Archives of Gerontology and Geriatrics 55 (2012) 744–748

Contents lists available at SciVerse ScienceDirect

Archives of Gerontology and Geriatrics

journal homepage: www.elsevier.com/locate/archger

Selective upregulation of p66-Shc gene expression in the liver and brain of aged rats

Kiyoaki Sone [a,1,2], Mari Mori [b,c,1], Nozomu Mori [a,c,*]

[a] Department of Molecular Genetics, National Institute for Longevity Sciences (NILS), Gengo 36-3, Morioka, oobu, Aichi 474-8522, Japan
[b] Department of Bioinformatics, Center for Biotechnology Education, Johns Hopkins University, USA
[c] Department of Anatomy and Neurobiology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan

ARTICLE INFO

Article history:
Received 1 September 2011
Received in revised form 2 November 2011
Accepted 4 November 2011
Available online 29 November 2011

Keywords:
Adapter
Anti-aging
Longevity
Phosphotyrosine
Signal transduction

ABSTRACT

The phosphotyrosine signaling followed by various receptor activations conforms a unique signaling platform during metazoan evolution, and is crucial for animal development, maturation, and aging. Shc is the most versatile bipartite phosphotyrosine signal adaptor harboring phosphotrosine-biding (PTB) and Src-homology2 (SH2) domains. Among the Shc adaptor family members, p66-Shc is of potential interest in aging studies, since its deletion in mice resulted in a longer lifespan and/or higher quality of life in later stages of life. However, a few studies have examined the gene expression profiles of p66-Shc in aging tissues. Here, we quantified the expression levels of transcripts of Shc-related isoforms in the liver and brain of young adult, middle-aged, and aged rats, and found that p66-Shc gene expression is specifically up-regulated in the aged liver and brain. In the aged liver tissue, p66-Shc expression was also evident at the protein level, and accumulated in the soluble fraction of the aged tissue. These results indicate that p66-Shc is not only related to animal longevity but also affected during aging, and thus the repression of p66-Shc could become a potential target for an anti-aging strategy.

1. Introduction

Aging affects various signal transduction events in cells and tissues, which may lead to the general decline in stress resistance and/or the deteriorative function in aged animals (Guarente and Kenyon, 2000; Mori et al., 2003). Although both the efficacy and specificity of these signaling events could be affected with aging, recent studies suggest that specific pathways are significantly affected by aging, and such signaling pathways in cellular metabolism seem to confirm the major mechanisms of determining organismal longevity (Finkel and Holbrook, 2000; Kenyon, 2001; Longo and Finch, 2003; Kenyon, 2010; Mori and Mori, 2011).

The phosphotyrosine signal adapter Shc is of particular interest with respect to aging-related cellular signaling because it is involved in the initial steps of intracellular signaling following the activation of various receptor tyrosine kinases (RTKs) (for review, see Bonfini et al., 1996; Luzi et al., 2000; Ravichandran, 2001), and yet at least one isoform of Shc, i.e. p66-Shc, is able to transmit a distinct signal using serine/threonine phosphorylation in response to oxidative stress, leading to cellular apoptosis (Migliaccio et al., 1999; Nemoto and Finkel, 2002; for reviews, see Mori et al., 2003; Purdom and Chen, 2003; Trinei et al., 2009). As an adapter or docking molecule, the members of the Shc family play important roles in cardiovascular development (Lai and Pawson, 2000; Cosentino et al., 2008), neuronal activation by neurotrophins (Nakamura et al., 1996, 1998; Liu and Meakin, 2002), oxidative stress-related redox response (Migliaccio et al., 1999; Nemoto and Finkel, 2002; Haga et al., 2010), cardiovascular complications (Cosentino et al., 2008; Francia et al., 2009), adiposity (Tomilov et al., 2011), and many other signaling routes including the main signal pathway of the so-called Ras-MAPK pathway via direct interaction with Grb2 (for reviews see Bonfini et al., 1996; Ravichandran, 2001). The fact that a mutant mouse deficient in p66-Shc lived longer than the wild-type, possibly due to increased tolerance against oxidative stress in the absence of p66-Shc (Migliaccio et al., 1999), has shed light on the specific involvement of this signaling molecule in the mechanisms of longevity determination and aging (Purdom and Chen, 2003; Orsini et al., 2006; Trinei et al., 2009).

