Final Common Molecular Pathways of Aging and Cardiovascular Disease

Role of the p66Shc Protein

Francesco Cosentino, Pietro Francia, Giovanni G. Camici, Pier Giuseppe Pelicci, Massimo Volpe, Thomas F. Lüscher

Abstract—Oxidative stress affects the availability of key-regulators of vascular homeostasis and controls a number of signaling pathways relevant to myocardial and vascular disease. Reactive oxygen species are generated by different intracellular molecular pathways principally located in mitochondria. The notion that mice carrying a targeted mutation of the p66Shc gene display prolonged lifespan, reduced production of intracellular oxidants, and increased resistance to oxidative stress–induced apoptosis prompted a series of studies aimed at defining the biochemical function of p66Shc and its possible implication in cardiovascular diseases. Indeed, p66Shc−/− mice are protected against vascular, cardiac, and renal impairment attributable to hypercholesterolemia, aging, diabetes, and ischemia/reperfusion. The present review focuses on the biochemical and physiological function of the p66Shc adaptor protein as well as on the mechanisms linking p66Shc-associated generation of free radicals to the pathophysiology of aging and cardiovascular disease. On the whole, the evidence so far reported and here discussed supports the concept that pharmacological modulation of p66Shc expression and activity may be a novel and effective target for the treatment of atherosclerotic vascular disease as well as myocardial adaptation to hypertrophic, inflammatory and neuro-hormonal stimuli in the overloaded heart. (Arterioscler Thromb Vasc Biol. 2008;28:622-628)

Key Words: p66Shc ■ aging ■ atherosclerosis ■ diabetes ■ energy metabolism

The mammalian Shc locus encodes for 3 different adaptor proteins with relative molecular masses of 46, 52, and 66 kDa, respectively, and identical modular structure. The 3 isoforms share an Src-homology 2 domain, a collagen-homology region, and a phosphotyrosine-binding domain (SH2-CH1-PTB). However, whereas p52Shc and p46Shc are involved in the transmission of mitogenic signals from tyrosine kinases to RAS proteins, the splice variant p66Shc, which contains a unique N-terminal region (CH2), functions as a redox enzyme implicated in mitochondrial reactive oxygen species (ROS) generation and translation of oxidative signals into apoptosis.1 In 1999, it was first noted2 that mice carrying a targeted mutation of the p66Shc gene display prolonged lifespan, reduced production of intracellular oxidants, and increased resistance to oxidative stress–induced apoptosis. This prompted a series of studies aimed at defining the biochemical and (patho)physiological role of p66Shc and possible implications of this newly defined pathway in cardiovascular disease in which ROS constitute a substantial triggering component. Indeed, because age is the most important risk factor, the identification of a novel mediator of age-associated changes of cardiovascular function could provide unforeseen strategies to fight myocardial infarction, heart failure, and stroke. The present review focuses on the biochemical function of p66Shc adaptor protein as well as on the mechanisms linking p66Shc redox system to the pathophysiology of major cardiovascular risk factors.

