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p66SHC: The apoptotic side of Shc proteins

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Initially identified as components of the signaling pathways triggered by receptor tyrosine kinases and leading to Ras activation, Shc proteins have been more recently implicated in the regulation of signals controlling not only cell proliferation, but also cell survival and apoptosis. Here we briefly review the current understanding of Shc proteins as promoters of apoptosis. Specifically, we focus on the 66 kDa isoform of ShcA, whose paramount importance in the regulation of oxidative stress responses leading to cell apoptosis and ageing has been by now firmly established.

Keywords: ageing; forkhead; mitochondria; oxidative stress; ROS; serine phosphorylation.

The Shc protein family

Shc proteins belong to a family which includes three members, known as ShcA, ShcB and ShcC, of which the best characterized to date is ShcA. All Shc proteins share a unique highly conserved domain organization, characterized by an N-terminal PTB domain and a C-terminal SH2 domain, which represents a signature of this family.[1] As opposed to ShcA, which is ubiquitously expressed, the expression of ShcB and ShcC appears restricted to neuronal cells.[2,3]

ShcA (which will be referred to from now on as Shc) was identified in 1992 as an adaptor coupling the activated EGF receptor to Ras and the MAP kinase cascade.[4,5] The existence of multiple Shc isoforms, as well as their apparent functional redundancy with Grb2, has however posed a puzzle which has only recently started to be unraveled. Shc is expressed as three isoforms of 66, 52 and 46 kDa respectively, all of which are encoded by the same genetic locus and display a common domain organization (Figure 1). The central proline-rich collagen homology (CH1) domain has three phosphorylatable tyrosine residues, 239/240 and 317 in human p52Shc, which in their phosphorylated state mediate Grb2/Sos recruitment to a membrane-proximal localization.[4,5] In some mammalian cell types these two Grb2 binding sites have been proposed to differentially couple cell surface receptors either to fos-dependent cell proliferation (Y317) or to myc-dependent survival (YY239/240).[6] Furthermore, the high proportion of proline residues in the CH1 domain make it a target of SH3 domain-dependent interactions,[7,8] further underlining the capacity of Shc proteins to function as molecular adaptors.

The longest isoform, p66Shc, has an additional N-terminal CH domain, termed CH2, containing a phosphorylatable serine residue at position 36 (S36). As opposed to the initial idea that p66Shc would also participate in receptor tyrosine kinase-dependent Ras activation, a dualism in the function of p52/p46Shc and p66Shc has emerged. Indeed, p66Shc has been shown to inhibit activation of the Ras/MAP kinase pathway triggered by the EGF and IGF-I receptors, as well as by the T-cell antigen receptor.[9–12] Furthermore, p66Shc-deficient T-cells have been recently reported to proliferate faster than their normal counterparts in response to limiting concentrations of ligand,[12] supporting an antagonistic activity of p66Shc on mitogenic signaling. Inhibition of Ras/MAP kinase activation by p66Shc requires phosphorylation on S36, which is triggered in response to receptor engagement in a MEK-dependent manner.[10] The negative effect of p66Shc on mitogenic signaling involves competitive inhibition of p52Shc binding to its upstream molecular partners and sequestering Grb2/Sos in a signaling-incompetent complex.[10,12] The mechanism whereby p66Shc-bound Grb2 becomes uncoupled from Ras remains to be determined. The finding that p66Shc participates in a complex which also includes RasGAP during early morphogenetic events in Xenopous gastrulation[13] suggests a possible clue to the negative control of Ras/MAP kinase activation by this protein. Whatever the mechanism, control of p66Shc expression provides the cell with an effective mechanism for fine-tuning the Ras/MAP kinase pathway. More recently, studies using a mouse selectively lacking p66Shc have unveiled an unexpected role of p66Shc in apoptotic responses to oxidative stress and in ageing.[14] This proapoptotic activity of p66Shc, which is unique to this isoform, will be discussed below.

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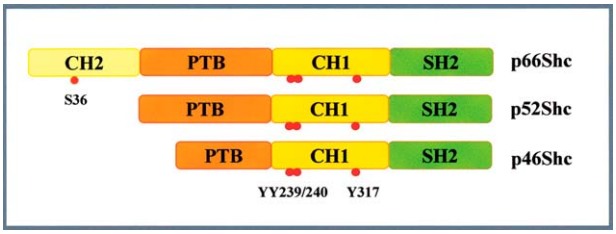
Figure 1. Schematic structure of ShcA proteins.



