ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 3 & 4, 2006

Forum Review

Apoptosis and Aging: Role of p66^{Shc} Redox Protein

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ABSTRACT

p66^{Shc} was the first mammalian gene whose mutation was demonstrated to increase resistance to oxidative stress and to prolong life span. Many hypotheses have been formulated to explain the biochemical and molecular basis of mammalian aging. Among them the free radical theory of aging, which was first proposed half a century ago by Harman, has received much attention by biomedical scientists. This theory proposed that, because of their high reactivity, reactive oxygen species (ROS) would lead to unavoidable and potentially deleterious by-products, and such an increasingly damaging process could be responsible for degenerative diseases and aging. Recent reports suggest an important role of p66^{Shc} protein in the regulation of cellular responses to oxidative stress, apoptosis, and aging. In this review we discuss what has been discovered about p66^{Shc} in the past 10 years and we focus particularly on its role in ROS regulation, which appears to be extremely promising to define mammalian aging processes. *Antioxid. Redox Signal.* 8, 600–608.

STRUCTURE OF p66^{Shc} PROTEINS

THE ADAPTOR Shc protein was initially identified as an SH2-containing protooncogene involved in growth factor signaling (6, 40). The Shc gene was identified in 1992 while searching for SH2 sequences. The Src homology 2 (SH2) domain is the prototype for protein–protein interaction modules that mediate the formation of multiprotein complexes during signaling (42, 55).

SH2 domains specifically function in protein tyrosine kinase (PTK) pathways, due to the dependence of their binding on tyrosine phosphorylation (42, 55). This ~100 amino acid domain is evolutionarily conserved in many eukaryotes and was found in a wide variety of proteins that participate in many different cellular processes including Ras-like GTPase regulation, phospholipid metabolism, transcription, and cytoskeletal reorganization.

The first *shc* transcript, isolated by screening cDNA libraries with a DNA probe representative of the c-Fes SH2 domain, displayed two in-frame ATGs that encode two polypeptides: the ubiquitously expressed p52^{Shc} and p46^{Shc} proteins. These two isoforms share an amino-terminal SH2 domain, followed by a proline-rich region (CH1) containing critical tyrosine phosphorylation sites implicated in Ras activation (40, 46), and a carboxy-terminal phosphotyrosine binding domain (PTB) (Fig. 1). p66^{Shc} is the third isoform encoded by the human and mouse *shc* loci, which originates from alternative promoter usage (31). It is composed of the entire p52^{Shc}/p46^{Shc} sequence and an additional amino-terminal proline-rich region, named CH2, which contains a serine phosphorylation site implicated in oxidative stress signaling.

A new functional region has been recently characterized within the p66^{Shc} CH2-PTB domains. It is named CB and it is essential for its function in ROS regulation (14) (Fig. 1).

SIGNALING VIA p66^{Shc}

Role in signaling from receptor tyrosine kinases to the ras-mapk of p66^{Shc}-fos pathway

Historically, the first function assigned to p52^{Shc}/p46^{Shc} protein was the involvement in Ras activation (6, 40). Upon growth factor stimulation, p52^{Shc}/p46^{Shc} proteins are rapidly

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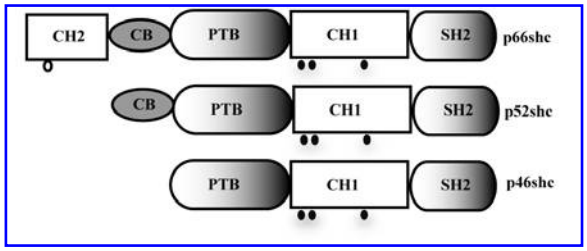

FIG. 1. Schematic representation of the modular structure of Shc proteins. The known phosphorylation sites are shown as *open circle* (Serine 36) or *black circles* (Tyrosines 239, 240, and 317).

and efficiently tyrosine-phosphorylated by all tyrosine kinases tested in three major tyrosine residues present in the CH1 domain, and recruit the Grb2-SOS complex on the plasma membrane (15, 46).