Biochemistry of the p66Shc Pathway: From ROS to Cellular Apoptosis

In any reaction involving a transfer of electrons, there is a change in the oxidation numbers of the atoms molecules involved. In general, a gain of electrons is termed reduction and a loss of electrons is termed oxidation. This process is termed oxidation/reduction or redox reaction. In a redox reaction the molecule undergoing reduction (and hence causing oxidation) is called oxidant, whereas the molecule undergoing oxidation (and hence causing reduction) is called reductant. The oxidant removes electrons from another molecule, and it is thus reduced. Because it accepts electrons it is also called an electron acceptor. Reactive oxygen species (ROS) are small molecules highly reactive because of the presence of unpaired electrons. They
are generated within the cell as by-products of several metabolic and enzymatic pathways. However, the majority of cellular ROS are generated within the mitochondrial oxidative phosphorylation, a process in which electrons are extracted from NADH and FADH and transferred to molecular oxygen through a chain of 4 enzymatic complexes ensuring phosphorylation of ADP in ATP and final reduction of molecular oxygen to water (Figure 1). Indeed, electrons derived from NADH or FADH can directly react with oxygen or other electron acceptors within the mitochondrial electron transport chain upstream the last enzymatic complex (complex IV, which is responsible for the reduction of molecular oxygen to water) and generate free radicals.3,4 At present, a body of evidence supports the concept that intracellular free oxidative stress are diminished in cells), and that systemic as well as intracellular markers of oxygen to water) and generate free radicals.3,4 At present, a body of evidence supports the concept that intracellular free oxidative stress are diminished in cells lacking the p66Shc gene (p66Shc−/− cells), and that systemic as well as intracellular markers of oxidative stress are diminished in p66Shc−/− mouse models exposed to high oxidative stress.2,5-7 Moreover, the reduced production of ROS provided by silencing the p66Shc gene is associated with resistance to apoptosis induced by a variety of different mediators, including hydrogen peroxide (H2O2), growth factor deprivation, ultraviolet radiation, and calcium ionophore.2,8,9 Because mitochondria play a key role in both ROS production and apoptosis, the p66Shc protein might be part of a complex mitochondrial system regulating the endogenous production of free radicals as well as the apoptotic program. As recently reported,10 a proportion of p66Shc is consistently present within mitochondria of mouse embryonic fibroblasts (MEFs) and participates in mitochondrial metabolism. Of note, oxygen consumption of immortalized p66Shc−/− MEFs is 30% to 50% lower compared with wild-type cells under unstimulated conditions, and shows only a modest rise under chemically induced uncoupling conditions.9 Given the fact that mitochondrial flow is the major source of ROS, the reduction of mitochondrial oxidative phosphorylation in the absence of p66Shc results in less oxygen consumption and lower levels of ROS generation.

Recent findings support a mechanistic model in which the p66Shc protein, which localizes within the mitochondrial intermembrane space, oxidizes cytochrome c rendering it unavailable to reduce oxygen to water. A fraction of the mitochondrial electron flow is therefore deviated to the production of H2O2, which induces opening of the high-conductance channel mitochondrial permeability transition pore (PTP). The resulting increase of mitochondrial membrane permeability to ions, solutes, and water promotes swelling and then disruption of the organelle, with consequent release of proapoptotic factors, such as cytochrome c, into the cytosol (Figure 1). This study demonstrated that p66Shc is a redox enzyme that generates mitochondrial H2O2 as a signaling molecule for apoptosis. In mitochondria isolated from mouse liver it was shown that p66Shc stimulates H2O2 production without affecting production of O2.−. Indeed, the oxidation of selective fluorescent probes for H2O2 and O2.− in WT and p66Shc−/− MEFs and purified mitochondria revealed decreased levels of H2O2 but not of O2.− in the p66Shc−/− samples.11

In keeping with these findings, gene delivery of Tim44, a component of the high-molecular weight complex that inactivates p66Shc within the mitochondria, was found to normalize increased ROS generation and proliferation of vascular smooth muscle cells exposed to high glucose and to improve inflammatory response and neointimal proliferation in balloon-injured carotid arteries of diabetic rats.12 Although this model gives an explanation for the redox and proapoptotic properties of the p66Shc protein, the signaling link between both endogenous and exogenous oxidative stress and p66Shc activation remained unclear. It was demonstrated only very recently13 that free radicals activate protein kinase C-β isoform to induce Ser16 phosphorylation of the p66Shc, allowing transfer of the protein from the cytosol to mitochondria.
via recognition and binding to prolyl isomerase Pin1. After such mitochondrial internalization, p66Shc causes blunting of Ca\(^{2+}\) responses and fragmentation of the 3-dimensional mitochondrial network, thus inducing ROS generation and apoptosis. In agreement with these findings, p66Shc mice are resistant to apoptosis induced by paraquat, hypercholesterolemia, and ischemia\(^2,5,14\) (Figure 2).