Regulation of p66Shc expression

The p52 and p46 isoforms of Shc are ubiquitously co-expressed by translation of a common transcript from two alternative initiation codons.[15] Conversely, p66Shc expression is more restricted. Specifically, p66Shc is absent or expressed at very low levels in hemopoietic cell lines, normal peripheral blood lymphocytes and brain, as well as in a subset of breast cancer cell lines.[15–18] This different pattern of expression of p66Shc compared to p52/p46Shc results from usage of two alternative promoters at the Shc locus, of which the proximal selectively controls transcription of the exon encoding the N-terminal CH2 domain. Silencing of p66Shc gene expression is controlled at least in part by epigenetic modifications of its promoter. Indeed, analysis of the p66Shc gene promoter in cells expressing different levels of the protein has revealed an inverse correlation between promoter methylation and p66Shc expression.[19] Accordingly, treatment with demethylating agents or with histone deacetylase inhibitors results in p66Shc expression in cells lacking p66Shc.[19]

Although promoter methylation is likely to be the main mechanism responsible for the absence of p66Shc in some cell types, post-transcriptional levels of control have been identified in cells which normally express p66Shc. Treatment of fibrobroblasts with oxidants, such as H_2O_2 or UV radiation, results in increased expression of p66Shc, which can be correlated to an increased half-life of the protein.[20] Interestingly, human peripheral blood lymphocytes, as well as mouse thymocytes and splenic T-cells, acquire the capacity to express p66Shc in response to apoptogenic stimulation.[12] Whether demethylation of the p66Shc gene promoter or additional mechanisms underlie induction of p66Shc expression in T-cells remains to be determined. It can nevertheless be hypothesized that p66Shc expression is limited in some cell types only to the time when the mechanisms responsible for cell cycle arrest or apoptosis must be activated. This notion is supported by the recent finding that, while p66Shc is undetectable in healthy cardiomyocytes, pacing-induced dilated cardiomyopathy, which is characterized by an increased production of reactive oxygen species (ROS) and apoptosis, is accompanied by a progressive accumulation of p66Shc in failing myocytes.[21]

p66Shc in oxidative stress and apoptosis

Oxidative stress results in the intracellular production of ROS by mitochondria. The increased levels of ROS interfere with many cellular processes, resulting in inhibition of cell proliferation and induction of apoptosis. ROS are furthermore major players in cell ageing.[22,23] Ablation of p66Shc gene expression in the mouse by homologous recombination has established a crucial role for this protein in oxidative stress responses and apoptosis. Indeed p66Shc$^{-/-}$ fibroblasts harbour increased resistance to treatment with oxidative stress-inducing agents, which can be correlated with a reduction in the apoptotic responses to these stimuli. Conversely, p66Shc overexpression results in enhanced stress-induced apoptosis.[14] A protective effect of p66Shc ablation on thymocyte and peripheral T-lymphocyte apoptosis induced by dexamethasone has also been recently reported.[12] Furthermore, p66Shc overexpression in Jurkat T-cells results in significantly inceased levels of both spontaneous and induced apoptosis.[12] Hence, p66Shc appears as a sensor of intracellular ROS concentrations which, by promoting apoptosis, would regulate the cellular lifespan.

The proapoptotic activity of p66Shc is strictly dependent on phosphorylation of S36 in the CH2 domain, as demonstrated by mutational analysis of this residue.[12,14] S36 phosphorylation is triggered by oxidative stress-inducing stimuli, such as H_2O_2 and UV radiation.[12,14] Other apoptogenic stimuli, both pharmacological and receptor-mediated, have been reported to induce S36 phosphorylation, including Fas ligation, T-cell antigen receptor engagement in the absence of costimulation, or treatment with a calcium ionophore in Jurkat T-cell transfectants overexpressing p66Shc.[12] Furthermore, treatment with taxol, an antimitogenic drug which also promotes apoptosis, has been shown to result in S36 phosphorylation in A549 human lung carcinoma cells and in RAW 264.7 murine macrophages.[24,25] Pharmacological studies in living cells, as well *in vitro* studies using purified kinases, indicate that, depending on the cellular context and on the identity of the stimulus, either the MAP kinases Erk1/2 or the stress-activated kinases JNK and p38 are responsible for S36 phosphorylation (refs.[10,24–26] and our unpublished results). Interestingly, mutational analysis of p66Shc in Jurkat T-cell transfectants showed that the apoptogenic activity of the protein requires not only S36, but also YY239/240 and Y317,[12] the tyrosine residues which when phosphorylated interact with Grb2, suggesting that p66Shc may need to assemble in a multimolecular complex to promote apoptosis.