In turn, SOS, through its guanine nucleotide exchange factor (GEF) activity, stimulates the conversion of the inactive Ras GDP to an active Ras GTP that subsequently activates the mitogen-activated protein kinase (MAPK) cascade (1). Recruitment of the Grb2/SOS complex by p52Shc/p46Shc and membrane relocalization of SOS is an event considered sufficient to induce Ras activation.

The hypothesis that Shc proteins are involved in the regulation of Ras is supported by the findings that overexpression of p52Shc/p46Shc induces increased proliferative response and enhances MAPK and fos activation upon stimulation with EGF (31, 46), GM-CSF (26), and PDGF (57).

There is, instead, no indication that p66Shc activates the Ras signaling pathway. Indeed, the demonstration that exists one different regulation between p66Shc and p52Shc/p46Shc is given from studies which have demonstrated that, although p66Shc is a target of receptor tyrosine kinases (EGFR, INSR, PDGFR) (9, 23, 31, 37) and it is able to bind the Grb2/SOS complex, p66Shc overexpression has a negative effect on the RAS-MAPK-fos pathway in response to EGF (31, 37) or to cytokines in lymphocytes (39).

Recently, p66Shc has been shown to exert an inhibitory effect on the Erk pathway, which is necessary for coordinated actin cytoskeleton polymerization and normal IGF-1 responsiveness of the MEK/ERK pathway in skeletal muscle myoblasts (34).

How p66Shc exerts this negative effect is not clear. It was proposed that it acts by competing with p52Shc for Grb2 binding, sequestering the Grb2/SOS complex therefore terminating Ras signaling (37).

Our results, however, suggest that p66Shc acts through a Ras-independent mechanism. The major finding of this sentence is the demonstration that the p66Shc inhibitory effect on the fos promoter is independent from the CH1 domain that contains crucial tyrosine residues for the p52Shc function on Ras signaling (31).

On the other hand, our opinion is that, although several reports have shown that p66Shc is a substrate for many different tyrosine kinases, our experience differently demonstrates that p66Shc tyrosine phosphorylation depends on the cellular context and may simply represent an experimental artifact consequent to the high doses of ligand used for stimulation. Therefore, studies concerning p66Shc functions that made use of very high concentrations of growth factors may need to be revisited.

p66Shc and stress signaling

The characterization of a p66Shc knockout mouse model revealed that p66Shc protein might serve different functions that are, apparently, independent of the Ras pathway. p66Shc null mouse fibroblasts have an enhanced resistance to apoptosis induced by a variety of signals, including hydrogen peroxide, ultraviolet radiation, staurosporine, growth factor deprivation, calcium ionophore, CD3-CD4 cross-linking, and taxol (32, 38, 39). Recently, we demonstrated that, in cultured cells and in tissues, a fraction of p66Shc is localized within mitochondria where it exerts its proapoptotic function (38).

The scenario that is developing from recent studies is that a series of posttranslation modification occurs in p66Shc that can affect its function in cellular stress response. These findings are in agreement with the observation that p66Shc is rapidly and persistently phosphorylated on serine 36 in the N-terminal CH2 domain (Fig. 1) in response to UV or H_2O_2 (32) and replacement of serine 36 residue with alanine prevent p66Shc pro-apoptotic functions. However, the mitochondrial pool of p66Shc is not phosphorylated (unpublished data) and the relative contribution of serine-phosphorylation on p66Shc mitochondrial function remains to be clarified.

Our hypothesis is that serine phosphorylation may influence other nonmitochondrial activities of p66Shc that are also necessary for its proapoptotic function. It has been shown that p66Shc can also be phosphorylated at serine residues in response to epidermal growth factor (EGF), insulin, TPA, FGF-2, taxol, and endothelin-1 (9, 10, 37, 54). EGF-induced serine phosphorylation of p66Shc has been implicated in negative regulation of the MAPK pathway (37). Consistent with this observation, it was shown that p66Shc serine phosphorylation precedes p66Shc tyrosine phosphorylation and that only the nonphosphorylated fraction of p66Shc was associated with EGF receptor (37).

