Basic Research in Cardiology (2019) 114:29
https://doi.org/10.1007/s00395-019-0738-x

INVITED REVIEW

P66shc and its role in ischemic cardiovascular diseases

Kerstin Boengler[1] · Julia Bornbaum[1] · Klaus-Dieter Schlüter[1] · Rainer Schulz[1]

Received: 30 April 2019 / Accepted: 29 May 2019 / Published online: 4 June 2019

Abstract
Oxidative stress caused by an imbalance in the formation and removal of reactive oxygen species (ROS) plays an important role in the development of several cardiovascular diseases. ROS originate from various cellular origins; however, the highest amount of ROS is produced by mitochondria. One of the proteins contributing to mitochondrial ROS formation is the adaptor protein p66shc, which upon cellular stresses translocates from the cytosol to the mitochondria. In the present review, we focus on the role of p66shc in longevity, in the development of cardiovascular diseases including diabetes, atherosclerosis and its risk factors, myocardial ischemia/reperfusion injury and the protection from it by ischemic preconditioning. Also, the contribution of p66shc towards cerebral pathologies and the potential of the protein as a therapeutic target for the treatment of the aforementioned diseases are discussed.

Keywords P66shc · Longevity · Diabetes · Atherosclerosis · Ischemia/reperfusion · Preconditioning · Mitochondria · Stroke

The p66shc protein

P66shc is a member of the spontaneous human combustion (shc) family. The human shcA locus not only encodes p66shc but also the isoforms p46shc and p52shc, which derive from the same transcript via usage of two distinct ATG start codons. The three isoforms share functional domains, namely the phosphotyrosine binding (PTB) domain, the adjacent proline- and glycine-rich collagen–homology (CH1) domain, as well as the carboxyterminal src-homology (SH2) domain. P66shc differs from p46shc and p52shc in the way that it contains an additional aminoterminal collagen–homology domain (CH2) consisting of 110 amino acids [69]. The shc proteins are adaptor proteins lacking intrinsic enzymatic activity and they function in the transduction of cellular signals [1]. Here, phosphorylated conserved tyrosine residues in the CH1 domain (tyrosine 239–240 and tyrosine 317) represent binding sites for other proteins. The proline- and glycin-rich stretches in the CH1 and CH2 domains mediate the interaction with SH3 domain-containing proteins. Serine (S) 36, which represents an important phosphorylation site within p66shc, is present in the p66shc-specific CH2 domain. At the carboxyterminal part of the CH2 domain, a region controlling the interaction with cytochrome *c* is localized [40]. Based on the differential structure of p46shc, p52shc and p66shc, the isoforms exert different functions: whereas p46shc and p52shc are involved in cell survival and the progression of the cell cycle [105], p66shc plays a role in apoptosis and contributes to oxidative stress [68]. In the present review, we will focus on the expression and function of p66shc.

P66shc: localization, phosphorylation and mitochondrial translocation

The intracellular localization of p66shc has first been analyzed in mouse embryonic fibroblasts (MEFs). Here, a predominantly cytosolic localization of the protein is demonstrated with fractions of p66shc present in the endoplasmic reticulum as well as in the mitochondria, even under basal conditions [80]. Cardiomyocytes contain different subpopulations of mitochondria, which differ in form and function, namely the subsarcolemmal and the interfibrillar mitochondria, [83, 84]. The quantification of p66shc in these subtypes of mitochondria reveals equal amounts of p66shc in the two subgroups [55]. The isolation of interfibrillar mitochondria involves the use of proteases, which may hamper the exact quantification of proteins in this mitochondrial subtype;

✉ Rainer Schulz
Rainer.schulz@physiologie.med.uni-giessen.de

[1] Institut für Physiologie, Justus-Liebig Universität Gießen, Aulweg 129, 35392 Giessen, Germany

however, in case of p66shc the protease treatment for mitochondrial isolation does not interfere with the amount of the protein in IFM [55]. The analysis of the exact submitochondrial localization of p66shc shows that in liver mitochondria the protein is present in the fraction enriched with inner membrane proteins [80]. A more detailed analysis of subfractionated MEF mitochondria indicates that the highest amount of p66shc immunoreactivity is found within the intermembrane space [40]. Mitochondrial p66shc is part of a high molecular weight complex (approximately 670 kDa), which, among other proteins, contains mitochondrial heat shock protein 70 (mtHSP70) with which p66shc directly interacts [80].

The translocation of p66shc from the cytosol to the mitochondria is dependent on the phosphorylation status of the protein. Treatment with a protein kinase C (PKC) activator induces a phosphorylation of p66shc at S36 [87]. The use of the PKCβ isoform-specific inhibitor hispidin reduces such phosphorylation, thereby demonstrating the importance of this isoform for p66shc phosphorylation [87]. This initial observation in MEFs is confirmed in other cell types such as airway epithelial cells [113] or endothelial cells [59]. However, another study excludes S36 as a residue phosphorylated by PKCβ in MEFs; instead, S139 and S213 are proposed to function as PKCβ-targeted amino acids [44]. It appears that S36 within p66shc can also be phosphorylated by JNK1/2 (c-Jun N-terminal kinases 1 and 2) [49], an observation confirmed in endothelial cells [81]. Also, S54 and threonine (T) 386 within p66shc become phosphorylated, presumably by p38 mitogen-activated protein kinase [50]. In addition to PKCβ, also PKCδ contributes to p66shc phosphorylation [113]. Hydrogen sulfide—which exerts multiple functions in the cardiovascular system [6]—inhibits mitochondrial ROS production via the sulfhydration of the cysteine 59 residue at p66shc, which in turn prevents the phosphorylation of p66shc [66, 109] (for review, also see Ref. [108]).

In endothelial cells, increased expression of NADPH oxidase (NOX) 4, potentially through increased canonical Wnt signaling [103], increases mitochondrial ROS; this increase in ROS formation is prevented by silencing of NOX2 [52]. Mechanistically, NOX2-induced hydrogen peroxide increases S36 phosphorylation of p66shc, which is inhibited by silencing NOX2. Moreover, NOX2 or NOX4 knockdown or overexpression of S36 phosphorylation-defective mutant of p66shc inhibits mitochondrial ROS formation; thus, NOX4, NOX2 and p66shc might contribute to the ROS-induced ROS release [52, 100]. Increased microRNA (miR) 200c expression might contribute to the ROS-induced increase in S36 phosphorylation of p66shc [20].

P66shc interacts with prolyl isomerase 1 (Pin1), an enzyme isomerizing phosphorylated serine/threonine-proline bonds in substrate proteins, in a phosphorylation-dependent manner. In Pin1-deficient MEFs, the mitochondrial amount of p66shc is reduced suggesting a contribution of this protein–protein interaction for the mitochondrial import of p66shc [87]. Also, the Pin1-inhibitor juglone decreases the mitochondrial translocation of p66shc in human umbilical vein endothelial cells (HUVECs) [112] and in intestinal cells [32]. Dimeric p66shc is imported into the mitochondria possibly via a disulfide relay system such as the mitochondrial intermembrane space import and assembly protein 40 (Mia40) [38]; however, the exact mechanism and pathway by which p66shc enters the mitochondria remain to be elucidated.

Other molecular targets of p66shc like the tumor suppressor p53, the kruppel like factor-2 (KLF2) transcription factor, or the forkhead box protein O (FOXO9) have been discussed in details recently [57]. Also, oxidation of protein tyrosine phosphatases is modified by p66shc, thereby controlling growth factor-induced signaling and migration of endothelial cells [35].

The role of p66shc in affecting mitochondrial function

The inner mitochondrial membrane forms extensive folds called cristae, and cristae structure is affected by p66shc [88]. Once p66shc has entered the mitochondria, the protein affects the function of the organelle, especially ROS formation. Mitochondrial p66shc oxidizes cytochrome *c* and thereby catalyzes the reduction of oxygen to hydrogen peroxide [40]. Accordingly, reduced levels of ROS in response to different stimuli are demonstrated in different cell types/organs devoid of p66shc, including mouse embryonic and adult fibroblasts [102], endothelial cells [81, 107], and myocardial tissue [21]. Whereas the prooxidant function of mitochondrial of p66shc is well documented, a cytosolic fraction of the protein may also exert antioxidative function [71].

Elevated levels of ROS function as trigger molecules to open the mitochondrial permeability transition pore (MPTP), a large conductance pore in the inner mitochondrial membrane, which is largely closed under physiological conditions. During the last years, progress has been made regarding the identification of proteins involved in MPTP formation. Whereas it is discussed that dimers of the F0F1ATP synthase build up the MPTP [41], other data showed that permeability transition occurs upon the dissociation of ATP synthase dimers [72]. Upon dissociation of the F1 and F0 subunits, the c subunits of the F0F1ATP synthase are considered to form the lumen of the MPTP [3, 8]. Opening of the MPTP induces mitochondrial swelling with subsequent rupture of the outer mitochondrial membrane. As a consequence, the activities of the complexes of the electron transport chain protein decline and pro-apoptotic factors are released [72]. Therefore, enhanced ROS formation by

p66shc would favor MPTP opening and indeed mitochondrial swelling is observed upon the addition of recombinant p66shc to isolated mitochondria [40].

In addition, the absence of p66shc is associated with reduced mitochondrial oxygen consumption in mouse embryonic fibroblasts, whereas the reconstitution of p66shc expression in p66shc-deficient cells increases respiration [77]. However, in mitochondria isolated from brown adipose tissue mitochondrial respiration is higher in p66shc-deficient compared to wildtype mitochondria and the authors suggest that p66shc-deficient mitochondria are in an uncoupled state [11]. Also, mitochondria isolated from aged mouse brains display higher complex 1-mediated respiration upon knockout of p66shc [86].

Mitophagy serves to eliminate dysfunctional mitochondria; in B cells, p66shc induces depolarization of the mitochondrial membrane potential and ubiquitination of outer mitochondrial membrane proteins inducing mitophagy. In addition, p66shc serves as a receptor for microtubule-associated protein 1A/1B-light chain 3 (LC3-II) and thereby contributes to the initiation of mitophagy [79].

Taken together, the expression of p66shc in a variety of cells/organs and its relevance for different aspects of mitochondrial function (including respiration, ROS formation and mitophagy) makes p66shc an interesting target to be studied in the context of cardiovascular diseases and we will highlight the role of the protein in this context in the following sections of the review.