Strong support for a pivotal role of p66Shc in oxidative stress responses comes from studies on mice deficient for p66Shc expression. p66Shc$^{-/-}$ mice show a longer survival after treatment with paraquat, a drug which

increases the production of superoxide anion, underlining *in vivo* the inverse correlation between p66Shc expression and resistance to oxidative stress.[14] A correlation between p66Shc expression and oxidative stress has also been established in animals subjected to a chronic high-fat dietary regime. Administration of a high-fat diet results in increased levels of serum cholesterol and in a concomitant systemic increase in the levels of ROS. Oxidized low density lipoproteins (LDL) and other redox-sensitive pathways are critically implicated in the development of vascular dysfunction and atherogenesis. p66Shc$^{-/-}$ mice fed with a high-fat diet develop hypercholesterolemia similar to wild-type mice. However, the absence of p66Shc is associated with an increased resistance to high-fat diet-induced atherogenesis, decreased levels of oxidized LDL in the arterial walls and reduced vascular apoptosis. Furthermore, p66Shc$^{-/-}$ mice show a constistent reduction in systemic oxidative stress and LDL oxidability,[27] supporting a causal role of p66Shc in oxidative stress-induced damage.

Remarkably, p66Shc$^{-/-}$ mice have a $\sim$30% longer lifespan compared to their normal counterparts.[14] Accumulation of oxidative damage to mitochondrial DNA, which represents a critical event during the ageing process, is significantly decreased in p66Shc$^{-/-}$ tissues.[20] In view of the role of ROS as key inducers of ageing, the increased capacity of p66Shc$^{-/-}$ mice to oppose oxidative stress is likely to underlie their increased longevity.

How does p66Shc work?