In contrast, TPA-induced serine phosphorylation of p66Shc is thought to be involved in ERK activation because it leads to an increase in p52Shc/Grb2 association. It has been recently reported that TPA specifically induces phosphorylation at Ser 36 and also at Ser 138 of p66Shc (10). Only two functional consequences of p66Shc serine phosphorylation have been reported: binding of the CH2 region to sequestering protein 14-3-3 upon endothelin-induced p66Shc Ser 36 phosphorylation (12) and increased binding of the PTB domain to tyrosine-phosphatase PTP-PEST upon TPA-induced Ser 138 phosphorylation (10).

These data agree with the finding that protein phosphorylation may result in conformational changes, and create binding modules involved in protein–protein interactions. However, even if the proline-rich CH2 region is able to bind many proteins *in vitro* (unpublished results), no correlation between the supposed binding proteins and p66Shc proapoptotic function has been found yet. Furthermore, the identification that p66Shc is target of a large number of serine/threonine kinases has greatly increased the complexity of interpreting the function of p66Shc serine phosphorylation.

The CH2 region of p66^{Shc} is rich in S/TP and S/P motifs that are known to form recognition sites for phosphorylation by proline-directed MAP/kinase (24), and has been shown as a good *in vitro* substrate for ERK, JNK, and p38 MAPK (27). It has become clear that p66^{Shc} can, in fact, functionally interact with the mitogen-activated protein kinase (MAPK) pathways, including ERK, JNK and p38 MAPK, also *in vivo* (27).

The complexity of this scenario may be linked to the function of p66^{Shc} in regulating the levels of reactive oxygen species (ROS), as discussed later. In fact, we cannot exclude that p66^{Shc} ROS regulation may also influence p66^{Shc} post-translational modifications, including serine and tyrosine phosphorylation, and further characterization of the interplay between intracellular redox status, p66^{Shc} induction, and function is likely to be an informative field of investigation in the future.

p66^{Shc} AND ROS SIGNALING

p66^{Shc} regulates intracellular ROS

p66^{Shc} modulates intracellular redox balance by increasing reactive oxygen species (ROS) concentration (14, 51). Many studies have clearly shown the involvement of ROS in the development of diverse pathologies such as radiation injury, carcinogenesis, aging, ischemia, and atherosclerosis. Low levels of ROS regulate cellular signaling and play an important role in normal cell proliferation. An excessive ROS production, instead, results in cell death and the balance between ROS production and antioxidant defences determines the degree of oxidative stress. Consequences of oxidative stress include modifications of cellular proteins, lipids, and DNA.

The following observations support the role of ROS in inducing apoptosis: (a) mitochondria are the principal source of production of ROS due to the leakage of the electron transport chain ETC, and elevated production of ROS leads to mitochondrial-mediated apoptosis (41); (b) antioxidants, such as *N*-acetylcysteine, can attenuate apoptosis (19). It has been shown that intracellular ROS concentration, as detected by an oxidation sensitive dye, is higher in WT than in p66^{Shc} −/− MEFs (51) and overexpression of p66^{Shc} in p66^{Shc} −/− or WT MEFs or in other cell lines (e.g., endothelia, p53−/− cells) increased ROS levels (35, 51).

Mitochondrial overproduction of ROS increases also upon activation of the tumor suppressor protein p53, which plays a pivotal role in regulating cell cycle arrest, differentiation, and apoptosis. However, recent data show that the activity of p66^{Shc} on ROS metabolism is linked to p53 that exerts its pro-apoptotic effect and aging properties by inducing oxidative damage through p66^{Shc}, a mechanism that does not interfere with other p53 functions (51).