Influence of p66shc on cardiac function and life span

The deletion of exon2a of p66shc by Migliaccio and coworkers [68] resulted in a mouse line with a normal development and without effects on fertility. These p66shc knockout (p66shc KO) mice are comparable to wildtype mice in terms of body weight, food consumption, and histology of different organs. However, some lung abnormalities are detected, although lung function is not affected. Others report that p66shc KO mice are leaner than wildtypes [23, 101] and that the observed difference is related to a modification of the composition of gut microbiota and their response to diet [22] (for detailed review: see Ref. [24]). A more detailed analysis of cardiovascular parameters reveals no differences between wildtype and p66shc KO mice regarding systolic blood pressure, heart rate, weights of the right and left ventricles. However, an increase in the number of cardiomyocytes is observed which is associated with a reduced volume of the left ventricular chamber [42]. In our own colony of p66shc KO mice, in contrast to the previous work [42], left ventricular end-diastolic and end-systolic volumes are increased compared to wildtype mice without affecting stroke volume or cardiac output (Fig. 1). In accordance, the length of cardiomyocytes isolated from p66shc KO mice is increased compared to the length of cardiomyocytes isolated from wildtype mice, while unloaded shortening is similar between both strains (Fig. 1).

In the context of the putative role of oxidative stress in mitochondria-dependent aging, p66shc represents an interesting target and the role of p66shc in life span is addressed in several studies. In wildtype hearts, the amount of p66shc increases with aging [110]. Initially, an increase in life span was shown in p66shc KO mice compared to wildtype mice [68], and others related the extended life span due to arginase II deficiency also to downregulation of p66shc [110]. However, this first report could not be confirmed when mice were housed in a large outdoor enclosure (with increased physical activity, temperature changes and food competition); here, even a deleterious effect of the p66shc knockout was described [39]. When life span is analyzed in p66shc KO mice on different genetic backgrounds, there is no indication that p66shc may function as a longevity protein [91]. However, when wildtype and p66shc KO mice are fed a calorie-restricted diet, the survival of p66shc KO mice early on is improved but without any difference in total life span compared to wildtype mice [91].

P66shc and cardiovascular risk factors

The importance of aging on p66shc and vascular dysfunction has been reviewed extensively elsewhere [18].

Hyperglycemia

High glucose induces acetylation of p66shc at lysine 81, which in turn promotes the phosphorylation of p66shc at S36 and thereby enhances the mitochondrial import of the protein, where ROS formation is stimulated [58]. A mouse line in which lysine 81 is not acetylable is protected from hyperglycemia-induced oxidative stress [58]. Augmented p66shc phosphorylation in response to high glucose is also shown in osteoclasts [90] and HUVECs [112] as well as in ventricular cardiomyocytes [63]. Again, enhanced phosphorylation of p66shc is associated with increased mitochondrial translocation of the protein and with a concomitant increase in ROS [63, 70]. The high glucose-induced enhancement of ROS is abrogated by silencing p66shc [70].

Apart from increased phosphorylation and mitochondrial translocation of p66shc, hyperglycemia induces an increased expression of p66shc in macrophages from apolipoprotein E (ApoE)-deficient mice [95], in HUVECs [104], retinal [70] and aortic endothelial cells [85], in the rat retina [114], and also in mouse hearts [25]. The transcription of p66shc is controlled by epigenetic modifications, and in human aortic

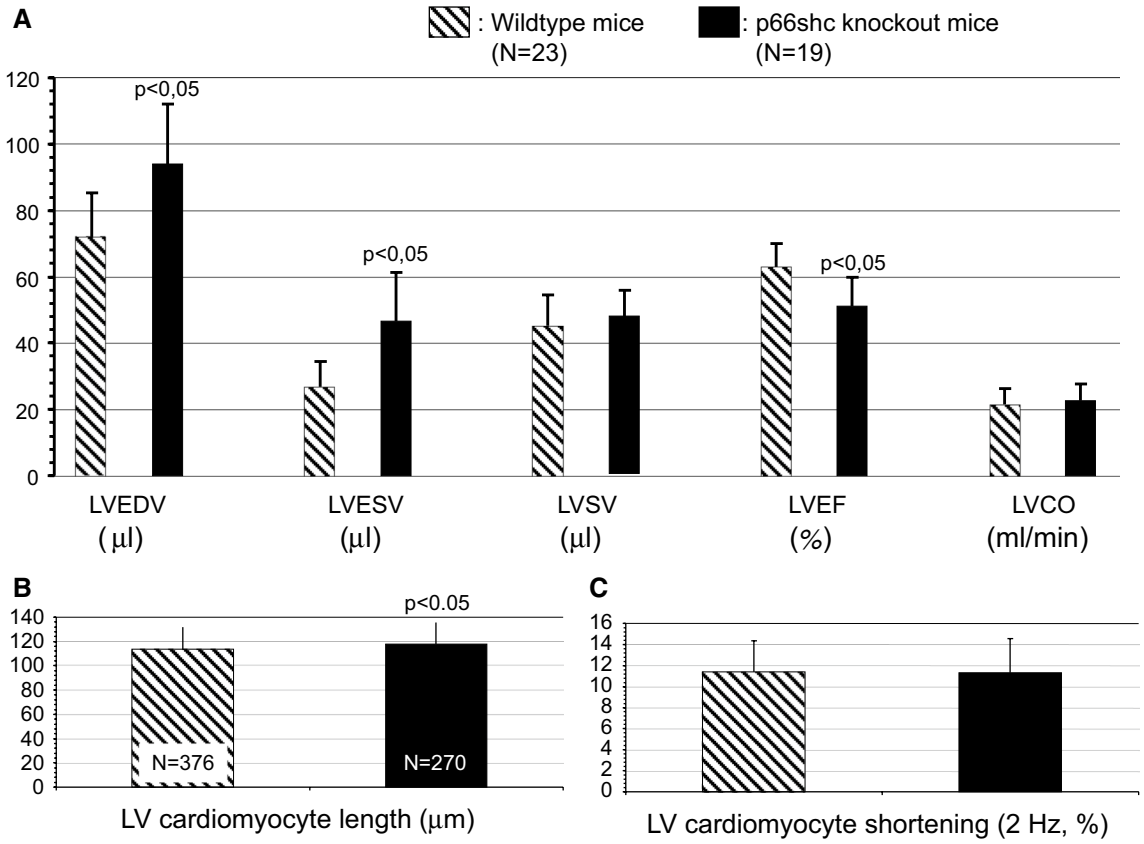


Fig. 1 **a** Echocardiographic assessment of wildtype and p66shc knockout (KO) mice at rest. Both the left ventricular (LV) end-diastolic (LVEDV) and end-systolic (LVESV) volumes were higher in p66shc KO mice than in wildtype mice, while LV stroke volume (LVSV) remained unchanged. LV ejection fraction decreased in p66shc KO mice compared to wildtype mice while cardiac output was similar between both strains of mice. **b** Cardiomyocytes isolated from p66shc KO mice hearts were somewhat longer than those isolated from wildtype mice hearts, while **c** unloaded cardiomyocyte shortening at 2 Hz pacing was identical. Statistical analysis was performed by unpaired Student's *t* test and a $p < 0.05$ was considered to indicate a significant difference between genotypes

endothelial cells high glucose causes acetylation of histone 3, which leads to an open chromatin structure and, therefore, promotes transcription and increases expression of the p66shc protein [85].

Diabetes

The overall role of ROS in the development of diabetes is reviewed in detail elsewhere [37, 78].

Sirtuin 1 (Sirt1, a member of the class III NAD^+-dependent histone deacetylases) is downregulated in diabetic mouse hearts, involving micro RNAs such as miR-34a [61]—and this is accompanied by reduced Sirt1-dependent deacetylation of the p66shc promoter [25], potentially leading to increased p66shc transcription (for review, see Ref. [56]). The tRNA methyltransferase NSUN2 can subsequently methylate SHC mRNA, thereby enhancing the translation of p66shc and subsequently the levels of ROS—for example—during hyperglycemia [17].

Sirt1 not only impacts on the p66shc promoter region but also directly acts on lysine 81 within p66shc [58], which might promote p66shc phosphorylation (see above). Indeed, diabetes (induced by streptozotocin) is associated with increased phosphorylation of p66shc in mouse hearts [25], and it is also detected in rat hearts [115], where it is accompanied by enhanced amounts of phosphorylated PKCβII. The mitochondrial amount of p66shc, its interaction with cytochrome *c*, and the amount of ROS are increased in hearts of diabetic mice; interestingly, glycemic control is unable to revert the increased translocation of p66shc into the mitochondria [25]. The in vivo silencing of p66shc during glycemic control, however, inhibits ROS formation and restores cardiac function. The finding that p66shc is of functional relevance in diabetes is confirmed in diabetic mice in which as a consequence of p66shc-mediated ROS formation a loss of viability occurs [31].

Streptozotocin-induced diabetic wildtype mice display a marked impairment of endothelium-dependent relaxations, increased peroxynitrite generation, nitrotyrosine expression,

and lipid peroxidation, all alterations being absent in p66shc KO mice [19].

Diabetes induced by streptozotocin also enhances the p66shc mRNA in the bone marrow [5]. Interestingly, the impairment of the mobilization of hematopoietic stem/progenitor cells from the bone marrow in diabetes is coupled to p66shc and oncostatin M [4] (for review, see Ref. [106]). In addition, an increased amount of p66shc mRNA is detected in peripheral blood mononuclear cells from diabetic patients and the increased p66sch expression correlates with a marker of oxidative stress [82].

Different from what has been described in diabetic hearts, in a prediabetic rat model the amount of p66shc is not affected [54].

Hypercholesterolemia

Hypercholesterolemia is characterized by an elevation of low-density lipoprotein cholesterol (LDL-C) and represents a major risk factor for atherosclerotic vascular disease. In LDL-C stimulated HUVECs, the amount of p66shc increases both at the mRNA and at the protein level [53]. The upregulation of p66shc by LDL-C is mediated by hypomethylation of two CpG dinucleotides and acetylation of histone 3 in the p66shc promoter, thereby increasing its activity. In addition to enhanced expression of p66shc after treatment with LDL-C, the phosphorylation of p66shc at S36 is stimulated in human and mouse aortic endothelial cells after incubation with oxidized LDL-C (oxLDL), together with increased phosphorylation of PKCβ2 [59, 96]. Such activation of p66shc is mediated by lectin-like oxLDL receptor-1, which is involved in oxLDL-induced ROS formation. In primary cultured HAEC treated with oxLDL, p66shc-mediated oxidative stress is derived from endothelial nitric oxide synthase (NOS) uncoupling and prevented by early treatment with tetrahydrobiopterin [97].