**P66\(^{Shc}\) and the Biology of Vascular Aging**

Aging vessels exhibit an increased production of reactive oxygen species and in turn undergo functional impairment as a result of loss of nitric oxide (NO) bioavailability.\(^15-19\) Indeed, while under physiological conditions the production of NO is not substantially affected by superoxide anion (O\(^2-\)), an excessive generation of O\(^2-\) rapidly inactivates NO leading to the formation of peroxynitrite (ONOO\(^-\)), a powerful oxidant.\(^20,21\) ONOO\(^-\) is able to penetrate across cellular membranes and inactivate by substrate nitration a number of regulatory receptors and enzymes, including free radical scavengers.\(^22,23\) The identification of molecular pathways modulating the endothelial cell redox state is therefore relevant to our understanding of mechanisms linking endothelial dysfunction, atherosclerosis, coronary plaque, and thrombus formation. In view of its originally reported role in determining the redox state of the cells and their responses to free radicals,\(^2\) p66 Shc mice have been regarded as part of a putative transduction pathway relevant to endothelial integrity. This hypothesis was further strengthened by the observation that p66\(^{Shc-/-}\) mice have an approximately 30% increase in life span compared with wild-type littermates.\(^2\) Accordingly, p66\(^{Shc-/-}\) mice are protected against age-dependent endothelial dysfunction.\(^6\) Indeed, wild-type mice display age-associated blunting of endothelium-dependent relaxation to acetylcholine, whereas p66\(^{Shc-/-}\) mice did not (Figure 2). Compared with age-matched wild-type mice, old p66\(^{Shc-/-}\) mice show increased endothelial bioavailability of NO, lower aortic O\(^2-\) levels and reduced aortic 3-nitrotyrosine content in the absence of any difference in the expression of Mn SOD and Cu/Zn SOD.\(^6\) Of interest, the expression of the inducible form of NO synthase (iNOS) increased significantly in the old WT mice, whereas no age-dependent changes were found in the p66\(^{Shc-/-}\) mice.\(^6\) This suggests a potential mechanism by which NO availability and vasorelaxant responses are preserved in aged p66\(^{Shc-/-}\) mice. Indeed, age-dependent upregulation of iNOS is involved in ONOO\(^-\) formation and, hence, may lead to increased oxidative vascular damage.\(^16,24\) Based on these findings, it is tempting to conclude that prevention of endothelial dysfunction and hence protection against aging-associated vascular diseases might contribute to the extended lifespan of p66\(^{Shc-/-}\) mice.

Accumulation of advanced glycation end-products (AGEs) has been associated with aging, diabetes, and other age-related disorders. Interestingly enough, tissue levels of p66\(^{Shc}\) were significantly lower in old mice exposed to a life-long low glycotoxin, AGE-restricted diet compared with mice exposed to high levels of prooxidant AGEs in normal diet.\(^25\) Lower p66\(^{Shc}\) levels were associated with reduced oxidative stress, less severe metabolic and kidney changes, and a longer lifespan.\(^25\)

The involvement of p66\(^{Shc}\) in aging was recently investigated in humans.\(^26\) p66\(^{Shc}\) protein and messenger RNA expression has been assessed in dermal fibroblast from young people, elderly, and centenarians. Under basal conditions, p66\(^{Shc}\) expression increased in an age-dependent manner, suggesting that p66\(^{Shc}\) expression increases in an age-dependent manner.\(^26\) Treatment of dermal fibroblasts with the prooxidant 2-deoxy-D-ribose (dRib) strongly induced p66\(^{Shc}\) expression in all age groups. Conversely, hypoxia caused p66\(^{Shc}\) downregulation only in fibroblasts from centenarians. Such a selective response deserves further clarification.\(^26\)

Similar Shc genomic organization and Shc transcript assembly exist in mice and in humans. Alignment of the predicted translation of the mouse p66\(^{Shc}\) sequence, as derived from analysis of p66 cDNAs with human p66\(^{Shc}\), showed a high degree of amino acid identity and identical overall organiza-
tion of the two proteins. However, the higher p66Shc expression in centenarians contrasts with the reported increase in life span observed in p66Shc−/− mice. To clarify this controversial issue, it would be necessary to determine the existence in humans of a p66Shc-dependent adaptive response to age-dependent cellular damage in cardiac and vascular tissue.