CONSEQUENCES OF p66^{Shc} ROS REGULATION

p66^{Shc} induces intracellular oxidative damage

There is strong evidence that increased levels of ROS during aging result in an increase of protein and DNA damage, and that mutations and deletions of both nuclear and mitochondrial DNA are much more frequent in older individuals of most species (47). Coherent with the finding that p66^{Shc} increases intracellular ROS concentration, oxidation of the C8 of guanine (8-oxo-dG), which is the most abundant type of oxidation-damaged nuclear DNA (18), and mutations of mitochondrial DNA, are decreased in cells derived from p66^{Shc} −/− mice (51). Furthermore, levels of oxidative damage correlate with the expression level of p66^{Shc}, which is higher in the lung, spleen, liver, and skin, whereas no significant oxidative damage is found in brain that does not expresses p66^{Shc} (51).

p66^{Shc} induces apoptosis

Apoptosis is a mechanism of controlled cell death that regulates many biological processes, including development, inflammation, immune system, and aging (41). Two main integration pathways exist and converge to a common execution phase: the death receptor pathway and the mitochondrial pathway (7). The Death Receptor Pathway is typically engaged in the immune system and is the method used to eliminate activated T-cells at the end of an immune response. The Mitochondrial Pathway is usually activated in response to other lethal stimuli such as DNA damage, oxidative stress, and hypoxia. Mitochondria-mediated apoptosis involves signaling pathways that induce various protein responses (e.g., post-translational modifications, conformational changes, and interorganelle translocation of specific proteins), alteration of mitochondrial membrane permeability, and release of apoptogenic factors (e.g., cytochrome *c* and apoptosis-inducing factor) and activation of cysteine protease caspases that induce biochemical and a morphological change in both cytoplasm and nucleus before cell death (21).

The Bcl-2 family members control the release of apoptogenic factors in the cytoplasm. The family consists of pro- and anti-apoptotic members, which regulate mitochondrial 'pores' by a yet uncharacterized mechanism. Upon apoptotic stimuli, cytochrome *c* is released in the cytosol, and associates with both Apaf-1 and procaspase 9 to form active caspase 9, the active enzyme complex is called the apoptosome. Apoptosome formation leads to activation of downstream caspases (such as caspase-3) that are responsible for the execution phase of apoptosis (specific degradation of proteins and DNA) (7).

We have shown that p66^{Shc}−/− cells are resistant to apoptosis induced by a variety of different signals, including hydrogen peroxide, ultraviolet radiation, staurosporine, growth factor deprivation, calcium ionophore, and CD3-CD4 cross-linking (32, 38, 39). Recent results demonstrated that p66^{Shc} regulates the mitochondrial pathway of apoptosis and acts upstream of the mitochondrial pore (PT) (31, 38). The relevance of the p66^{Shc} role in the mitochondria pathway is supported by several lines of evidence (38, 51): (a) p66^{Shc} protein is partly localized in the mitochondria; (b) cyclosporine A, an inhibitor of mitochondrial pore (PT), prevents the ability of p66^{Shc} to induce H_2O_2-induced apoptosis; (c) p66^{Shc} is required for the collapse of the mitochondrial transmembrane potential induced by oxidative stress; (d) cytochrome *c* release and caspase activation are impaired in the absence of

p66Shc. The strong implication of these results is that the consequences of ROS accumulation (apoptosis and oxidative damage) during aging are genetically determined and controlled by a stress-induced signal transduction pathway, which involves p53 and p66Shc.

THE INVOLVEMENT OF p66Shc IN CANCER DEVELOPMENT

The Shc gene is related to cell proliferation and carcinogenesis (16, 48). In fact the p52Shc and p46Shc isoforms of Shc were found to be overexpressed (5, 8, 56, 58) or hyperphosphorylated (28, 49) in many tumor types. Clearly the two shortest isoforms of Shc play a role in the proproliferating functions of many cellular and viral oncogenes (4, 43, 44). On the contrary the involvement of p66Shc in tumorigenesis is still controversial. Indeed different levels of p66Shc were found in some tumors but the results seem contradictory. For example, in breast cancer cells and primary tumors, p66Shc was described to be both overexpressed (16, 22, 29) and downregulated (49, 59). Indeed the ablation of p66Shc does not increase spontaneous or induced tumor incidence in mice (32, 51). Furthermore the transformation rate and phenotype of primary embryonic fibroblast derived from p66−/− or from the WT mice are comparable (51; unpublished data). Thereby although p66Shc is a key component of the apoptotic execution machinery, it seems that its proapoptotic role is dispensable for any relevant tumor suppressive function.