While several studies have examined the signaling efficacy of Shc in aged tissues, particularly using fibroblasts, hepatocytes, muscular and neural tissues (Palmer et al., 1999; Hutter et al., 2000; Jiang et al., 2003; Pandolfi et al., 2005), few attempts have

* Corresponding author at: Department of Anatomy and Neurobiology, Nagasaki University School of Medicine, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan. Tel.: +81 95 819 7017; fax: +81 95 819 7017.
E-mail address: morinosm@nagasaki-u.ac.jp (N. Mori).
[1] These authors contributed equally to this work.
[2] Present address: Department of Engineering, Yamagata University, 4-3-16 Johnan, Yonezawa, Yamagata 992-8510, Japan.

0167-4943/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.archger.2011.11.001

been made to quantify the age-difference in the gene expression of p66-Shc and other Shc-related proteins such as neuronal Shc (N-Shc, also designated ShcC) and Sck (also ShcB). The latter two are Shc-related homologues derived from distinct genes on different chromosomes (Luzi et al., 2000; Kojima et al., 2001), and are expressed preferentially in the nervous system (Nakamura et al., 1996, 1998; O'Bryan et al., 1996; Mori and Mori, 2011). Therefore, we should determine the mRNA and protein levels of Shc-related genes in young and old animals using a real-time polymerase chain reaction (PCR) and Western blot analysis in order to clarify whether the gene expression of either Shc, N-Shc, Sck, or particularly p66-Shc is affected during aging.

2. Materials and methods

2.1. Animals

Male Fischer 344 rats were used throughout the study. The rats were grouped into young (6 month-old), middle-aged (12 month-old), and aged (24–26 month-old) animals. The rats were maintained at 23 ± 1 °C under artificial lighting for 12 h per day on a standard show diet (CLEA Japan Inc.) and tap water provided ad libitum in the animal facility. All experiments were performed in accordance with the guidelines for animal experiments of National Institute for Longevity Science (NILS) and/or Nagasaki University.

2.2. RNA analysis

Tissue RNA was extracted and cDNA was generated using reverse transcriptase as described previously (Nakamura et al., 1998; Kojima et al., 2001; Mori et al., 2002; Kontani et al., 2005). To quantify mRNA levels in aged tissues, we employed a real-time PCR using a LightCycler (Roche Diagnostics). Specific primers used for the measurement of each transcript are as follows: Shc-S (sense strand), 5′-AATGGTGACTTCTTGGTGCGAGAG-3′, Shc-AS (antisense strand) 5′-TTTCCGATCCACGGGTTGCTGTAG-3′; p66Shc-S, 5′-AGCCATG-GATCTTCTACCCC-3′, p66Shc-AS, 5′-CCCTGCACTCCCTTCCCCAT-3′; N-Shc-S, 5′-AGATGGCCCCGATCACCCGTACTA-3′, N-Shc-AS, 5′-GGGCTCAGCATTAAGCTCCTCCAG-3′; Sck-S, 5′-GTTACTGCAGAC-CACGATTACTAC-3′, Sck-AS, 5′-GGGCTCCTGCCTCAGTTGCTCCTC-3′; G3PDH-S, 5′-ACCACAGTCCATGCCATCAC-3′, G3PDH-AS, 5′-TCCAC-CACCCTGTTGCTGTA-3′. These probes were selected for their individual specificity in the amplification of complementary DNA (cDNA) of either total Shc, p66-Shc, Sck or N-Shc as shown in Fig. 1. The real-time PCR was performed in a capillary containing a total volume of 20 μl in the presence of appropriate PCR primers and a cDNA template. A reaction without the cDNA template was used for background subtraction, and a reaction with cDNA derived from glycelaldehyde 3-phosphate dehydrogenase (G3PDH) was used for normalization of the mRNA levels of each sample.