In visceral fat arteries (VFAs) isolated from obese subjects dysregulation of methyltransferase SUV39H1, demethylase JMJD2C and acetyltransferase SRC-1 occurs compared to VFAs from normal weight patients and these changes are associated with reduced di-(H3K9me2) and trimethylation (H3K9me3) as well as acetylation (H3K9ac) of histone 3 lysine 9 (H3K9) on p66shc promoter. Reprogramming SUV39H1, JMJD2C, and SRC-1 in isolated endothelial cells as well as in aortas from obese mice suppressed p66shc-derived ROS, restored nitric oxide levels, and rescued endothelial dysfunction [26].

There is also a link between hyperlipidemia and diabetes in that high concentration of LDL-C [7] or free fatty acids [73] induce pancreatic β-cell dysfunction and/or apoptosis. Free fatty acid-induced β-cell apoptosis is abrogated in islets from p66shcKO mice; by contrast, overexpression of p66shc, but not that of the phosphorylation-defective p66shc mutant, enhances palmitate-induced apoptosis of pancreatic β-cells [76]. The underlying mechanism includes CD36, c-Jun-N-terminal kinase activation, and p66shc S36 phosphorylation causing beta-cell apoptosis via mitochondrial dysfunction [48].

Hyperhomocysteinemia

Hyperhomocysteinemia represents a risk factor for atherosclerotic vascular disease and homocysteine is known to impact on DNA methylation. The p66shc promoter is hypomethylated in response to homocysteine and in turn the amount of the p66shc mRNA increases [51]. *S*-adenosylhomocysteine (SAH) is a precursor of homocysteine and the *S*-adenosylhomocysteine hydrolase (SAHH) catalyzes the breakdown of SAH to homocysteine. Inhibition of SAHH leads to hypomethylation of the p66shc promoter and subsequently enhances the expression of p66shc in the mouse aorta and in human endothelial cells [107]. SAHH inhibition is also accompanied by an induction of ROS formation, which is reduced after downregulation of p66shc by small interfering RNAs.

Taking into account that LDL-C and homocysteine induce the expression of p66shc, it is hypothesized that inhibition or deletion of p66shc would decrease the development of atherosclerosis (for review, also see Ref. [43]). Indeed, p66shc KO mice fed a high-fat diet not only demonstrate reduced levels of oxidative stress, but also develop less early atherosclerotic lesions than wildtype mice [75]. In addition, mice deficient for both ApoE and p66shc have smaller advanced atherosclerotic lesions compared to ApoE knockout mice [65].

In patients with coronary heart disease (CHD), expression of the p66shc mRNA in peripheral blood mononuclear cells positively correlates with the serum LDL-C and homocysteine levels and inversely with flow-mediated vasodilation, highlighting the pivotal role for the expression of p66shc in CHD and endothelial dysfunction [67]. P66shc might be most important for the transition of a stable coronary artery disease to an acute coronary syndrome patient [34]. The search for variants in the p66shc gene in patients with early-onset coronary artery disease indicates that variations appear to be rare and are presumably not linked to the genetic susceptibility of the disease [94]. Also, according to current databases (Online Mendelian Inheritance in Man, ClinVar, Human Genome Mutation Database), the gene encoding p66shc, *SHC1,* has not yet been linked to a cardiovascular disease condition.

Taken together, hyperglycemia induces the expression and phosphorylation of p66shc, which leads to an accumulation of the protein into the mitochondria, where it induces ROS formation leading to cell and/or tissue damage. The finding that p66shc-deficient cells or mice are protected

from hyperglycemia/diabetes-induced cellular/organ damage emphasizes the important role of the protein in the development of diabetes. LDL-C and homocysteine induce epigenetic modifications and lead to hypomethylation of the p66shc promoter, which stimulates the expression of p66shc. As a consequence, all risk factors increase oxidative stress through p66shc and finally result in endothelial dysfunction, inflammation and the development of atherosclerosis.

P66shc and myocardial ischemia/reperfusion injury

During myocardial ischemia, ROS formation is initiated [14]; however, the majority of ROS is generated at the onset of reperfusion [36, 74, 118]. The detrimental effects of high amounts of ROS are mediated by oxidative modifications on proteins, lipids and on the histone-free mitochondrial DNA. Therefore, a reduction of ROS at the onset of reperfusion is proposed to decrease myocardial ischemia/reperfusion (I/R) injury and indeed anti-oxidative therapies exert cardioprotective effects (reviewed in Ref. [13]). However, ROS are not only causing myocardial damage; if present in low amounts they are important signaling molecules in the cardioprotection by ischemic conditioning strategies (for review see Refs. [15, 27, 30, 45]). Indeed, ROS scavenging during the preconditioning ischemia abrogates the cardioprotection by ischemic preconditioning (IPC, here, infarct size is reduced by short, non-lethal periods of I/R applied before a longer lasting phase of I/R) [98]. In this context, the ROS-generating protein p66shc represents an interesting protein to be studied in models of I/R injury and protection from it by preconditioning.

Indeed, the phosphorylation of p66shc at S36 increases in isolated guinea pig hearts undergoing I/R; however, the extent of phosphorylation of the protein depends on the duration of I/R. Whereas the p66shc phosphorylation is not altered after 20 or 30 min ischemia without reperfusion, 5 or 10 min ischemia followed by 20 min reperfusion increases p66shc phosphorylation. When 30 min of ischemia are followed by 10 min of reperfusion such enhanced phosphorylation is maintained for up to 1 h of reperfusion [111]. The enhanced phosphorylation of p66shc is accompanied by a translocation of the protein into the mitochondria and inhibition of PKCβII diminishes such translocation of p66shc. Translocation of p66shc after 30 min ischemia and 10 min reperfusion, however, is enhanced only in SSM isolated from wildtype mice hearts [12]; such enhanced translocation of p66shc is associated with increased mitochondrial ROS formation in SSM compared to IFM. In contrast, ROS formation is comparable in SSM and IFM isolated after I/R from p66shc KO hearts [12]. Interestingly, the I/R-induced increased mitochondrial translocation of p66shc into SSM is abrogated after IPC [12].

Malondialdehyde formation and tropomyosin oxidation—indicators of oxidative stress—as well as irreversible injury decreases following I/R in p66shc-deficient compared to wildtype hearts [21]. However, these in vitro results cannot easily be transferred to the in vivo situation, where myocardial infarct size after I/R is not affected [12] or even increased [2] by the ablation of p66shc. The authors of the aforementioned study also investigated the involvement of signaling pathways involved in I/R injury and in the protection from it, namely the RISK (reperfusion injury salvage kinase) and SAFE (survivor activating factor enhancement) pathway. In p66shc-deficient hearts, the phosphorylation of Akt kinase (also known as protein kinase B) at T308 and the phosphorylation of the signal transducer and activator of transcription 3 (STAT3) at S727 is blunted following 30 min ischemia and 5 min of reperfusion. Our own results demonstrate that neither the phosphorylation of the p38 mitogen-activated protein kinase (p38) nor the phosphorylation of the AMP-activated protein kinase (AMPK)—proteins suggested to be involved in I/R injury [46, 62, 89]—differs in wildtype and p66shc KO hearts after I/R without or with IPC in vitro (Fig. 2). Therefore, we hypothesize that the absence of reduced I/R injury in p66shc KO hearts in our model in vitro (30 min ischemia/10 min reperfusion) is due to a lacking activation of cardioprotective signaling pathways. In contrast, expression of heat shock protein 60 (HSP60) increased in p66shc KO hearts after IPC; however, since IPC was equally effective in reducing myocardial infarct size in vitro [21] and in vivo [12] in wildtype and p66shc KO hearts this upregulation of HSP60 is not a prerequisite for cardioprotection. Thus, it is unlikely that increased p66shc-mediated mitochondrial translocation and subsequent ROS formation are a prerequisite for IPC-induced cardioprotection (for review, also see Ref. [47]).

Post-myocardial infarction

In mice with a coronary artery ligation, p66shc expression and phosphorylation increase transiently during the first week post-infarction. P66shc KO mice, despite a similar infarct size compared to wildtype mice, show improved survival and reduced occurrence of heart rupture post-myocardial infarction. In p66shc KO mice, the expression of cardiac matrix metalloproteinase 2 is reduced, fibroblast activation and collagen accumulation are facilitated and oxidative stress is attenuated early post-myocardial infarction resulting in reduced reactive fibrosis and left ventricular dilatation. Thus, p66shc activation is involved in adverse cardiac remodeling post-myocardial infarction [10].