MECHANISM OF p66Shc ROS REGULATION

We can therefore assert that the key functions of p66Shc are in the intracellular pathway(s) that regulate(s) ROS metabolism and apoptosis. In aerobic organisms, ROS are generated in different compartments within the cell: in mitochondria and peroxisomes, as result of normal intracellular metabolism (36).

A complex enzymatic and nonenzymatic antioxidant defense system including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (Gpx) neutralizes and controls overall ROS levels to maintain physiological homeostasis (50).

The effect of p66Shc expression on ROS scavenging was studied with the aim of understanding the mechanisms underlying the regulatory effect of p66Shc on mitochondrial ROS generation. Two alternative mechanisms have been proposed: Nemoto and Finkel, using an immortal clone of p66Shc −/− fibroblasts have shown that p66Shc is required for Akt-mediated phosphorylation and inactivation of the transcription factor Foxo3a/FKHRL1 in response to serum starvation or oxidative stress (35). These authors also showed that one of the genes activated by Foxo3 is a catalase, whose function is to remove ROS. Based on these data, they propose that in p66Shc null cells, Foxo3 is constitutively active, induces higher levels of catalase that, in turn, is responsible for the reduced ROS concentrations.

In contrast with these findings, we recently showed that the reduced ROS levels in p66Shc −/− cells and tissues are not the consequences of increased scavenging activity (14). Thanks to sophisticated and complex experiments, the molecular mechanism through which p66Shc regulates ROS has recently been characterized and reveals that p66Shc has the amazing property to directly stimulate mitochondrial ROS generation by an enzymatic activity.

The major source of mitochondrial ROS is oxidative phosphorylation. In the respiratory chain, the mitochondrial electron transport system receives electrons from NADH or from flavoprotein-linked dehydrogenases and ultimately reduces oxygen to water by an electron transfer from cytochrome *c* to cytochrome *c* oxidase. An estimated 2–4% of the total oxygen consumed during electron transport is not reduced to water by cytochrome c oxidase but rather to superoxide anion ($O_2^{\bullet -}$), by reduced semiquinone and by reducing equivalents that derive from complex III or II (2, 3). Superoxide dismutase (SOD) converts superoxide enzymatically into hydrogen peroxide, which, in turn, may be converted into water by the enzymes catalase or glutathione peroxidase.

In studies of signaling mechanism by ROS, it is often difficult or impossible to determine the specific forms of reactive oxygen that produces specific ROS ($O_2^{\bullet -}$ or H_2O_2). Routinely, experiments with purified mitoplasts and H_2DCFDA, a fluorescent probe that is oxidized by H_2O_2 (30) can be used to measure ROS production. Experiments with recombinant p66Shc on purified mitoplasts indicated that the effect of p66Shc on mitochondrial ROS production is to directly stimulate mitochondrial H_2O_2 generation (14). The specificity in this experimental system was confirmed by the findings that basal and p66Shc-induced H_2DCFDA oxidation by mitoplasts was inhibited by catalase (an H_2O_2 scavenger), but not by superoxide dismutase (SOD) (which generates H_2O_2 from $O_2^{\bullet -}$).

Next, elaborate experiments with cyclic voltammetry established that p66Shc is capable itself of successfully executing an electron transfer reaction (14).