2.3. Protein analysis

Rats were sacrificed and decapitated under deep anesthesia with diethylether. Following the decapitation, the brains (cerebral cortex and cerebellum) and livers were isolated and dissected on ice. Each dissected region was homogenized in the lysis buffer (50 mM Tris–HCl, pH8.0/150 mM NaCl/1.0%NP-40/0.5% sodium deoxycholate/0.1% SDS/0.5 mM PMSF/10 mM NaF/2 mM Na_3VO_4/1× protease inhibitor complete; Roche Diagnostics GmbH, Mannheim, Germany). Aliquot of this total homogenate was stored, and was further sub-fractionated by a conventional procedure for separation of post-nuclear supernatant (S1), membraneous pellet (P1), and remaining soluble (S2) fractions. Samples containing equal amounts of protein (10 μg eq.) of each fraction were mixed with 2× sample buffer, heated for 1 min at 95 °C, and subjected to

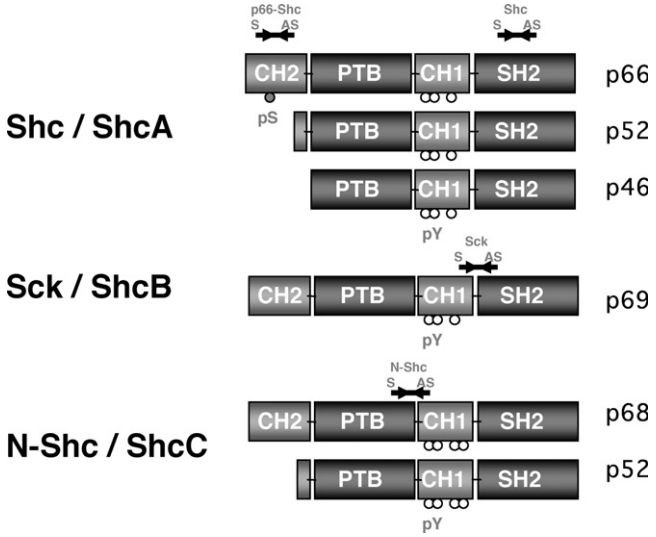

Fig. 1. Schematic drawing of domain features of Shc and related isoforms. The Shc family contains two homologues, Sck/ShcB and N-Shc/ShcC, which are predominantly expressed in the nervous system. Sck has only one form, p69, but N-Shc has large (p68) and small (p52) isoforms. Shc retains a large (p66) and smaller (p52/p46) forms. p66-Shc releases a unique signal from the N-terminally located CH2 domain via serine phosphorylation (pS), whereas the other forms send signals through tyrosine phosphorylation (pY) at the CH1 domain, which is present between two phosphotyrosine-binding domains, PTB and SH2. Regions used for PCR-amplification are marked by thick horizontal bars with arrowheads, and the positions of primer sets (sense (S) and anti-sense (AS) oligo-nucleotides) for each amplification is indicated above the schematic structures. These areas were selected for less homology among the family members.

electrophoresis on 5–20% gradient PAGE, and then transferred onto polyvinylidene difluoride (PVDF) membranes (GE Healthcare). Membranes were blocked with 5% blocking agent (GE Healthcare) in 1× Tris-buffered saline/Tween-20 buffer for 1 h at room temperature and were incubated with primary antibodies, e.g., anti-Shc antibody (1:1000; C-20, Santa Cruz Biotechnology or 1:1000; 2432S, Cell Signal Technology); anti-N-Shc/ShcC antibody (1:5000; 610643, BD Transduction Laboratories) for overnight at 4 °C. The membrane was also blotted for Na^+K^+ ATPase subunit α-1 (a marker for the plasma membrane fraction) in order to evaluate the validity of cellular fractionation and/or for Grb2 (G16720, purchased from Transduction Laboratories), another signaling molecule, for normalization. The membranes were rinsed with Tris-buffered saline/Tween-20 and incubated with secondary antibodies (anti-rabbit IgG antibody, Amersham Pharmacia) for 1 h at room temperature. Signals were detected with ECL (Amersham Pharmacia) or ECL Plus (GE Healthcare) on X-ray films.