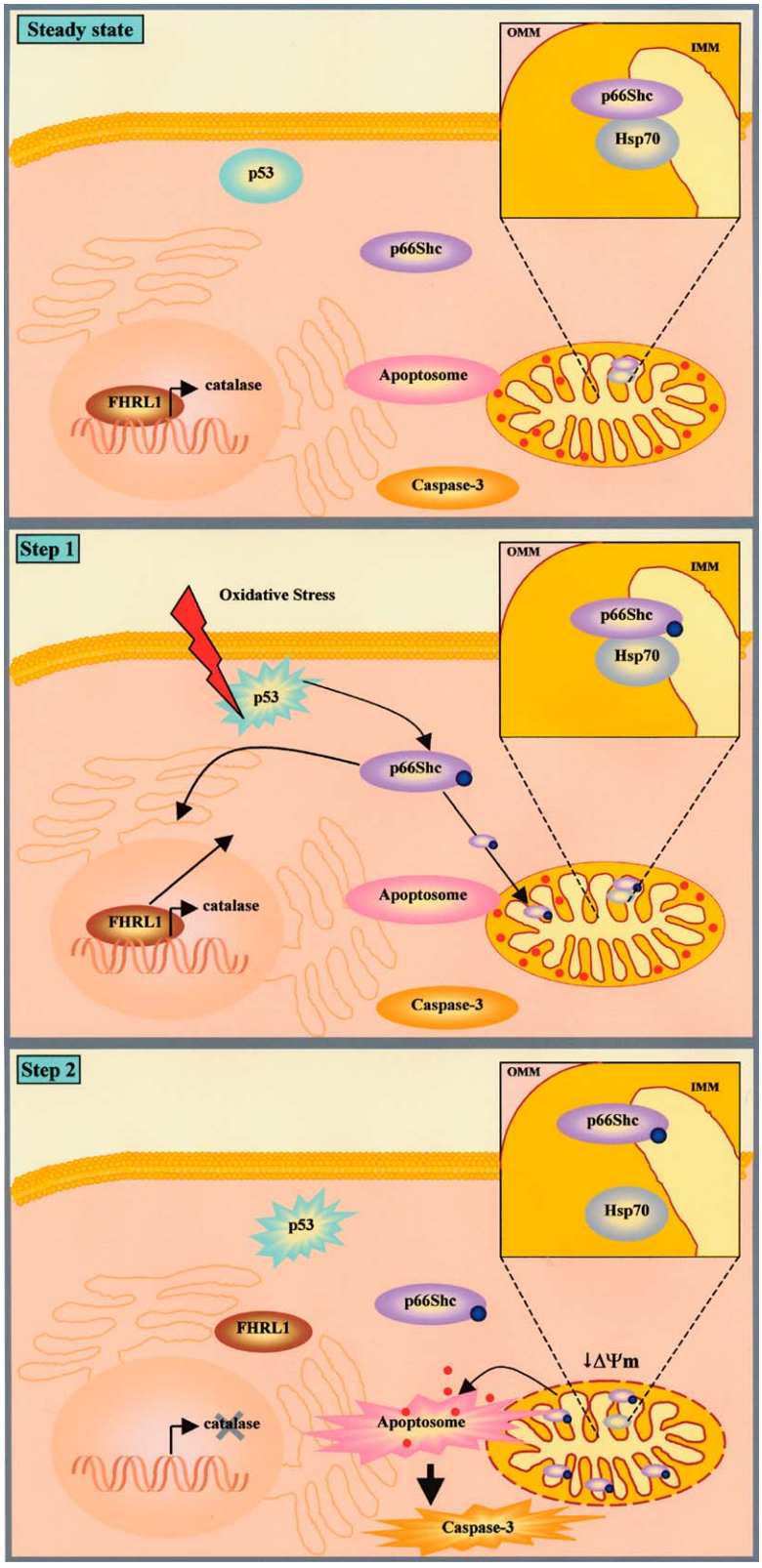
While the causal role of p66Shc in apoptotic responses to oxidative stress is at this stage firmly established, the mechanisms underlying this activity are only beginning to be elucidated. Two recent reports, both based on the analysis of embyonic fibroblasts from p66Shc$^{-/-}$ mice (MEF), have contributed a significant advance to our understanding of this issue. The tumor suppressor p53 is critically involved in oxidative stress-dependent apoptosis.[14,20] One of the principal effects of p53 activation is release from mitochondria of cytochrome c, which interacts with caspase-3, thereby promoting apoptosome assembly.[28–30] Trinei *et al.*[20] have shown that p66Shc is a downstream effector of p53 in stress-induced apoptosis. First, activated p53 induces and is required for upregulation of p66Shc expression. This effect is the result of increased p66Shc protein stability. Second, p66Shc is essential for p53-dependent apoptosis, but not for other activities of p53, such as its capacity to induce cell cycle arrest. Indeed, release of cytochrome c and caspase-3 activation fail to occur in p66Shc$^{-/-}$ MEFs treated with oxidative stress-inducing stimuli, a defect which can be fully reverted by reconstitution of p66Shc expression in these cells.[20] The capacity of p66Shc to mediate p53-dependent apoptotic responses requires phosphorylation of S36 which, as indicated above, is triggered by oxidative stress.[20]

The release of cytochrome c from mitochondria in oxidative stress is the endpoint of the p53-dependent transcriptional activation of redox-related genes. The resulting sustained rise of intracellular ROS levels affects the mitochondrial membrane potential, leading to membrane permeability transition and cytochrome c release.[28–30]. Cyclosporin A, an inhibitor of the mitochondrial permeability transition pore which blocks oxidative stress-induced apoptosis of wild-type MEFs, is able to prevent the ability of re-expressed p66Shc to restore apoptotic responses to oxidants in p66Shc$^{-/-}$ MEFs, suggesting that p66Shc may regulate mitochondrial pemeability transition, and hence cytochrome c release, by modulating the production of ROS.[31] This notion as been substantiated by two independent reports, which demonstrate that the increase in intracellular ROS levels triggered by oxidative stress is drastically reduced in p66Shc$^{-/-}$ cells. Conversely, p66Shc overexpression results in enhanced ROS production.[31,32] Remarkably, p66Shc appears to also regulate steady-state levels of intracellular ROS,[31] which is likely to underlie the accumulation of mitochondrial DNA damage in wild-type mice compared to p66Shc$^{-/-}$ mice.[20]