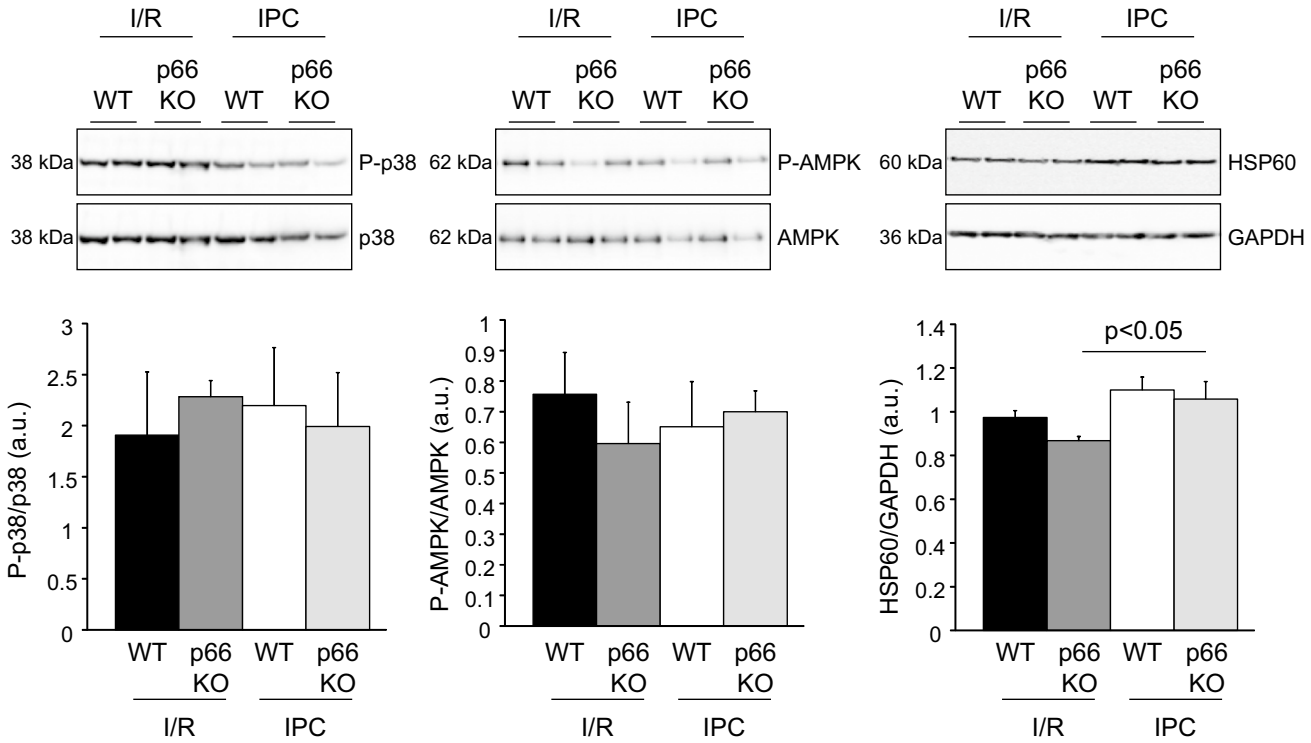


Fig. 2 Activation of signaling molecules in wildtype and p66shc KO hearts undergoing I/R injury or IPC. Wildtype or p66shc KO (p66 KO, $n=4$ per group) hearts were Langendorff-perfused for 30 min under ischemic conditions and subsequently reperfused for 10 min. IPC was performed by three preceding cycles of 3 min ischemia and 5 min reperfusion. The left ventricular tissue was homogenized and subjected to western blot analysis for total and phosphorylated p38 (Thr180, Tyr182), total and phosphorylated (Thr172) AMPK and the expression of HSP60. Immunoreactivities of the phosphorylated proteins were normalized to the total forms of the proteins, whereas the expression of HSP60 was normalized to that of the housekeeping protein GAPDH (glyceraldehyd-3-phosphate dehydrogenase). Statistical analysis was performed by two-way ANOVA and a $p<0.05$ was considered to indicate a significant difference between groups

Drug-induced cardiotoxicity

Cancer patients treated with chemotherapy often suffer from cardiotoxicity of the drugs [33], which is induced by mitochondrial dysfunction and cardiomyocyte apoptosis leading to heart failure [60]. In the rat cardiomyoblast H9c2 cell line doxorubicin (Dox) treatment induces activation of p66shc [92] which is in part responsible for increased cellular oxidative stress. In rat cardiomyoblast, H9c2 cells as well as in rats in vivo, Dox treatment increases miR-34a-5p in the myocardium and plasma; similarly in patients with diffuse large B cell lymphoma miR-34a-5p is enhanced after epirubicin therapy. MiR-34a-5p modulates Sirt1 expression and enhances p66shc expression, accompanied by an increase of Bax and activation of caspase-3. MiR-34a-5p enhances cardiomyocyte apoptosis by targeting Sirt1 and activation of miR-34a-5p/Sirt1/p66shc pathway contributes to Dox-induced cardiotoxicity [116], which can be attenuated by endurance exercise training [64].

Taken together, p66shc becomes activated by I/R and translocates to mitochondria, most importantly the subsarcolemmal ones. Such activation and translocation can be abolished by IPC. Despite increased translocation and subsequent ROS formation, p66shc does neither contribute to infarct size development during I/R nor to cardioprotection by IPC. However, p66shc activation appears to be important for post-myocardial infarction remodeling and cardiotoxicity elicited by cancer drugs. Here, abrogation of p66shc signaling might be of therapeutic interest.

The contribution of p66shc towards brain injury

The role of p66shc is not restricted to the heart, since a contribution of the protein towards brain injury is also described. In Alzheimer's disease (AD), abnormally folded amyloid β proteins accumulate in the brain and induce cytotoxicity, e.g., via increased ROS formation. In C6 glioma cells, amyloid β induces the phosphorylation of p66shc at S36 whereas the knockdown of p66shc is associated with reduced amyloid β-induced ROS formation and subsequently less apoptotic cell death [9]. P66shc-ablated PSAPP mice (a double transgenic mouse line expressing mutant amyloid

precursor protein and presenilin-1, used as AD model) show improved survival and less cognitive defects, the latter being mediated by improved function of brain mitochondria (including enhanced respiration, adenosine triphosphate production as well as reduced ROS formation) [28]. Increased respiration and diminished ROS formation are also detected in brain mitochondria isolated from aged p66shc-KO mice compared to aged wildtype mice [86]. The induction of oxidative stress and apoptosis in mouse hippocampal HT22 cells by angiotensin II increases the amount of PKCβII, the phosphorylation of p66shc at S36 and the mitochondrial translocation of the protein, which is accompanied by enhanced ROS formation and cell death, effects which are reversed by propofol [117].

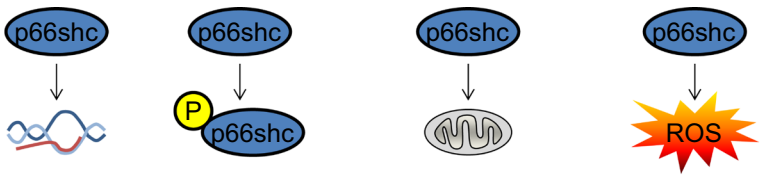
An involvement of p66shc on neuronal cell death is shown in p66shc KO mice, in which the development of experimental autoimmune encephalomyelitis is delayed, an effect suggested to be mediated by delayed MPTP opening [93].

Post-ischemic silencing of p66shc in the brain of wildtype mice undergoing I/R injury by occlusion of the middle cerebral artery decreases neurological deficits, reduces stroke volume and finally increases survival 6 days after stroke [99]. In acute ischemic stroke patients, the amount of the p66shc mRNA in peripheral blood monocytes increases 6 h after initial stroke symptoms and positively correlates with short-term neurological outcome [99].

Taken together, pathological conditions in the brain are often associated with activation of p66shc, and enhanced oxidative stress and consequently downregulation and/or inhibition of p66shc reduce or delay these pathologies and make the protein an interesting therapeutic target. However, preconditioning of cortical cells results in a phosphorylation and subsequent mitochondrial translocation of p66shc and, different from the heart, the cytoprotection afforded by preconditioning is blocked by inhibition of p66shc [16]. Alterations of p66shc expression, phosphorylation, translocation into mitochondria and ROS formation in aging, cardiovascular risk factors and pathologies as well as in brain injury are summarized in Fig. 3.

Conclusion

Risk factors for the development of atherosclerosis increase the expression of p66shc, a process involving epigenetic modifications. The protein becomes phosphorylated, translocates into the mitochondria and contributes to



risk factor/ pathology	mRNA, protein	phosphorylation	mitochondrial translocation	ROS formation
aging	↑			
diabetes	↑	↑	↑	↑
hyperglycemia	↑	↑	↑	↑
hypercholesterolemia	↑	↑		↑
hyperhomocysteinemia	↑			↑
ischemia/ reperfusion		↑	↑	↑
post-myocardial infarction/heart failure	↑	↑		↑
cardiotoxicity	↑			↑
brain injury	↑	↑	↑	↑

Fig. 3 P66shc and cardiovascular risk factors and pathologies. The figure summarizes the role of p66shc in relation to cardiovascular risk factors and pathologies, especially towards the expression (mRNA, protein), phosphorylation and translocation of the protein into the mitochondria as well as reactive oxygen species (ROS) formation. ↑ Indicates a p66shc-dependent increase in the respective process. For further details, see text

ROS formation. Consequently, the inhibition or ablation of p66shc may represent a therapeutic target for the treatment of these diseases. The role of p66shc in myocardial I/R is less clear and further studies are required to establish whether p66shc increases, decreases or has no effect on I/R injury. A causative role for p66shc in ischemic brain injury is also suggested; however, more in vivo studies in the context of conditioning phenomena are required. Whereas the pharmacological modulation of p66shc activity is generally feasible [29], more specific inhibitors or activators of p66shc would help to elucidate the function of p66shc in cardiovascular diseases.

Acknowledgements RS was funded by the German Research Foundation: Project number 268555672-SFB-B05 and the Cardio-Pulmonary Institute (CPI), EXC 2026, Project ID: 390649896.

Compliance with ethical standards

Conflict of interest Rainer Schulz received honoraries for lectures provided to AstraZeneca, Recordati and Sanofi.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The study was approved by local authorities ("Regierungspräsidium Giessen" (G91-2017) and the animal welfare office of the Justus-Liebig-University Giessen). This article does not contain any studies with human participants performed by any of the authors.