It has been largely demonstrated in different experimental systems that a fraction of p66Shc is localized within mitochondria, and almost 35% of mitochondrial p66Shc localizes within the mitochondrial intermembrane space (14). Another protein that localizes in the mitochondrial intermembrane space and that is involved in electron transfer reactions and apoptosis is cytochrome *c* (21). Interestingly, p66Shc interacts with and oxidizes cytochrome *c* generating hydrogen peroxide (14) (Fig. 2).

The redox centre of p66Shc has been mapped to the CH2 domain, within a region that presents the highest degree of identity in sequence alignments of the known p66Shc vertebrate orthologues. This region (designated CB) contains three glutamic (E125, E132, E133) and two tryptophan (W134 and W148) conserved residues (Fig. 1). The same residues are found within the cytochrome *c* pocket region of COX IV and yeast cytochrome *c* peroxidase, which represent the two known redox enzymes that utilize cytochrome *c* as a substrate. In both cases, the negatively charged glutamic residues are critical for the interaction with the positively charged cytochrome *c,* and the aromatic tryptophan residues are essential for the electron transfer reaction with reduced cytochrome *c* (61). Mutations in the CB region prove that the redox centre of p66Shc acts to stimulate H_2O_2 generation *in vitro* and *in vivo,* and to mediate cellular apoptosis after stress.

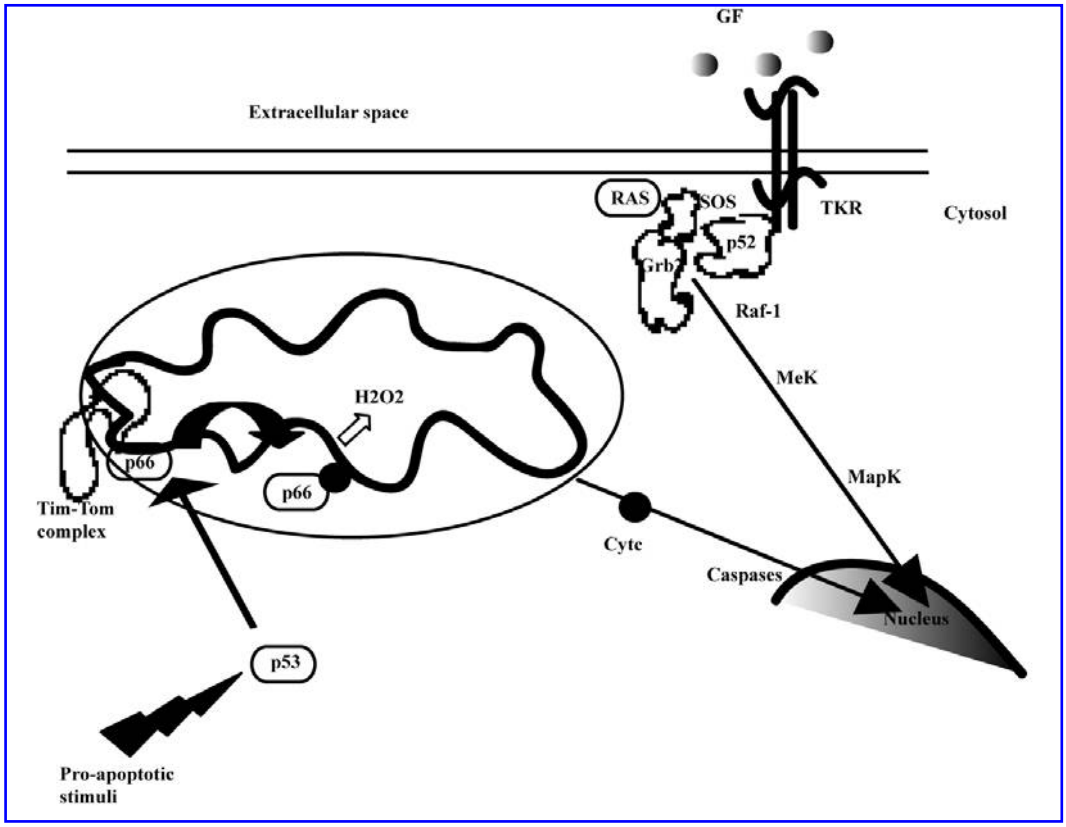

FIG. 2. Schematic description of contributions of the Shc proteins in survival and death. p52^{Shc} participates in receptor tyrosine kinase-dependent Ras activation. Proapoptotic signals induce on mitochondria release of p66^{Shc} from a putative inhibitory complex including mtHsp70. Active p66^{Shc} then oxidized reduced cyt*c* (*black circle*) and generates H_2O_2 that leads to mitochondrial swelling and apoptosis.