The capacity of p66Shc to modulate the levels of intracellular ROS has furthermore been linked to the transcriptional activity of the forkhead family transcription factor, FKHRL1, a mammalian homogue of DAF-16, which controls lifespan in *C. elegans*.[32] FKHRL1 is prevalently localized in the nucleus of quiescent cells, where it positively controls the expression of genes encoding proteins, such as catalase, implicated in ROS scavenging.[32] Oxidative stress promotes FKHRL1 phosphorylation and in its relocalization to the cytoplasm, resulting in a reduction of its trascriptional activity. Phosphorylation of FKHRL1 in response to H_2O_2 fails to occur in p66Shc$^{-/-}$ MEFs and, accordingly, FKHRL1-dependent trascriptional activity is augmented in these cells, supporting a pivotal role of p66Shc in the redox-dependent inactivation of FKHRL1 and thereby in the control of intracellular ROS.[32] Although the mechanisms whereby p66Shc regulates FKHRL1 phosphorylation remain to be understood, p66Shc has been shown to modulate gene expression also in Jurkat T-cell transfectants overexpressing p66Shc, where a decreased expression of the antiapoptotic gene *Bcl-xL*, and an increased expression of the proapoptotic genes *bax*, *GADD45* and *FasL*, has been observed.[12]

The capacity of p66Shc to control the release of cytochrome c from mitochondria and the subsequent activation of caspase-3, as well as the recent demonstration that p66Shc induces mitochodrial membrane depolarization following oxidative stress, strongly supports the notion that the mitochondrion is a target of p66Shc. Intracellular localization studies have revealed that a fraction of p66Shc

Figure 2. p66Shc in oxidative stress-induced apoptosis. *Top panel,* Steady state condition. *Middle panel,* Oxidative stress-induced apoptosis, step 1. *Bottom panel,* Oxidative stress-induced apoptosis, step 2. Phosphorylation of p66Shc on serine 36 is shown as a small blue circle. Cytochrome c is shown as small red circles. OMM, outer mitochondrial membrane; IMM, inner mitochondrial membrane; $\Delta\Psi m$, mitochondrial membrane potential difference.



is constitutively associated with mitochondria, and that oxidative stress induces an increase in mitochondrial p66Shc. Of note, mitochondrial p66Shc forms a constitutive complex with mitochondrial Hsp70, a chaperonin which has been implicated in protection from oxidative stress-induced mitochondrial damage.[31] This complex is released upon stimulation with UV radiation,[31] suggesting that Hsp70 may inhibit the apoptogenic activity of p66Shc. The present understanding of how p66Shc participates in oxidative stress-dependent apoptosis is summarized in Figure 2.

Conclusions

Since its first identification in 1999 as a cellular mediator of oxidative stress, p66Shc has come to be considered as a master regulator of cell survival and ageing. The proapoptotic and progeric activity of p66Shc is closely related to its capacity to control both steady-state and acute production of intracellular ROS. Furthermore, its pivotal role in the mitochondrial pathway of apoptosis controlled by p53 has been convicingly established. How p66Shc controls the production of ROS remains however to be fully understood. The inverse correlation between expression of p66Shc and transcriptional activation of genes implicated in ROS scavenging, while providing a clear-cut mechanism for the control of intracellular ROS production, does not explain how p66Shc is coupled to gene trascription. On the other hand, the finding that a pool of p66Shc is localized in mitochondria, where an increase in ROS levels resulting from the age-related impairment in respiratory enzyme function has been demonstrated[22,23] suggests the exciting possibility that p66Shc may be directly implicated in the control of ROS production by mitochondria.

An important open question is the potential impact of p66Shc expression on tumorigenesis. Although both the proapoptotic activity of p66Shc and its capacity to inhibit mitogenic signaling through Ras suggest that it could function as a tumor suppressor, the available evidence, which at present has been largely obtained on tumor-derived cell lines, appears conflicting.[16,18,33–36] On the other hand, studies on p66Shc$^{-/-}$ MEFs show that p66Shc selectively regulates p53-dependent apoptosis, while leaving unaffected p53-dependent cell cycle arrest.[20] Together with the finding that p66Shc$^{-/-}$ mice, notwithstanding their increased lifespan, are not more susceptible to either sponeaneous or carcinogen-induced tumorigenesis,[14,20] these data would argue against a tumor suppressor function of p66Shc. A related question, which has been raised by the demonstration that p66Shc is expressed in T-cells primed to undergo apotosis, is the potential impact of p66Shc expression on negative selection of self-reactive lymphocytes -a process occurring by apoptosis- and hence on the development of autoimmunity. More work is clearly required to fully clarify these issues. At this stage, the enviable state of p66Shc$^{-/-}$ mice make the loss of p66Shc appear as the achievement of Faust's aspirations to eternal youth. Where is the rub?