References

1. Ahmed SBM, Prigent SA (2017) Insights into the Shc family of adaptor proteins. J Mol Signal 12:2. https://doi.org/10.5334/1750-2187-12-2
2. Akhmedov A, Montecucco F, Braunersreuther V, Camici GG, Jakob P, Reiner MF, Glanzmann M, Burger F, Paneni F, Galan K, Pelli G, Vuilleumier N, Belin A, Vallee JP, Mach F, Luscher TF (2015) Genetic deletion of the adaptor protein p66Shc increases susceptibility to short-term ischaemic myocardial injury via intracellular salvage pathways. Eur Heart J 36:516–526a. https://doi.org/10.1093/eurheartj/ehu400
3. Alavian KN, Beutner G, Lazrove E, Sacchetti S, Park HA, Licznerski P, Li H, Nabili P, Hockensmith K, Graham M, Porter GA Jr, Jonas EA (2014) An uncoupling channel within the c-subunit ring of the F1FO ATP synthase is the mitochondrial permeability transition pore. Proc Natl Acad Sci USA 111:10580–10585. https://doi.org/10.1073/pnas.1401591111
4. Albiero M, Ciciliot S, Tedesco S, Menegazzo L, D'Anna M, Scattolini V, Cappellari R, Zuccolotto G, Rosato A, Cignarella A, Giorgio M, Avogaro A, Fadini GP (2019) Diabetes-associated myelopoiesis drives stem cell mobilopathy through an OSM-p66Shc signaling pathway. Diabetes. https://doi.org/10.2337/db19-0080
5. Albiero M, Poncina N, Tjwa M, Ciciliot S, Menegazzo L, Ceolotto G, Vigili de Kreutzenberg S, Moura R, Giorgio M, Pelicci P, Avogaro A, Fadini GP (2014) Diabetes causes bone marrow autonomic neuropathy and impairs stem cell mobilization via dysregulated p66Shc and Sirt1. Diabetes 63:1353–1365. https://doi.org/10.2337/db13-0894
6. Andreadou I, Iliodromitis EK, Rassaf T, Schulz R, Papapetropoulos A, Ferdinandy P (2015) The role of gasotransmitters NO, H_2S and CO in myocardial ischaemia/reperfusion injury and cardioprotection by preconditioning, postconditioning and remote conditioning. Br J Pharmacol 172:1587–1606. https://doi.org/10.1111/bph.12811
7. Asalla S, Girada SB, Kuna RS, Chowdhury D, Kandagatla B, Oruganti S, Bhadra U, Bhadra MP, Kalivendi SV, Rao SP, Row A, Ibrahim A, Ghosh PP, Mitra P (2016) Restoring mitochondrial function: a small molecule-mediated approach to enhance glucose stimulated insulin secretion in cholesterol accumulated pancreatic beta cells. Sci Rep 6:27513. https://doi.org/10.1038/srep27513
8. Azarashvili T, Odinokova I, Bakunts A, Ternovsky V, Krestinina O, Tyynela J, Saris NE (2014) Potential role of subunit c of F0F1-ATPase and subunit c of storage body in the mitochondrial permeability transition. Effect of the phosphorylation status of subunit c on pore opening. Cell Calcium 55:69–77. https://doi.org/10.1016/j.ceca.2013.12.002
9. Bashir M, Parray AA, Baba RA, Bhat HF, Bhat SS, Mushtaq U, Andrabi KI, Khanday FA (2014) beta-Amyloid-evoked apoptotic cell death is mediated through MKK6-p66shc pathway. Neuromolecular Med 16:137–149. https://doi.org/10.1007/s12017-013-8268-4
10. Baysa A, Sagave J, Carpi A, Zaglia T, Campesan M, Dahl CP, Bilbija D, Troitskaya M, Gullestad L, Giorgio M, Mongillo M, Di Lisa F, Vaage JI, Valen G (2015) The p66ShcA adaptor protein regulates healing after myocardial infarction. Basic Res Cardiol 110:13. https://doi.org/10.1007/s00395-015-0470-0
11. Berniakovich I, Trinei M, Stendardo M, Migliaccio E, Minucci S, Bernardi P, Pelicci PG, Giorgio M (2008) p66Shc-generated oxidative signal promotes fat accumulation. J Biol Chem 283:34283–34293. https://doi.org/10.1074/jbc.M804362200
12. Boengler K, Bencsik P, Paloczi J, Kiss K, Pipicz M, Pipis J, Ferdinandy P, Schluter KD, Schulz R (2017) Lack of contribution of p66shc and its mitochondrial translocation to ischemia–reperfusion injury and cardioprotection by ischemic preconditioning. Front Physiol 8:733. https://doi.org/10.3389/fphys.2017.00733
13. Boengler K, Lochnit G, Schulz R (2018) Mitochondria "THE" target of myocardial conditioning. Am J Physiol Heart Circ Physiol 315:H1215–H1231. https://doi.org/10.1152/ajpheart.00124.2018
14. Bolli R, Jeroudi MO, Patel BS, DuBose CM, Lai EK, Roberts R, McCay PB (1989) Direct evidence that oxygen-derived free radicals contribute to postischemic myocardial dysfunction in the intact dog. Proc Natl Acad Sci USA 86:4695–4699
15. Botker HE, Hausenloy D, Andreadou I, Antonucci S, Boengler K, Davidson SM, Deshwal S, Devaux Y, Di Lisa F, Di Sante M, Efentakis P, Femmino S, Garcia-Dorado D, Giricz Z, Ibanez B, Iliodromitis E, Kaludercic N, Kleinbongard P, Neuhauser M, Ovize M, Pagliaro P, Rahbek-Schmidt M, Ruiz-Meana M, Schluter KD, Schulz R, Skyschally A, Wilder C, Yellon DM, Ferdinandy P, Heusch G (2018) Practical guidelines for rigor and reproducibility in preclinical and clinical studies on cardioprotection. Basic Res Cardiol 113:39. https://doi.org/10.1007/s00395-018-0696-8
16. Brown JE, Zeiger SL, Hettinger JC, Brooks JD, Holt B, Morrow JD, Musiek ES, Milne G, McLaughlin B (2010) Essential role of the redox-sensitive kinase p66shc in determining energetic and oxidative status and cell fate in neuronal preconditioning. J Neurosci 30:5242–5252. https://doi.org/10.1523/jneurosci.6366-09.2010
17. Cai X, Hu Y, Tang H, Hu H, Pang L, Xing J, Liu Z, Luo Y, Jiang B, Liu T, Gorospe M, Chen C, Wang W (2016) RNA methyltransferase NSUN2 promotes stress-induced HUVEC

senescence. Oncotarget 7:19099–19110. https://doi.org/10.18632/oncotarget.8087
18. Camici GG, Savarese G, Akhmedov A, Luscher TF (2015) Molecular mechanism of endothelial and vascular aging: implications for cardiovascular disease. Eur Heart J 36:3392–3403. https://doi.org/10.1093/eurheartj/ehv587
19. Camici GG, Schiavoni M, Francia P, Bachschmid M, Martin-Padura I, Hersberger M, Tanner FC, Pelicci P, Volpe M, Anversa P, Luscher TF, Cosentino F (2007) Genetic deletion of p66(Shc) adaptor protein prevents hyperglycemia-induced endothelial dysfunction and oxidative stress. Proc Natl Acad Sci USA 104:5217–5222. https://doi.org/10.1073/pnas.0609656104
20. Carlomosti F, D'Agostino M, Beji S, Torcinaro A, Rizzi R, Zaccagnini G, Maimone B, Di Stefano V, De Santa F, Cordisco S, Antonini A, Ciarapica R, Dellambra E, Martelli F, Avitabile D, Capogrossi MC, Magenta A (2017) Oxidative stress-induced miR-200c disrupts the regulatory loop among SIRT1, FOXO1, and eNOS. Antioxid Redox Signal 27:328–344. https://doi.org/10.1089/ars.2016.6643
21. Carpi A, Menabo R, Kaludercic N, Pelicci P, Di Lisa F, Giorgio M (2009) The cardioprotective effects elicited by p66(Shc) ablation demonstrate the crucial role of mitochondrial ROS formation in ischemia/reperfusion injury. Biochim Biophys Acta 1787:774–780. https://doi.org/10.1016/j.bbabio.2009.04.001
22. Ciciliot S, Albiero M, Campanaro S, Poncina N, Tedesco S, Scattolini V, Dalla Costa F, Cignarella A, Vettore M, Di Gangi IM, Bogialli S, Avogaro A, Fadini GP (2018) Interplay between gut microbiota and p66Shc affects obesity-associated insulin resistance. FASEB J 32:4004–4015. https://doi.org/10.1096/fj.201701409R
23. Ciciliot S, Albiero M, Menegazzo L, Poncina N, Scattolini V, Danesi A, Pagnin E, Marabita M, Blaauw B, Giorgio M, Trinei M, Foletto M, Prevedello L, Nitti D, Avogaro A, Fadini GP (2015) p66Shc deletion or deficiency protects from obesity but not metabolic dysfunction in mice and humans. Diabetologia 58:2352–2360. https://doi.org/10.1007/s00125-015-3667-8
24. Ciciliot S, Fadini GP (2019) Modulation of obesity and insulin resistance by the redox enzyme and adaptor protein p66(Shc). Int J Mol Sci 20:E985. https://doi.org/10.3390/ijms20040985
25. Costantino S, Paneni F, Mitchell K, Mohammed SA, Hussain S, Gkolfos C, Berrino L, Volpe M, Schwarzwald C, Luscher TF, Cosentino F (2018) Hyperglycaemia-induced epigenetic changes drive persistent cardiac dysfunction via the adaptor p66(Shc). Int J Cardiol 268:179–186. https://doi.org/10.1016/j.ijcard.2018.04.082
26. Costantino S, Paneni F, Virdis A, Hussain S, Mohammed SA, Capretti G, Akhmedov A, Dalgaard K, Chiandotto S, Pospisilik JA, Jenuwein T, Giorgio M, Volpe M, Taddei S, Luscher TF, Cosentino F (2019) Interplay among H3K9-editing enzymes SUV39H1, JMJD2C and SRC-1 drives p66Shc transcription and vascular oxidative stress in obesity. Eur Heart J 40:383–391. https://doi.org/10.1093/eurheartj/ehx615
27. Davidson SM, Ferdinandy P, Andreadou I, Botker HE, Heusch G, Ibanez B, Ovize M, Schulz R, Yellon DM, Hausenloy DJ, Garcia-Dorado D (2019) Multitarget strategies to reduce myocardial ischemia/reperfusion injury: JACC review topic of the week. J Am Coll Cardiol 73:89–99. https://doi.org/10.1016/j.jacc.2018.09.086
28. Derungs R, Camici GG, Spescha RD, Welt T, Tackenberg C, Spani C, Wirth F, Grimm A, Eckert A, Nitsch RM, Kulic L (2017) Genetic ablation of the p66(Shc) adaptor protein reverses cognitive deficits and improves mitochondrial function in an APP transgenic mouse model of Alzheimer's disease. Mol Psychiatry 22:605–614. https://doi.org/10.1038/mp.2016.112
29. Di Lisa F, Giorgio M, Ferdinandy P, Schulz R (2017) New aspects of p66Shc in ischaemia reperfusion injury and other cardiovascular diseases. Br J Pharmacol 174:1690–1703. https://doi.org/10.1111/bph.13478
30. Egea J, Fabregat I, Frapart YM, Ghezzi P, Gorlach A, Kietzmann T, Kubaichuk K, Knaus UG, Lopez MG, Olaso-Gonzalez G, Petry A, Schulz R, Vina J, Winyard P, Abbas K, Ademowo OS, Afonso CB, Andreadou I, Antelmann H, Antunes F, Aslan M, Bachschmid MM, Barbosa RM, Belousov V, Berndt C, Bernlohr D, Bertran E, Bindoli A, Bottari SP, Brito PM, Carrara G, Casas AI, Chatzi A, Chondrogianni N, Conrad M, Cooke MS, Costa JG, Cuadrado A, My-Chan Dang P, De Smet B, Debelec-Butuner B, Dias IHK, Dunn JD, Edson AJ, El Assar M, El-Benna J, Ferdinandy P, Fernandes AS, Fladmark KE, Forstermann U, Giniatullin R, Giricz Z, Gorbe A, Griffiths H, Hampl V, Hanf A, Herget J, Hernansanz-Agustin P, Hillion M, Huang J, Ilikay S, Jansen-Durr P, Jaquet V, Joles JA, Kalyanaraman B, Kaminskyy D, Karbaschi M, Kleanthous M, Klotz LO, Korac B, Korkmaz KS, Koziel R, Kracun D, Krause KH, Kren V, Krieg T, Laranjinha J, Lazou A, Li H, Martinez-Ruiz A, Matsui R, McBean GJ, Meredith SP, Messens J, Miguel V, Mikhed Y, Milisav I, Milkovic L, Miranda-Vizuete A, Mojovic M, Monsalve M, Mouthuy PA, Mulvey J, Munzel T, Muzykantov V, Nguyen ITN, Oelze M, Oliveira NG, Palmeira CM, Papaevgeniou N et al (2017) European contribution to the study of ROS: a summary of the findings and prospects for the future from the COST action BM1203 (EU-ROS). Redox Biol 13:94–162. https://doi.org/10.1016/j.redox.2017.05.007
31. Fadini GP, Albiero M, Menegazzo L, Boscaro E, Pagnin E, Iori E, Cosma C, Lapolla A, Pengo V, Stendardo M, Agostini C, Pelicci PG, Giorgio M, Avogaro A (2010) The redox enzyme p66Shc contributes to diabetes and ischemia-induced delay in cutaneous wound healing. Diabetes 59:2306–2314. https://doi.org/10.2337/db09-1727
32. Feng D, Yao J, Wang G, Li Z, Zu G, Li Y, Luo F, Ning S, Qasim W, Chen Z, Tian X (2017) Inhibition of p66Shc-mediated mitochondrial apoptosis via targeting prolyl-isomerase Pin1 attenuates intestinal ischemia/reperfusion injury in rats. Clin Sci (Lond) 131:759–773. https://doi.org/10.1042/cs20160799
33. Ferdinandy P, Baczko I, Bencsik P, Giricz Z, Gorbe A, Pacher P, Varga ZV, Varro A, Schulz R (2018) Definition of hidden drug cardiotoxicity: paradigm change in cardiac safety testing and its clinical implications. Eur Heart J. https://doi.org/10.1093/eurheartj/ehy365
34. Franzeck FC, Hof D, Spescha RD, Hasun M, Akhmedov A, Steffel J, Shi Y, Cosentino F, Tanner FC, von Eckardstein A, Maier W, Luscher TF, Wyss CA, Camici GG (2012) Expression of the aging gene p66Shc is increased in peripheral blood monocytes of patients with acute coronary syndrome but not with stable coronary artery disease. Atherosclerosis 220:282–286. https://doi.org/10.1016/j.atherosclerosis.2011.10.035
35. Frijhoff J, Dagnell M, Augsten M, Beltrami E, Giorgio M, Ostman A (2014) The mitochondrial reactive oxygen species regulator p66Shc controls PDGF-induced signaling and migration through protein tyrosine phosphatase oxidation. Free Radic Biol Med 68:268–277. https://doi.org/10.1016/j.freeradbiomed.2013.12.022
36. Garlick PB, Davies MJ, Hearse DJ, Slater TF (1987) Direct detection of free radicals in the reperfused rat heart using electron spin resonance spectroscopy. Circ Res 61:757–760
37. Gerber PA, Rutter GA (2017) The role of oxidative stress and hypoxia in pancreatic beta-cell dysfunction in diabetes mellitus. Antioxid Redox Signal 26:501–518. https://doi.org/10.1089/ars.2016.6755
38. Gertz M, Fischer F, Wolters D, Steegborn C (2008) Activation of the lifespan regulator p66Shc through reversible disulfide bond formation. Proc Natl Acad Sci USA 105:5705–5709. https://doi.org/10.1073/pnas.0800691105