2.4. Statistical analysis

Data were expressed as the mean ± S.E. Significant differences among groups were assessed by analysis of variance (ANOVA) Fischer's PLSD test ($p < 0.01$) using StatView-J 4.02 as described previously (Mori et al., 2002; Kontani et al., 2005).

3. Results

Comparing the relative mRNA levels of Shc, N-Shc and Sck in the three age groups, no significant difference was observed in the real-time PCR analyses (Fig. 2A, C, and D); however, we noted significant increase in the p66-Shc gene transcript being found in the brain tissue of aged rats (Fig. 2B). Although the Shc, N-Shc and Sck mRNA levels tended to decrease during aging, the mRNA level of p66-Shc revealed a gradual increase. Since N-Shc and Sck are more abundantly expressed in the adult brain than Shc and further,

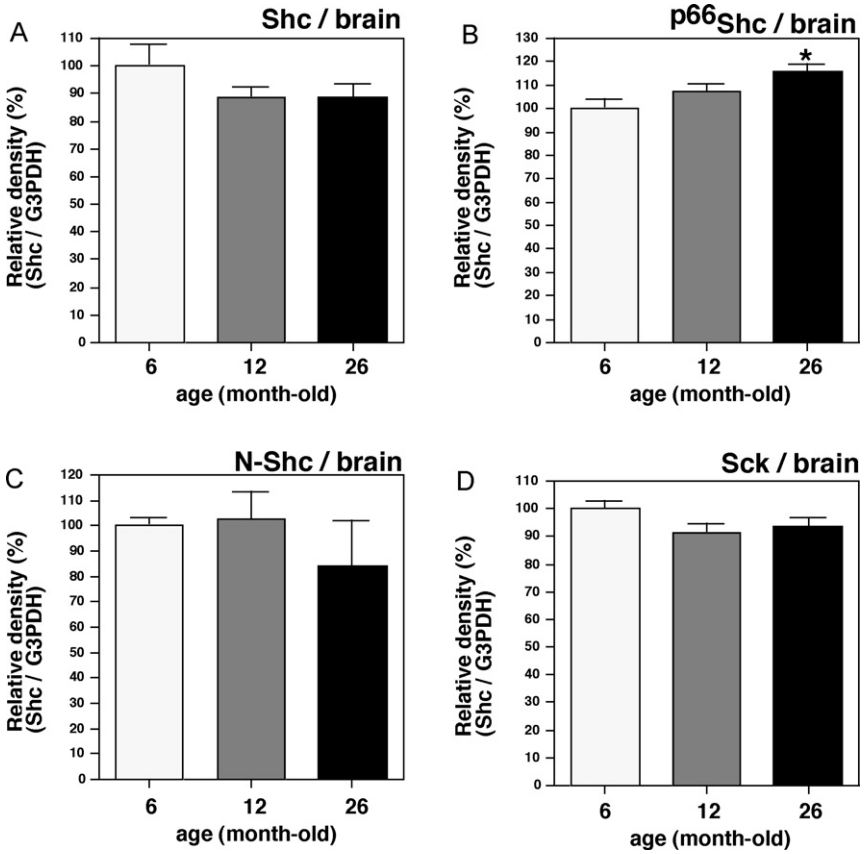

Fig. 2. Quantification of Shc-related mRNA levels in the brains of young (6 month-old, $n = 6$), middle-aged (12 month-old, $n = 6$), and old (26 month-old, $n = 5$) rats as determined by real time PCR using a LightCycler (Roche Diagnostics). Values are relative to the levels of G3PDH mRNA in each tissue. (A) Shc, (B) p66-Shc, (C) N-Shc, and (D) Sck. The mRNA level of p66-Shc was significantly higher in the aged rat brain than in the young rat brain ($p = 0.01$).

p66-Shc is a minor isoform of the Shc gene transcripts, it is possible that no significant difference was observed in abundant gene transcripts due to technical limits.