We believe that the discrepancy between our report and the data presented by Nemoto *et al.* is at least partly explained by the use of different model systems. Nemoto and Finkel, in fact, performed their study using an immortalized clone of p66^{Shc} −/− mouse embryo fibroblast, which has a high probability of carrying multiple alterations that could have affected the FKHRL1 pathway. In addition, we have established a new and surprising redox property of p66^{Shc} protein.

In conclusion, the p66^{Shc}/cytochrome *c* electron transfer reaction might give an alternative mechanism of generating ROS in mitochondria, representing an intriguing example of the link between ROS generation and biology of aging, as proposed by free radical theory of aging (17).

MECHANISM OF p66^{Shc} REGULATION: TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL

The results mentioned above demonstrate that the main function of p66^{Shc} is to produce proapoptotic H_2O_2 through an electron transfer reaction with cytochrome *c*. Nevertheless, p66^{Shc} is a stress response protein that does not influence the mitochondrial function under steady state conditions, but is indispensable for mitochondria-mediated apoptosis following a proapoptotic signal (14, 38, 51). These findings are compatible with the existence of an inactive form of p66^{Shc} under basal conditions, and an active form after proapoptotic signals. A series of posttranslation modifications occur in p66^{Shc}, subsequent to treatment with proapoptotic signals, which can affect its level, its stability, its localization, its heterooligomerization, and its monomerization, and that probably modulate its activity: (a) Upon stress stimuli, p66^{Shc} is serine phosphorylated and, as mentioned before, this modification is required for its ability to mediate apoptosis (32); (b) a small quantity of p66^{Shc} translocates from the cytosol to mitochondria upon apoptotic stimuli (38); (c) p53 induces an increase in p66^{Shc} protein levels by increasing its stability (38); (d) in basal conditions, the mitochondrial p66^{Shc} fraction is found within multimolecular complexes including mtHsp70 and components of TIM-TOM import complex, and becomes monomeric only following proapoptotic stimuli (14, 38) (Fig. 2).

However, future studies will be required to elucidate the precise role of p66^{Shc} posttranslation modifications that might work in a concerted manner to regulate ROS production and apoptosis.

Many results suggest that, in addition to posttranslational modifications, transcriptional regulation could also play a

role in regulating p66Shc function. First of all, levels of p66Shc expression correlate with lifespan in mice, since heterozygote mice p66Shc +/− display an intermediate lifespan compared with wt and p66Shc −/−. Further, p66Shc (mRNA and protein) is upregulated in skeletal muscle, spinal cord, and forebrain of aged rats and the extent of this behavior increases with age (20). In accordance with p66Shc promoter regulation by epigenetic modifications (52), p66Shc (mRNA and protein) is expressed at different levels in specific tissues, such as lung, spleen, liver, heart, and kidney, but is absent in brain (51).

Nevertheless, transcriptional levels of control of p66Shc have been identified in cells, such as peripheral blood lymphocytes, mouse thymocytes, and splenic T-cells that acquire the capacity to express p66Shc in response to apoptogenic stimuli (39).

Although the involvement of p66Shc in cellular senescence may require further investigation, few recent reports show a correlation between high levels of p66Shc expression (mRNA and protein) and senescence, increase of ROS, and oxidative stress (11, 20, 25). Another interesting study showed that the p66Shc promoter is sensitive to treatment with aurintricarboxylic acid (ATA), which protects cells against a variety of death stimuli and in *Drosophila* induces an increase of lifespan (45). However, a treatment with ATA decreases level in p66Shc protein in mouse lung (45).