**Oxidative Stress Pathways in Atherosclerosis Involve p66Shc**

Oxidative stress is central to the pathogenesis of atherosclerosis. Reactive oxygen and nitrogen species produced from endothelial as well as vascular smooth muscle cells and infiltrating macrophages promote oxidative modification of low-density lipoproteins (LDLs), a key step preceding their transfer into the subendothelial space of the arterial wall where they initiate atherosclerosis.28,29 Also, scavenging of NO induced by free radicals leads to endothelial dysfunction, promotes smooth muscle cell proliferation, leukocyte adhesion, and inflammation. Together these processes contribute to vascular remodeling and plaque formation. Moreover, oxidized LDLs promote apoptosis of the cellular constituents of the atherosclerotic plaque through a mitochondrial-dependent pathway which involves opening of the PTP and cytosolic release of cytochrome c and other apoptogenic proteins.30,31 Such high rate of programmed cell death is a critical step for plaque erosion and rupture.32,33

The original concept that p66Shc might be at the crossroad of ROS production prompted one of the earliest investigations on its role in atherosclerosis.3 Systemic and tissue levels of oxidative stress, as well as the development of early vascular lesions, were investigated in wild-type and p66Shc−/− mice chronically fed a normocholesterolemic diet or a 21% high-fat diet containing 0.15% cholesterol and 19.5% casein. Despite a comparable lipid profile both under low fat conditions as well as after high-fat diet in both strains, wild-type animals displayed increased early aortic lesion formation, whereas p66Shc−/− were protected. Furthermore, p66Shc−/− mice also exhibited a marked decrease in the accumulation of intimal macrophage-derived foam cells, arterial oxidation-specific epitopes of oxidized LDLs (tissue oxidative stress), reduced plasma isoprostanes (systemic oxidative stress), and diminished susceptibility of LDLs to ex vivo oxidation, as assessed by TBARS. Of relevance, poor predisposition to atherogenesis and reduced oxidative stress were coupled with reduced apoptosis in aortic lesions (Figure 2).

A mutual relationship between lipids, oxidative stress, and p66Shc is also suggested by a recent study evaluating p66Shc mRNA in peripheral white blood cells (WBCs) and subcutaneous adipose specimens of patients with high and low LDL plasma levels.34 In this study, WBC and adipose tissue p66Shc mRNA levels were significantly higher in high as compared with low LDL patients. Moreover, in a multiple regression analysis among a number of serological variables, LDL plasma levels were the only variable affecting p66Shc mRNA expression.34

**P66Shc as a Common Pathway in Diabetes-Related Cardiovascular Disease**

Diabetes is estimated to affect more than 150 million people worldwide, with an expected doubling number in the next 25 years, reaching 5.4% of the total adult population.35 In the United States 17 million people are diabetics, 95% of whom have type 2 diabetes. Among these, 5 to 6 million are unaware of their condition and do not receive treatment.36,37 An additional 35 million—20% of all people in the middle-adult years and 35% of the entire old population—have some degree of abnormal glucose tolerance and show signs of insulin resistance; this higher-risk group will account for a significant proportion of cardiovascular disease and premature mortality in the coming years. The increasing frequency of obesity and sedentary life-styles, major underlying risk factors for type 2 diabetes in both developed and developing countries, portends that diabetes will continue to be a growing worldwide entity. Hyperglycemia plays a central role in causing diabetic vascular complications. Among the full spectrum of biochemical effects of high glucose, generation of ROS has been advocated as one of the main pathophysiological mechanisms linking glucose metabolism to endothelial dysfunction and atherosclerosis.38,39 Indeed, high glucose induces a series of cellular events that increase the production of free radicals, which scavenge NO to form peroxynitrite hence decreasing NO bioavailability.40,41

As discussed, p66Shc oxidizes cytochrome C and generates proapoptotic ROS in response to stress signals through a PKC-β dependent pathway.7,10 Indeed, mitochondrial O2− production has been recognized as a crucial mediator of hyperglycemic vascular damage.42 In line with these notions, p66Shc mRNA expression is increased in peripheral blood monocytes from patients with diabetes mellitus and correlates with plasma isoprostanes, an established in vivo marker of oxidative stress.43 The putative role of p66Shc in hyperglycemia-induced ROS-mediated cardiovascular complications has been further investigated by 3 independent studies focusing on diabetic glomerulopathy,44 endothelial dysfunction,7 and cardiomyopathy.45 The former study, which addressed the question whether p66Shc−/− mice are protected against diabetic glomerulopathy, a leading cause of chronic renal failure, showed that changes in renal function and structure were significantly less pronounced or virtually absent in diabetic p66Shc−/− mice compared with diabetic wild-type controls.44 Indeed, p66Shc−/− mice did not show high glucose-induced increase in glomerular cell apoptosis, nor increase in extracellular matrix deposition, thus supporting the concept of a major role of p66Shc in mediating in vivo stress-induced apoptosis (Figure 2).