39. Giorgio M, Berry A, Berniakovich I, Poletaeva I, Trinei M, Stendardo M, Hagopian K, Ramsey JJ, Cortopassi G, Migliaccio E, Notzli S, Amrein I, Lipp HP, Cirulli F, Pelicci PG (2012) The p66Shc knocked out mice are short lived under natural condition. Aging Cell 11:162–168. https://doi.org/10.1111/j.1474-9726.2011.00770.x
40. Giorgio M, Migliaccio E, Orsini F, Paolucci D, Moroni M, Contursi C, Pelliccia G, Luzi L, Minucci S, Marcaccio M, Pinton P, Rizzuto R, Bernardi P, Paolucci F, Pelicci PG (2005) Electron transfer between cytochrome *c* and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. Cell 122:221–233. https://doi.org/10.1016/j.cell.2005.05.011
41. Giorgio V, von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M, Glick GD, Petronilli V, Zoratti M, Szabo I, Lippe G, Bernardi P (2013) Dimers of mitochondrial ATP synthase form the permeability transition pore. Proc Natl Acad Sci USA 110:5887–5892. https://doi.org/10.1073/pnas.1217823110
42. Graiani G, Lagrasta C, Migliaccio E, Spillmann F, Meloni M, Madeddu P, Quaini F, Padura IM, Lanfrancone L, Pelicci P, Emanueli C (2005) Genetic deletion of the p66Shc adaptor protein protects from angiotensin II-induced myocardial damage. Hypertension 46:433–440. https://doi.org/10.1161/01.HYP.0000174986.73346.ba
43. Grimaldi V, Vietri MT, Schiano C, Picascia A, De Pascale MR, Fiorito C, Casamassimi A, Napoli C (2015) Epigenetic reprogramming in atherosclerosis. Curr Atheroscler Rep 17:476. https://doi.org/10.1007/s11883-014-0476-3
44. Haller M, Khalid S, Kremser L, Fresser F, Furlan T, Hermann M, Guenther J, Drasche A, Leitges M, Giorgio M, Baier G, Lindner H, Troppmair J (2016) Novel Insights into the PKCbeta-dependent regulation of the oxidoreductase p66Shc. J Biol Chem 291:23557–23568. https://doi.org/10.1074/jbc.M116.752766
45. Hausenloy DJ, Garcia-Dorado D, Botker HE, Davidson SM, Downey J, Engel FB, Jennings R, Lecour S, Leor J, Madonna R, Ovize M, Perrino C, Prunier F, Schulz R, Sluijter JPG, Van Laake LW, Vinten-Johansen J, Yellon DM, Ytrehus K, Heusch G, Ferdinandy P (2017) Novel targets and future strategies for acute cardioprotection: position paper of the European Society of Cardiology Working Group on Cellular Biology of the Heart. Cardiovasc Res 113:564–585. https://doi.org/10.1093/cvr/cvx049
46. Hausenloy DJ, Yellon DM (2006) Survival kinases in ischemic preconditioning and postconditioning. Cardiovasc Res 70:240–253. https://doi.org/10.1016/j.cardiores.2006.01.017
47. Heusch G (2015) Mitochondria at the heart of cardiovascular protection: p66shc-friend or foe? Eur Heart J 36:469–471. https://doi.org/10.1093/eurheartj/ehu409
48. Karunakaran U, Elumalai S, Moon JS, Won KC (2019) CD36 dependent redoxosomes promotes ceramide-mediated pancreatic beta-cell failure via p66Shc activation. Free Radic Biol Med 134:505–515. https://doi.org/10.1016/j.freeradbiomed.2019.02.004
49. Khalid S, Drasche A, Thurner M, Hermann M, Ashraf MI, Fresser F, Baier G, Kremser L, Lindner H, Troppmair J (2016) cJun N-terminal kinase (JNK) phosphorylation of serine 36 is critical for p66Shc activation. Sci Rep 6:20930. https://doi.org/10.1038/srep20930
50. Khanday FA, Yamamori T, Mattagajasingh I, Zhang Z, Bugayenko A, Naqvi A, Santhanam L, Nabi N, Kasuno K, Day BW, Irani K (2006) Rac1 leads to phosphorylation-dependent increase in stability of the p66shc adaptor protein: role in Rac1-induced oxidative stress. Mol Biol Cell 17:122–129. https://doi.org/10.1091/mbc.e05-06-0570
51. Kim CS, Kim YR, Naqvi A, Kumar S, Hoffman TA, Jung SB, Kumar A, Jeon BH, McNamara DM, Irani K (2011) Homocysteine promotes human endothelial cell dysfunction via site-specific epigenetic regulation of p66shc. Cardiovasc Res 92:466–475. https://doi.org/10.1093/cvr/cvr250
52. Kim YM, Kim SJ, Tatsunami R, Yamamura H, Fukai T, Ushio-Fukai M (2017) ROS-induced ROS release orchestrated by Nox4, Nox2, and mitochondria in VEGF signaling and angiogenesis. Am J Physiol Cell Physiol 312:C749–C764. https://doi.org/10.1152/ajpcell.00346.2016
53. Kim YR, Kim CS, Naqvi A, Kumar A, Kumar S, Hoffman TA, Irani K (2012) Epigenetic upregulation of p66shc mediates low-density lipoprotein cholesterol-induced endothelial cell dysfunction. Am J Physiol Heart Circ Physiol 303:H189–H196. https://doi.org/10.1152/ajpheart.01218.2011
54. Koncsos G, Varga ZV, Baranyai T, Boengler K, Rohrbach S, Li L, Schluter KD, Schreckenberg R, Radovits T, Olah A, Matyas C, Lux A, Al-Khrasani M, Komlodi T, Bukosza N, Mathe D, Deres L, Bartekova M, Rajtik T, Adameova A, Szigeti K, Hamar P, Helyes Z, Tretter L, Pacher P, Merkely B, Giricz Z, Schulz R, Ferdinandy P (2016) Diastolic dysfunction in prediabetic male rats: role of mitochondrial oxidative stress. Am J Physiol Heart Circ Physiol 311:H927–H943. https://doi.org/10.1152/ajpheart.00049.2016
55. Koncsos G, Varga ZV, Baranyai T, Ferdinandy P, Schulz R, Giricz Z, Boengler K (2018) Nagarse treatment of cardiac subsarcolemmal and interfibrillar mitochondria leads to artefacts in mitochondrial protein quantification. J Pharmacol Toxicol Methods 91:50–58. https://doi.org/10.1016/j.vascn.2018.01.004
56. Kong X, Guan J, Li J, Wei J, Wang R (2017) P66(Shc)-SIRT1 regulation of oxidative stress protects against cardio-cerebral vascular disease. Mol Neurobiol 54:5277–5285. https://doi.org/10.1007/s12035-016-0073-2
57. Kumar S (2019) P66Shc and vascular endothelial function. Biosci Rep. https://doi.org/10.1042/bsr20182134
58. Kumar S, Kim YR, Vikram A, Naqvi A, Li Q, Kassan M, Kumar V, Bachschmid MM, Jacobs JS, Kumar A, Irani K (2017) Sirtuin1-regulated lysine acetylation of p66Shc governs diabetes-induced vascular oxidative stress and endothelial dysfunction. Proc Natl Acad Sci USA 114:1714–1719. https://doi.org/10.1073/pnas.1614112114
59. Lee SK, Chung JI, Park MS, Joo HK, Lee EJ, Cho EJ, Park JB, Ryoo S, Irani K, Jeon BH (2011) Apurinic/apyrimidinic endonuclease 1 inhibits protein kinase C-mediated p66shc phosphorylation and vasoconstriction. Cardiovasc Res 91:502–509. https://doi.org/10.1093/cvr/cvr095
60. Li M, Sala V, De Santis MC, Cimino J, Cappello P, Pianca N, Di Bona A, Margaria JP, Martini M, Lazzarini E, Pirozzi F, Rossi L, Franco I, Bornbaum J, Heger J, Rohrbach S, Perino A, Tocchetti CG, Lima BHF, Teixeira MM, Porporato PE, Schulz R, Angelini A, Sandri M, Ameri P, Sciarretta S, Lima-Junior RCP, Mongillo M, Zaglia T, Morello F, Novelli F, Hirsch E, Ghigo A (2018) Phosphoinositide 3-kinase gamma inhibition protects from anthracycline cardiotoxicity and reduces tumor growth. Circulation 138:696–711. https://doi.org/10.1161/circulationaha.117.030352
61. Li Q, Kim YR, Vikram A, Kumar S, Kassan M, Gabani M, Lee SK, Jacobs JS, Irani K (2016) P66Shc-induced microRNA-34a causes diabetic endothelial dysfunction by downregulating sirtuin1. Arterioscler Thromb Vasc Biol 36:2394–2403. https://doi.org/10.1161/atvbaha.116.308321
62. Lin KM, Lin B, Lian IY, Mestril R, Scheffler IE, Dillmann WH (2001) Combined and individual mitochondrial HSP60 and HSP10 expression in cardiac myocytes protects mitochondrial function and prevents apoptotic cell deaths induced by simulated ischemia-reoxygenation. Circulation 103:1787–1792
63. Malhotra A, Vashistha H, Yadav VS, Dube MG, Kalra SP, Abdellatif M, Meggs LG (2009) Inhibition of p66ShcA redox activity in cardiac muscle cells attenuates hyperglycemia-induced