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References

1. Luzi L, Confalonieri S, Di Fiore PP, Pelicci PG. Evolution of Shc functions from nematode to human. *Curr Opin Genet Dev* 2000; **10**: 668–674.
2. Pelicci G, Dente L, De Giuseppe A, *et al.* A family of Shc releted proteins with conserved PTB, CH1 and SH2 regions. *Oncogene* 1996; **13**: 633–641.
3. Nakamura T, Muraoka S, Sanokawa R, Mori N. N-Shc and Sck, two neuronally expressed Shc adapter homologs. Their differential regional espression in the brain and roles in neurotrophin and Src signaling. *J Biol Chem* 1998; **273**: 6960–6967.
4. Bonfini L, Migliaccio E, Pelicci G, Lanfrancone L, Pelicci PG. Not all Shc's roads lead to Ras. *Trends Biochem Sci* 1996; **21**: 257–261.
5. Ravichandran KS. Signaling via Shc family adapter proteins. *Oncogene* 2001; **20**: 6322–6330.
6. Gotoh N, Tojo A, Shibuya M. A novel pathway from phosphorylation of tyrosine residues 239/240 of Shc, contributing to suppress apoptosis by IL-3. *EMBO J* 1996; **15**: 6197–6204.
7. Weng Z, Thomas SM, Rickles RJ, *et al.* Identification of Src, Fyn, and Lyn SH3-binding proteins: Implication for a function of SH3 domains. *Mol Cell Biol* 1994; **14**: 4509–4521.
8. Ulivieri C, Pacini S, Bartalini S, Valensin S, Telford JL, Baldari CT. Obligatory cross-talk with the tyrosine kinases assembled with the TCR/CD3 complex in CD4 signal transduction. *Eur J Immunol* 1999; **29**: 2625–2635.
9. Migliaccio E, Mele S, Salcini AE, *et al.* Opposite effects of the p52shc/p46shc and p66shc splicing isoforms on the EGF receptor-MAP kinase-fos signalling pathway. *EMBO J* 1997; **16**: 706–716.
10. Okada S, Kao AW, Ceresa BP, Blaikie P, Margolis B, Pessin JE. The 66-kDa Shc isoform is a negative regulator of the epidermal growth factor-stimulated mitogen-activated protein kinase pathway. *J Biol Chem* 1997; **272**: 28042–28049.
11. Natalicchio A, Laviola L, De Tullio C, *et al.* Role of p66SHC isoform in IGF-I receptor signal through MEK/ERK and regulation of actin cytoskeleton in rat muoblasts. *J Biol Chem* 2004; [Epub ahead of print].
12. Pacini S, Pellegrini M, Migliaccio E, *et al.* p66SHC promotes apoptosis and antagonizes mitogenic signaling in T cells. *Mol Cell Biol* 2004; **24**: 1747–1757.
13. Dupont H, Blancq M. Formation of complexes involving RasGAP and p190RhoGAP during morphogenetic events of the gastulation in xenopus. *Eur J Biochem* 1999; **265**:530–538.
14. Migliaccio E, Giorgio M, Mele S, *et al.* The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* 1999; **402**: 309–313.