The role of p66Shc in mediating hyperglycemia-induced NO-dependent endothelial dysfunction has been recently investigated7 in a model of streptozotocin-induced type 1 diabetes. Unlike diabetic wild-type mice, p66Shc−/− diabetic mice did not develop impairment of acetylcholine-induced vasorelaxation by virtue of an unaltered NO bioavailability.7 In this setting, p66Shc−/− diabetic mice showed an enhanced antioxidant defense and lower ROS generation, which accounted for the preserved NO bioavailability (Figure 2). Indeed, whereas expression of MnSOD and Cu/ZnSOD was comparable in control and diabetic wild-type and p66Shc−/− mice, HO-1 expression and activity was significantly upregulated in control and diabetic p66Shc−/− mice. As already emerged,46 this result further underlines the significance of...
HO-1—a potent antioxidant enzyme which may exert a protective effect in hyperglycemic conditions. Of note, the expression of p66Shc protein was increased in aortas from wild-type diabetic mice as compared with normoglycemic controls, thus underlining a causal relationship between high glucose and p66Shc.

Interestingly enough, treatment of human aortic endothelial cells exposed to elevated glucose with PKC β inhibitor LY379196 blunted glucose-induced p66Shc up-regulation (G.G. Camici and F. Cosentino, unpublished data, 2007). These findings suggest that p66Shc acts as a downstream target after glucose-induced PKC β activation. Thus, a unifying PKC β/p66Shc-dependent mechanism may contribute to endothelial dysfunction and oxidative stress under hyperglycaemic conditions.

Diabetes mellitus is known to promote a specific form of cardiomyopathy which is unrelated to coronary artery disease. Data from both diabetic patients and streptozotocin-induced diabetic mice suggest that loss of myocyte viability largely occurs as a consequence of oxidative stress–triggered cell apoptosis. Thus, oxidative stress rather than hyperglycaemia per se may account for subcellular remodeling, cardiomyocyte apoptosis, and in turn the development of cardiomyopathy. Accordingly, a significant increase in 3-nitrotyrosine containing proteins, typical end products of the reaction between peroxynitrite and biological compounds, has been reported in cardiomyocytes from diabetic patients and streptozotocin-induced diabetic animals. In a model of type 1 diabetes cardiomyopathy, ROS promote premature myocyte senescence and death as well as loss of cardiac progenitor cell (CPC) viability leading to an impairment of cardiac and vascular cell turnover. In this setting, ablation of the p66Shc gene almost completely prevents oxidative damage in CPCs and myocytes. Indeed, ROS-mediated cytoplasmic and DNA damage, assessed by nitrotyrosine and 8-OH-deoxyguanosine labeling, was found only in left ventricular CPCs and myocytes isolated from WT mice 28 days after streptozotocin injection. Cellular response to ROS is known to be dose-dependent; low, intermediate, and high free radical levels elicit cell growth, apoptosis, and necrosis, respectively. Accordingly, CPC replication was found to predominate in diabetic p66Shc−/− mice, whereas CPC and myocyte apoptosis or necrosis prevailed in diabetic wild-type animals (Figure 2). Hence, the expansion of CPCs and developing myocytes may explain the preservation of cardiac geometry as well as diastolic and systolic function in diabetic p66Shc−/− mice. These animals, unlike their diabetic wild-type littermates, displayed unaltered wall thickness, chamber volume, left ventricular end-diastolic pressure, systolic pressure, and diastolic wall stress 28 days after induction of diabetes.