oxidative stress and apoptosis. Am J Physiol Heart Circ Physiol 296:H380–H388. https://doi.org/10.1152/ajpheart.00225.2008
64. Marques-Aleixo I, Santos-Alves E, Mariani D, Rizo-Roca D, Padrao AI, Rocha-Rodrigues S, Viscor G, Torrella JR, Ferreira R, Oliveira PJ, Magalhaes J, Ascensao A (2015) Physical exercise prior and during treatment reduces sub-chronic doxorubicin-induced mitochondrial toxicity and oxidative stress. Mitochondrion 20:22–33. https://doi.org/10.1016/j.mito.2014.10.008
65. Martin-Padura I, de Nigris F, Migliaccio E, Mansueto G, Minardi S, Rienzo M, Lerman LO, Stendardo M, Giorgio M, De Rosa G, Pelicci PG, Napoli C (2008) p66Shc deletion confers vascular protection in advanced atherosclerosis in hypercholesterolemic apolipoprotein E knockout mice. Endothelium 15:276–287. https://doi.org/10.1080/10623320802487791
66. Meng G, Zhao S, Xie L, Han Y, Ji Y (2018) Protein S-sulfhydration by hydrogen sulfide in cardiovascular system. Br J Pharmacol 175:1146–1156. https://doi.org/10.1111/bph.13825
67. Miao Q, Wang Q, Dong L, Wang Y, Tan Y, Zhang X (2015) The expression of p66shc in peripheral blood monocytes is increased in patients with coronary heart disease and correlated with endothelium-dependent vasodilatation. Heart Vessels 30:451–457. https://doi.org/10.1007/s00380-014-0497-4
68. Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, Pelicci PG (1999) The p66shc adaptor protein controls oxidative stress response and life span in mammals. Nature 402:309–313. https://doi.org/10.1038/46311
69. Migliaccio E, Mele S, Salcini AE, Pelicci G, Lai KM, Superti-Furga G, Pawson T, Di Fiore PP, Lanfrancone L, Pelicci PG (1997) Opposite effects of the p52shc/p46shc and p66shc splicing isoforms on the EGF receptor-MAP kinase-fos signalling pathway. EMBO J 16:706–716. https://doi.org/10.1093/emboj/16.4.706
70. Mishra M, Duraisamy AJ, Bhattacharjee S, Kowluru RA (2018) Adaptor protein p66Shc: a link between cytosolic and mitochondrial dysfunction in the development of diabetic retinopathy. Antioxid Redox Signal. https://doi.org/10.1089/ars.2018.7542
71. Miyazawa M, Tsuji Y (2014) Evidence for a novel antioxidant function and isoform-specific regulation of the human p66Shc gene. Mol Biol Cell 25:2116–2127. https://doi.org/10.1091/mbc.E13-11-0666
72. Morciano G, Bonora M, Campo G, Aquila G, Rizzo P, Giorgi C, Wieckowski MR, Pinton P (2017) Mechanistic role of mPTP in ischemia–reperfusion injury. Adv Exp Med Biol 982:169–189. https://doi.org/10.1007/978-3-319-55330-6_9
73. Mukhuty A, Fouzder C, Mukherjee S, Malick C, Mukhopadhyay S, Bhattacharya S, Kundu R (2017) Palmitate induced Fetuin-A secretion from pancreatic beta-cells adversely affects its function and elicits inflammation. Biochem Biophys Res Commun 491:1118–1124. https://doi.org/10.1016/j.bbrc.2017.08.022
74. Murphy E, Steenbergen C (2008) Mechanisms underlying acute protection from cardiac ischemia–reperfusion injury. Physiol Rev 88:581–609. https://doi.org/10.1152/physrev.00024.2007
75. Napoli C, Martin-Padura I, de Nigris F, Giorgio M, Mansueto G, Somma P, Condorelli M, Sica G, De Rosa G, Pelicci P (2003) Deletion of the p66Shc longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet. Proc Natl Acad Sci USA 100:2112–2116. https://doi.org/10.1073/pnas.0336359100
76. Natalicchio A, Tortosa F, Labarbuta R, Biondi G, Marrano N, Carchia E, Leonardini A, Cignarelli A, Bugliani M, Marchetti P, Fadini GP, Giorgio M, Avogaro A, Perrini S, Laviola L, Giorgino F (2015) The p66(Shc) redox adaptor protein is induced by saturated fatty acids and mediates lipotoxicity-induced apoptosis in pancreatic beta cells. Diabetologia 58:1260–1271. https://doi.org/10.1007/s00125-015-3563-2
77. Nemoto S, Combs CA, French S, Ahn BH, Fergusson MM, Balaban RS, Finkel T (2006) The mammalian longevity-associated gene product p66shc regulates mitochondrial metabolism. J Biol Chem 281:10555–10560. https://doi.org/10.1074/jbc.M511626200
78. Newsholme P, Cruzat VF, Keane KN, Carlessi R, de Bittencourt PI Jr. (2016) Molecular mechanisms of ROS production and oxidative stress in diabetes. Biochem J 473:4527–4550. https://doi.org/10.1042/bcj20160503c
79. Onnis A, Cianfanelli V, Cassioli C, Samardzic D, Pelicci PG, Cecconi F, Baldari CT (2018) The pro-oxidant adaptor p66SHC promotes B cell mitophagy by disrupting mitochondrial integrity and recruiting LC3-II. Autophagy 14:2117–2138. https://doi.org/10.1080/15548627.2018.1505153
80. Orsini F, Migliaccio E, Moroni M, Contursi C, Raker VA, Piccini D, Martin-Padura I, Pelliccia G, Trinei M, Bono M, Puri C, Tacchetti C, Ferrini M, Mannucci R, Nicoletti I, Lanfrancone L, Giorgio M, Pelicci PG (2004) The life span determinant p66Shc localizes to mitochondria where it associates with mitochondrial heat shock protein 70 and regulates trans-membrane potential. J Biol Chem 279:25689–25695. https://doi.org/10.1074/jbc.M401844200
81. Oshikawa J, Kim SJ, Furuta E, Caliceti C, Chen GF, McKinney RD, Kuhr F, Levitan I, Fukai T, Ushio-Fukai M (2012) Novel role of p66Shc in ROS-dependent VEGF signaling and angiogenesis in endothelial cells. Am J Physiol Heart Circ Physiol 302:H724–H732. https://doi.org/10.1152/ajpheart.00739.2011
82. Pagnin E, Fadini G, de Toni R, Tiengo A, Calo L, Avogaro A (2005) Diabetes induces p66shc gene expression in human peripheral blood mononuclear cells: relationship to oxidative stress. J Clin Endocrinol Metab 90:1130–1136. https://doi.org/10.1210/jc.2004-1283
83. Palmer JW, Tandler B, Hoppel CL (1977) Biochemical properties of subsarcolemmal and interfibrillar mitochondria isolated from rat cardiac muscle. J Biol Chem 252:8731–8739
84. Palmer JW, Tandler B, Hoppel CL (1986) Heterogeneous response of subsarcolemmal heart mitochondria to calcium. Am J Physiol 250:H741–H748
85. Paneni F, Mocharla P, Akhmedov A, Costantino S, Osto E, Volpe M, Luscher TF, Cosentino F (2012) Gene silencing of the mitochondrial adaptor p66(Shc) suppresses vascular hyperglycemic memory in diabetes. Circ Res 111:278–289. https://doi.org/10.1161/circresaha.112.266593
86. Perez H, Finocchietto PV, Alippe Y, Rebagliati I, Elguero ME, Villalba N, Poderoso JJ, Carreras MC (2018) p66(Shc) inactivation modifies RNS production, regulates Sirt3 activity, and improves mitochondrial homeostasis, delaying the aging process in mouse brain. Oxid Med Cell Longev 2018:8561892. https://doi.org/10.1155/2018/8561892
87. Pinton P, Rimessi A, Marchi S, Orsini F, Migliaccio E, Giorgio M, Contursi C, Minucci S, Mantovani F, Wieckowski MR, Del Sal G, Pelicci PG, Rizzuto R (2007) Protein kinase C beta and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc. Science 315:659–663. https://doi.org/10.1126/science.1135380
88. Plecita-Hlavata L, Jezek P (2016) Integration of superoxide formation and cristae morphology for mitochondrial redox signaling. Int J Biochem Cell Biol 80:31–50. https://doi.org/10.1016/j.biocel.2016.09.010
89. Qi D, Young LH (2015) AMPK: energy sensor and survival mechanism in the ischemic heart. Trends Endocrinol Metab 26:422–429. https://doi.org/10.1016/j.tem.2015.05.010
90. Qu B, Gong K, Yang H, Li Y, Jiang T, Zeng Z, Cao Z, Pan X (2018) SIRT1 suppresses high glucose and palmitate-induced osteoclast differentiation via deacetylating p66Shc.