The upregulation of p66-Shc gene expression was also evident in the liver of aged rats even though total Shc messenger levels remained unchanged (Fig. 3A and B). A relative increase of p66-Shc gene products over p52 and p46 isoforms in the liver of aged rats was also confirmed at the level of protein by Western blot analysis (Fig. 3C and D). Much of the p66-Shc protein seemed present in the soluble fraction (or cytoplasm) rather than in membranous compartments (Fig. 4). These results demonstrate that p66-Shc gene transcription is upregulated relative to p52/46-Shc in both tissues during aging, and further suggest the accumulation of p66-Shc protein in the soluble fraction of liver, in particular, of the aged animals.

4. Discussion

Overall, our results indicate that there is an imbalance in the gene expression of Shc isoforms during aging, and the relative abundance of p66-Shc gene products may reflect cellular conditions in aged animals with limited growth and/or differentiation, and a greater defense against extrinsic and intrinsic stress. When we initiated this study, a similar study on the gene expression of Shc-related proteins during aging in the brain, spinal cord, and hind limb muscles of young and aged rats (BKI strain of Harlan Sprague-Dawley rats; female) was published (Jiang et al., 2003). In their aging study, comparisons were made between young (2–3 month-old) vs. aged (30 month-old) female Sprague-Dawley rats. Interestingly, their data indicated that Shc/ShcA gene expression was upregulated in aged tissues; p46-Shc and p66-Shc were increased in the spinal cord and muscles, respectively, of aged animals. In contrast, Sck/ShcB mRNA expression tended to be downregulated, and the expression of N-Shc/ShcC was unaffected by age. While they performed Western blots for p46/p52 and p66 Shc protein isoforms using protein samples extracted from young and aged thoracic spinal cord tissues, the changes in the expression of these genes were not confirmed at the protein level; no significant upregulation of p66-Shc protein was observed. In our current investigation, however, a specific upregulation of the expression of the p66-Shc isoform was confirmed at both the mRNA and protein levels in the liver. Thus, age-related accumulation of p66-Shc may be evident in the liver tissue, while in the nervous system, where the expression of ShcA is limited (Mori and Mori, 2011), accumulation of p66-Shc protein may be hardly detected in either spinal cord (Jiang et al., 2003), cerebral cortex or cerebellum (this study).

Our current study may be viewed as a confirmatory study of the previous study by the Swedish group (Jiang et al., 2003) with minor differences in methods. While we examined male Fischer 344 rats, the Ulfhake's group utilized female Sprague-Dawley rats. There were also some differences in age groups; we compared 6-month vs. 24–26-month-old animals whereas they compared 2–3-month vs. 30-month-old animals. Yet both studies demonstrated selective upregulation of p66-Shc in the muscle, liver, spinal cord, and brain, at least at the mRNA level. As aforementioned, we further revealed that p66 Shc protein accumulated in the soluble fraction of the liver of aged animals. While the p66-Shc protein is claimed to serve as the radical generator in the mitochondrial inner membrane (Giorgio et al., 2005; Trinei et al., 2009), there might be additional role(s) of p66-Shc in the cytoplasm, comparable to other short forms including p52 and p46.