### P66Shc-Dependent Pathways in Myocardial Hypertrophy and Remodeling

As many other signaling molecules regulating growth and apoptosis, p66Shc is prominent in neonatal cardiomyocytes and downregulated during growth and maturation of the heart. Many signaling proteins downregulated with development are reexpressed during cardiac hypertrophy. Therefore, it was recently investigated whether hypertrophic stimuli may induce p66Shc expression in cardiomyocytes, Pasteurella multocida toxin (PMT), a direct activator of the endogenous free monomeric Goq subunit, which promotes hypertrophy in cardiomyocytes through a PKC- and extracellular signal regulated kinase (ERK)-dependent pathway, induced a significant increase in p66Shc expression, ERK activation, and p66Shc-Ser36 phosphorylation in cardiomyocyte cultures. Interestingly enough, MEK inhibitor U0126 and PKC inhibitor GF109203X abolished the PMT-dependent increase in p66Shc expression. These findings indicate that in cardiomyocytes p66Shc is regulated through the MEK-ERK pathway. Noteworthy, thrombin activates signaling pathways relevant to cardiomyocyte growth and extracellular matrix remodeling via protease-activated receptor-1 (PAR-1), and in turn triggers MEK-dependent p66Shc-Ser36 phosphorylation in cardiomyocytes. The emerging concept of a direct link between the activation of PAR-1 and p66Shc-Ser36 phosphorylation sheds some light into putative mechanisms underlying the transition from cardiomyocyte hypertrophy to apoptosis and myocardial failure.

A role of p66Shc as a mediator of cardiomyocyte hypertrophy and apoptosis is further supported by in vivo evidence using continuous infusion of suppressor doses of Angiotensin II (Ang II) in mice. Ang II causes left ventricular hypertrophy and cardiomyocyte apoptosis in wild-type but not in p66Shc−/− mice. Cardiomyocyte cultures obtained from p66Shc−/− mice exhibit a higher number of cycling cells under unstimulated conditions compared with cells from wild-type littersmates. Ang II increased the number of cycling myocytes in cultures obtained from both p66Shc−/− and WT mice. However, in cells derived from wild-type mice apoptosis exceeded proliferation (Figure 2). Beyond its well-known vasomotor, proliferative, and profibrotic effects, Ang II induces free radical production through AT1 receptor. As CPC replication predominates in diabetic p66Shc−/− mice exposed to high glucose attributable to the blunted generation of free radicals, it is tempting to speculate that lack of p66Shc protein downregulates Ang II–induced ROS generation in cardiomyocytes thereby favoring cardiomyocyte proliferation over apoptosis.

All these findings are in keeping with previous observation that oxidative stress and p66Shc may be critical for activation of apoptosis in the overloaded heart. Indeed, in a dog model of pacing-induced left ventricular dysfunction, cardiomyocyte expression of p66Shc increased with pacing duration and was associated with nitrotyrosine formation, apoptosis, and ventricular failure.

### Perspectives

Mitochondria have long been known to play a critical role in maintaining the bioenergetic status of cells under physiological conditions. However, it is also recognized that reduction of oxygen to generate ROS occurs at various sites in the mitochondrial respiratory chain. The loss of control of free radical formation from the mitochondrion affects the availability of key regulators of vascular homeostasis triggering a number of redox signals relevant to vascular and myocardial remodeling in response to hypertrophic and proapoptotic stimuli. In evolution, ROS pathways have developed for energy metabolism and host defense. However, in today’s
environment (eg, fewer infections, high caloric intake), ROS appear detrimentally involved in aging and age-associated cardiovascular diseases. Thus, cellular mechanisms crucial for survival during ancient times may contribute to degenerative processes in long-living organisms today. Mitochondria and redox-sensitive molecular pathways are therefore under intensive investigation as the "bottle neck" of the pathophysiology of several cardiovascular risk factors. In this view, the concept that p66Shc regulates ROS production and determines cell susceptibility to apoptosis rises the question whether pharmacological modulation of its expression and activity may be effective in fighting atherosclerotic vascular disease as well as myocardial adaptation to hypertrophic, inflammatory, and neuro-hormonal stimuli in the overloaded heart.

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Disclosures

None.

References


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In the article “Final Common Molecular Pathways of Aging and Cardiovascular Disease: Role of the p66\textsuperscript{Shc} Protein” by Consentino et al, which appeared in the April 2008 issue of the journal (Arterioscler Thromb Vasc Biol. 2008;28:622–628), an author’s name was inadvertently left out of the manuscript.

Massimo Volpe should be added to the list of authors.

The publisher regrets this error.