15. Pelicci G, Lanfrancone L, Grignani F, *et al.* A novel transforming protein (SHC) with an SH2 domain is implicated in mitogenic signal transduction. *Cell* 1992; **70**: 93–104.
16. Xie Y, Hung MC. p66shc isoform down-regulated and not required for HER-2/neu signaling pathway in human breast cancer cell lines with HER-2/neu overexpression. *Biochem Biophys Res Commun* 1996; **221**: 140–145.
17. Stevenson LE, Frackelton AR. Constitutively tyrosine phosphorylated p52 Shc in breast cancer cell: correlation with ErbB2 and p66 Shc expression. *Breast Cancer Res Treat* 1998; **49**: 119–128.
18. Jackson JG, Yoneda T, Clark GM, Yee D. Elevated levels of p66 Shc are found in breast cancer cell lines and primary tumors with high metastatic potential. *Clin Cancer Res* 2000; **6**: 1135–1139.
19. Ventura A, Luzi L, Pacini S, Baldari CT, Pelicci PG. The p66Shc longevity gene is silenced through epigenetic modifications of an alternative promoter. *J Biol Chem* 2002; **277**: 22370–22376.
20. Trinei M, Giorgio M, Cicalese A, *et al.* A p53-p66Shc signalling pathway controls intracellular redox status, levels of oxidation-damaged DNA and oxidative stress-induced apoptosis. *Oncogene* 2002; **21**: 3872–3878.
21. Cesselli D, Jakoniuk I, Barlucchi L, *et al.* Oxidative stress-mediated cardiac cell death is a major determinant of ventricular dysfunction and failure in dog dilated cardiomyopathy. *Circ Res* 2001; **89**: 198–200.
22. Inoue M, Sato EF, Nishikawa M, *et al*. Mitochondrial generation of reactive oxygen species and its role in aerobic life. *Curr Med Chem* 2003; **10**: 2495–2505.
23. Sastre J, Pallardo FV, Vina J. The role of mitochondrial oxidative stress in aging. *Free Radic Biol Med* 2003; **35**: 1–8.
24. Yang CP, Horwitz SB. Taxol mediates serine phosphorilation of the 66-Kda Shc isoform. *Cancer Res* 2000; **60**: 5171–5178.
25. Yang CP, Horwitz SB. Distinct mechanisms of taxol-induced serine phosphorylation of the 66-kDa Shc isoform in A549 and RAW 264.7 cells. *Biochim Biophys Acta* 2002; **1590**: 76–83.
26. Le S, Connors TJ, Maroney AC. c-Jun N-terminal Kinase specifically phosphorylates p66 ShcA at serine 36 in respose to ultraviolet irradiation. *J Biol Chem* 2001; **276**: 48332–48336.
27. Napoli C, Martin-Padura I, de Nigris F, *et al.* 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* 2003; **100**: 2112–2116.
28. Polyak K, Xia Y, Zweier JL, Kinzler KW, Vogelstein B. A model for p53-induced apoptosis. *Nature* 1997; **389**: 300–305.
29. Li PF, Dietz R, von Harsdorf R. p53 regulates mitochondrial membrane potential through reactive oxygen species and induces cytochrome c-indipendent apoptosis blocked by Bcl-2. *EMBO J* 1999; **18**: 6027–6036.
30. Schuler M, Bossy-Wetzel E, Goldstein JC, Fitzgerald P, Green DR. P53 induces apoptosis by caspase activation through mitochondrial cytochrome c release. *J Biol Chem* 2000; **275**: 7337–7342.
31. Orsini F, Migliaccio E, Moroni M, *et al.* The life span determinant p66Shc localizes to mitochondria where it associates with mtHsp70 and regulates trans-membrane potential. *J Biol Chem* 2004; **279**: 25689–25695.
32. Nemoto S, Finkel T. Redox regulation of Forkhead proteins through p66Shc-dependent signaling pathway. *Science* 2002; **295**: 2450–2452.
33. Liu JF, Chevet E, Kebache S, *et al.* Functional Rac-1 and Nck signaling networks are required for FGF-2-induced DNA synthesis in MCF-7 cells. *Oncogene* 1999; **18**: 6425–6433.
34. Kwok Fung Lo A, Liu Y, Wang X, *et al.* Identification of downstream target genes of latent membrane protein 1 in nasopharyngeal carcinoma cells by suppression subtractive hybidization. *Biochim Biophys Acta* 2001; **1520**: 131–140.
35. Abdollahi A, Gruver BN, Patriotis C, Hamilton TC. Identification of epidermal growth factor-responsive genes in normal rat ovarian surface epithelial cells. *Biochem Biophys Res Commun* 2003; **307**: 188–197.
36. Lee MS, Igawa T, Chen SJ, *et al*. p66Shc protein is upregulated by steroid hormones in hormone-sensitive cancer cells and primary prostate carcinomas. *Int J Cancer* 2004; **108**: 672–678.