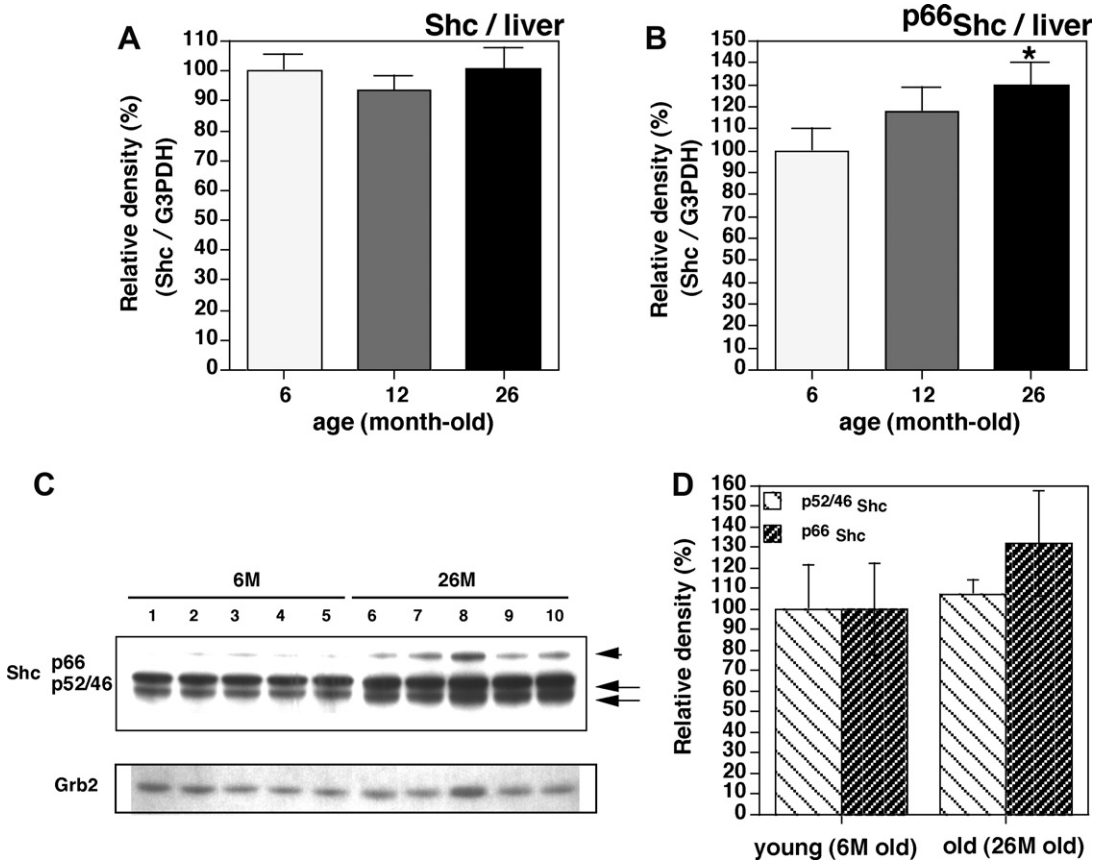

Fig. 3. Quantification of total Shc and p66-Shc mRNA and protein levels in the liver of young (6 month-old, $n = 6$), middle-aged (12 month-old, $n = 6$), and old (26 month-old, $n = 5$) rats as determined by real time PCR and Western blotting. (A, B) Relative mRNA levels of total Shc and p66-Shc mRNAs. Values are relative to the levels of G3PDH mRNA in each tissue. The p66-Shc mRNA level was significantly higher in the liver of old rats young rats ($p = 0.01$). (C) Western blot analysis of p66-Shc and p52/46-Shc protein levels in the young (6 month-old, lanes 1–5) and old (26 month-old, lanes 6–10) rats using polyclonal anti-Shc antibody. For compariosn, the blot was re-probed with anti-Grb2 antibody, showing that the Grb2 level was relatively unchanged during aging. (D) Comparison of p52/46 Shc and p66-Shc protein levels in the young and old rat liver as determined by densitometry of the Western blot shown in panel C ($n = 5$ each).

The major forms of Shc, i.e. p52 and p46, play an essential role in cellular growth and differentiation via the activation of the Ras-MAPK signaling route (Bonfini et al., 1996; Ravichandran, 2001), whereas p66-Shc, the minor component of Shc, functions negatively against this signaling pathway (Migliaccio et al., 1997). Therefore, the relative increase in p66-Shc may be relevant to the reduced cell division and differentiation in tissues of aged animals. Alternatively, since p66-Shc is implicated in oxidative stress-activated signaling and cellular apoptosis in conditions of stress (Migliaccio et al., 1999; Giorgio et al., 2005), the higher levels of p66-Shc may be responsible for the susceptibility of aged tissues to cellular deterioration including cell death following various stress-related insults during aging.