Mol Cell Endocrinol 474:97–104. https://doi.org/10.1016/j.mce.2018.02.015
91. Ramsey JJ, Tran D, Giorgio M, Griffey SM, Koehne A, Laing ST, Taylor SL, Kim K, Cortopassi GA, Lloyd KC, Hagopian K, Tomilov AA, Migliaccio E, Pelicci PG, McDonald RB (2014) The influence of Shc proteins on life span in mice. J Gerontol A Biol Sci Med Sci 69:1177–1185. https://doi.org/10.1093/gerona/glt198
92. Sampaio SF, Branco AF, Wojtala A, Vega-Naredo I, Wieckowski MR, Oliveira PJ (2016) p66Shc signaling is involved in stress responses elicited by anthracycline treatment of rat cardiomyoblasts. Arch Toxicol 90:1669–1684. https://doi.org/10.1007/s00204-015-1583-9
93. Savino C, Pelicci P, Giorgio M (2013) The P66Shc/mitochondrial permeability transition pore pathway determines neurodegeneration. Oxid Med Cell Longev 2013:719407. https://doi.org/10.1155/2013/719407
94. Sentinelli F, Romeo S, Barbetti F, Berni A, Filippi E, Fanelli M, Fallarino M, Baroni MG (2006) Search for genetic variants in the p66Shc longevity gene by PCR-single strand conformational polymorphism in patients with early-onset cardiovascular disease. BMC Genet 7:14. https://doi.org/10.1186/1471-2156-7-14
95. Shahzad K, Gadi I, Nazir S, Al-Dabet MM, Kohli S, Bock F, Breitenstein L, Ranjan S, Fuchs T, Halloul Z, Nawroth PP, Pelicci PG, Braun-Dullaeus RC, Camerer E, Esmon CT, Isermann B (2018) Activated protein C reverses epigenetically sustained p66(Shc) expression in plaque-associated macrophages in diabetes. Commun Biol 1:104. https://doi.org/10.1038/s42003-018-0108-5
96. Shi Y, Cosentino F, Camici GG, Akhmedov A, Vanhoutte PM, Tanner FC, Luscher TF (2011) Oxidized low-density lipoprotein activates p66Shc via lectin-like oxidized low-density lipoprotein receptor-1, protein kinase C-beta, and c-Jun N-terminal kinase kinase in human endothelial cells. Arterioscler Thromb Vasc Biol 31:2090–2097. https://doi.org/10.1161/atvbaha.111.229260
97. Shi Y, Luscher TF, Camici GG (2014) Dual role of endothelial nitric oxide synthase in oxidized LDL-induced, p66Shc-mediated oxidative stress in cultured human endothelial cells. PLoS One 9:e107787. https://doi.org/10.1371/journal.pone.0107787
98. Skyschally A, Schulz R, Gres P, Korth HG, Heusch G (2003) Attenuation of ischemic preconditioning in pigs by scavenging of free oxyradicals with ascorbic acid. Am J Physiol Heart Circ Physiol 284:H698–H703. https://doi.org/10.1152/ajpheart.00693.2002
99. Spescha RD, Klohs J, Semerano A, Giacalone G, Derungs RS, Reiner MF, Rodriguez Gutierrez D, Mendez-Carmona N, Glanzmann M, Savarese G, Krankel N, Akhmedov A, Keller S, Mocharla P, Kaufmann MR, Wenger RH, Vogel J, Kulic L, Nitsch RM, Beer JH, Peruzzotti-Jametti L, Sessa M, Luscher TF, Camici GG (2015) Post-ischaemic silencing of p66Shc reduces ischaemia/reperfusion brain injury and its expression correlates to clinical outcome in stroke. Eur Heart J 36:1590–1600. https://doi.org/10.1093/eurheartj/ehv140
100. Tomilov AA, Bicocca V, Schoenfeld RA, Giorgio M, Migliaccio E, Ramsey JJ, Hagopian K, Pelicci PG, Cortopassi GA (2010) Decreased superoxide production in macrophages of long-lived p66Shc knock-out mice. J Biol Chem 285:1153–1165. https://doi.org/10.1074/jbc.M109.017491
101. Tomilov AA, Ramsey JJ, Hagopian K, Giorgio M, Kim KM, Lam A, Migliaccio E, Lloyd KC, Berniakovich I, Prolla TA, Pelicci P, Cortopassi GA (2011) The Shc locus regulates insulin signaling and adiposity in mammals. Aging Cell 10:55–65. https://doi.org/10.1111/j.1474-9726.2010.00641.x
102. Trinei M, Giorgio M, Cicalese A, Barozzi S, Ventura A, Migliaccio E, Milia E, Padura IM, Raker VA, Maccarana M, Petronilli V, Minucci S, Bernardi P, Lanfrancone L, Pelicci PG (2002) A p53-p66Shc signalling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis. Oncogene 21:3872–3878. https://doi.org/10.1038/sj.onc.1205513
103. Vikram A, Kim YR, Kumar S, Naqvi A, Hoffman TA, Kumar A, Miller FJ Jr, Kim CS, Irani K (2014) Canonical Wnt signaling induces vascular endothelial dysfunction via p66Shc-regulated reactive oxygen species. Arterioscler Thromb Vasc Biol 34:2301–2309. https://doi.org/10.1161/atvbaha.114.304338
104. Wang J, Qi J, Wu Q, Jiang H, Yin Y, Huan Y, Zhao Y, Zhu M (2019) Propofol attenuates high glucose-induced P66shc expression in human umbilical vein endothelial cells through Sirt1. Acta Biochim Biophys Sin (Shanghai) 51:197–203. https://doi.org/10.1093/abbs/gmy167
105. Wary KK, Mainiero F, Isakoff SJ, Marcantonio EE, Giancotti FG (1996) The adaptor protein Shc couples a class of integrins to the control of cell cycle progression. Cell 87:733–743
106. Wils J, Favre J, Bellien J (2017) Modulating putative endothelial progenitor cells for the treatment of endothelial dysfunction and cardiovascular complications in diabetes. Pharmacol Ther 170:98–115. https://doi.org/10.1016/j.pharmthera.2016.10.014
107. Xiao Y, Xia J, Cheng J, Huang H, Zhou Y, Yang X, Su X, Ke Y, Ling W (2019) Inhibition of *S*-adenosylhomocysteine hydrolase induces endothelial dysfunction via epigenetic regulation of p66shc-mediated oxidative stress pathway. Circulation. https://doi.org/10.1161/circulationaha.118.036336
108. Xie ZZ, Liu Y, Bian JS (2016) Hydrogen sulfide and cellular redox homeostasis. Oxid Med Cell Longev 2016:6043038. https://doi.org/10.1155/2016/6043038
109. Xie ZZ, Shi MM, Xie L, Wu ZY, Li G, Hua F, Bian JS (2014) Sulfhydration of p66Shc at cysteine59 mediates the antioxidant effect of hydrogen sulfide. Antioxid Redox Signal 21:2531–2542. https://doi.org/10.1089/ars.2013.5604
110. Xiong Y, Yepuri G, Montani JP, Ming XF, Yang Z (2017) Arginase-II deficiency extends lifespan in mice. Front Physiol 8:682. https://doi.org/10.3389/fphys.2017.00682
111. Yang M, Stowe DF, Udoh KB, Heisner JS, Camara AK (2014) Reversible blockade of complex I or inhibition of PKCbeta reduces activation and mitochondria translocation of p66Shc to preserve cardiac function after ischemia. PLoS One 9:e113534. https://doi.org/10.1371/journal.pone.0113534
112. Zhang M, Lin L, Xu C, Chai D, Peng F, Lin J (2018) VDR agonist prevents diabetic endothelial dysfunction through inhibition of prolyl isomerase-1-mediated mitochondrial oxidative stress and inflammation. Oxid Med Cell Longev 2018:1714896. https://doi.org/10.1155/2018/1714896
113. Zhang M, Tang J, Shan H, Zhang Q, Yang X, Zhang J, Li Y (2018) p66Shc mediates mitochondrial dysfunction dependent on PKC activation in airway epithelial cells induced by cigarette smoke. Oxid Med Cell Longev 2018:5837123. https://doi.org/10.1155/2018/5837123
114. Zhao MH, Hu J, Li S, Wu Q, Lu P (2018) P66Shc expression in diabetic rat retina. BMC Ophthalmol 18:58. https://doi.org/10.1186/s12886-018-0724-3
115. Zheng W, Li D, Gao X, Zhang W, Robinson BO (2019) Carvedilol alleviates diabetic cardiomyopathy in diabetic rats. Exp Ther Med 17:479–487. https://doi.org/10.3892/etm.2018.6954
116. Zhu JN, Fu YH, Hu ZQ, Li WY, Tang CM, Fei HW, Yang H, Lin QX, Gou DM, Wu SL, Shan ZX (2017) Activation of miR-34a-5p/Sirt1/p66shc pathway contributes to

doxorubicin-induced cardiotoxicity. Sci Rep 7:11879. https://doi.org/10.1038/s41598-017-12192-y

117. Zhu M, Chen J, Wen M, Sun Z, Sun X, Wang J, Miao C (2014) Propofol protects against angiotensin II-induced mouse hippocampal HT22 cells apoptosis via inhibition of p66Shc mitochondrial translocation. Neuromolecular Med 16:772–781. https://doi.org/10.1007/s12017-014-8326-6
118. Zweier JL, Flaherty JT, Weisfeldt ML (1987) Direct measurement of free radical generation following reperfusion of ischemic myocardium. Proc Natl Acad Sci USA 84:1404–1407