,12} Vascular and cardiac levels of ecSOD are dependent on endogenous NO production and are improved by exercise. It is therefore likely that conditions associated with a loss of NO might be associated with a decrease in ecSOD. Finally, hydrogen peroxide is known to inactivate the copper-containing SODs, and it is possible that increased production of hydrogen peroxide in the extracellular space could lead to loss of ecSOD activity.

Previously, a substantial debate centered on whether cardiac hypertrophy in response to challenges like aortic occlusion or hypertension was a necessary compensatory mechanism or an untoward pathologic process.\textsuperscript{13} Studies such as that by Lu et al\textsuperscript{4} reinforce the concept that a portion of hypertrophic response is unnecessary and deleterious. Likewise, Takimoto et al\textsuperscript{7} showed that tetrahydrobiopterin treatment could prevent cardiac hypertrophy in this same model with beneficial outcomes. Taken together, these studies have begun to provide a glimpse of strategies that might be helpful in slowing the progression of pressure-induced heart failure. In addition to the administration of agents such as tetrahydrobiopterin, efforts to augment levels of ecSOD might be useful. Although it might not be possible to repeatedly administer ecSOD to humans, strategies to increase its levels, eg, by increasing endogenous production of NO, could be effective therapy. In this regard, statins have been shown to increase the expression of the endothelial NO synthase and might augment ecSOD levels. As mentioned above, exercise training also increases endogenous NO and might have a protective effect in this setting, despite the long-held notion that exercise should be avoided in humans with aortic stenosis. Taken together, Lu et al\textsuperscript{4} findings and other recent studies point to new understanding of oxidative mechanisms in heart failure and provide new ideas about how to prevent and treat this devastating disease.

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