CONSEQUENCES OF p66Shc ROS REGULATION *IN VIVO*: p66Shc AND AGE-RELATED DEGENERATIVE PATHOLOGY

Many reports since 1999 confirmed that p66Shc −/− mice are a helpful mammalian model to study the molecular mechanism of aging. The mice lacking p66Shc live 30% longer than control animals, and experiments using paraquat (a free radical generating compound) represented the first evidence that ablation of p66Shc expression enhances resistance to oxidative stress also *in vivo* (32).

The finding that p66Shc deletion delays aging, and its implication in age-associated pathology was supported by much evidence: (a) a high-fat diet has no effect on p66Shc −/− mice, whereas this diet in wt mice results in signs of early atherogenesis (increased of aortic cumulative early lesion, apoptosis in vascular cells, and tissue oxidative stress) (33); (b) p66Shc −/− mice do not show significant age-dependent decrease of ROS-mediated endothelial function, such as aortic relaxation that is impaired in old wild-type mice, but not in p66Shc −/− mice (13); (c) p66Shc −/− mice show decrease of tissue damage and apoptosis induced by acute ischemia (60); (d) p66Shc deletion does not increase incidence of cancer.

Therefore, the p66Shc −/− mouse model might also be an excellent model for vascular disease as well as longevity. The relevance of this model is related to p66Shc and H_2O_2 production, which now represent a new key to revisiting the known theory of aging.

In summary, insulin-like signaling, modulation of forkhead activity, and p53 attenuation are a major mechanism for increasing lifespan and now one interesting open question is how p66Shc-mediated H_2O_2 production works with these pathways.

CONCLUSIONS

Shc proteins were initially characterized as signal transduction adapters involved in the cytoplasmic propagation of mitogenic stimuli, through Ras.

Recent findings, however, indicate a more general role of Shc proteins in regulating cellular and organism homeostasis. Genetic and biological evidence indicate that the three isoforms are not functionally redundant and regulate growth (p52Shc), apoptosis (p66Shc), and an unknown signaling transduction pathway involving the mitochondria (p46Shc) (53) (Fig. 2). The surprising new message is that p66Shc is an atypical signal transducer which can be regulated by oxidative stress and that the p66Shc/cytochrome *c* electron transfer reaction represents an integrative model for H_2O_2 generation.

Further studies are required to elucidate the p66Shc role in regulation of intracellular redox balance and its role in integration of cellular pathways, that are relevant to the process, which regulate Ras (growth) and p53 (apoptosis). Remarkably, the p66Shc −/− mouse model provides direct support for the involvement of ROS in aging (17), and the discovery of a p53-p66Shc signaling pathway could initiate a complex but exciting new area of research in apoptosis and aging.

ACKNOWLEDGMENTS

The authors thank M. Alcalay, C. Contursi, L. Luzi, N. Offenhäuser, and F. Orsini for helpful discussions.

ABBREVIATIONS

CAT, catalase; CB, cytochrome *c* binding; CH1, collagen-homology domain 1; CH2, collagen-homology domain 2; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; ETC, electron transport chain; FGF-2, fibroblast growth factor-2; GEF, guanine nucleotide exchange factor; GM-CSF granulocyte-macrophage colony-stimulating factor; Gpx, glutathione peroxidase; H_2DCFDA, dichlorodihydrofluorescein; H_2O_2, hydrogen peroxide; IGF-1, insulin-like growth factor-I; INR, insulin receptor; MEF, mouse embryo fibroblast; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; PTB, phosphotyrosine-binding domain; PTK, protein tyrosine kinase; ROS, reactive oxygen species; SH2, Src homology 2; SOD, superoxide dismutase; TPA, tissue plasminogen activator.

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Received after revision October 6, 2006; accepted October 7, 2006.

This article has been cited by:

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