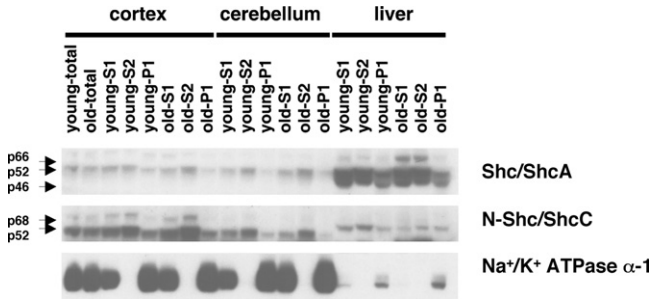

Fig. 4. Accumulation of p66-Shc protein in soluble fractions of the liver of aged rats. Representative Western blot of Shc and N-Shc proteins in the liver and brain (cerebral cortex and cerebellum) of young (6 month-old) and old (24 month-old) rats is shown ($n = 3$ each). Protein samples (10 μg) from each sample of total homogenate (total), or post-nuclear supernatant (S1), membranous pellet subfraction (P1), and 100,000 × g supernatant (S2) from each tissue were resolved on 5–20% polyacrylamide gradient gel, and probed with anti-Shc (upper panel), anti-N-Shc (middle panel), and anti-sodium-potassium ATPase α-1 subunit (lower panel) antibodies. Note that p66-Shc is abundant in the soluble fractions (S1 and S2) of the aged liver. No difference was observed in the level of p68-N-Shc in the cerebral cortex between the young and old rats.

It is worth to note that p66-Shc is highly abundant in fibroblasts of centenarians (Pandolfi et al., 2005). The abundance of p66-Shc in the liver of aged animals and centenarian fibroblasts *per se* might be merely the outcome of aging rather than the pre-requisite to acquire a superior longevity. Given that the p66-Shc gene-deficient mouse strain resulted in a longer lifespan (Migliaccio et al., 1999), accumulation of p66-Shc should be considered non-beneficial or rather deteriorative to longevity. Thus, we assume that the upregulation of p66-Shc gene expression in the aged tissue and also in the centenarians' fibroblasts could be due to the outcome of accumulation during aging.

The accumulation of p66-Shc could thus be the outcome of aging. If so, p66-Shc in the aged tissue could be a potential target for anti-aging strategy. Accumulative evidence clearly indicates that the deletion and/or suppression of p66-Shc function should be beneficial for longer lifespan or higher QOL (Camici et al., 2011). As we showed herein, the p66-Shc gene expression does increase, if not diminish, during aging, making it a reasonable target for anti-aging therapy. Small molecular compounds that would selectively inhibit the phosphorylation of Ser36 at the CH2 domain of p66-Shc

could be a candidate for therapeutic intervention in the very elderly population in developed countries.

Conflict of interest statement

Nothing declared.

Acknowledgements

We thank Ms. Y. Kadokawa and K. Onga for technical assistance. This research was supported by a Grant-in-Aid for Scientific Research (Kiban-B) from MEXT, Japan, and also in part by the Asian CORE Program from JSPS and Smoking Research Foundation (to NM).

References

Bonfini, L., Migliaccio, E., Pelicci, G., Lanfrancone, L., Pelicci, P.G., 1996. Not all Shc's roads lead to Ras. Trends Biochem. Sci. 21, 257–261.

Camici, G.G., Shi, Y., Cosentino, F., Francia, P., Lüscher, T.F., 2011. Anti-aging medicine: molecular basis for endothelial cell-targeted strategies – a mini-review. Gerontology 57, 101–108.

Cosentino, F., Francia, P., Camici, G.G., Pelicci, P.G., Lüscher, T.F., Volpe, M., 2008. Final common molecular pathways of aging and cardiovascular disease: role of the p66Shc protein. Arterioscler. Thromb. Vasc. Biol. 28, 622–628.

Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.

Francia, P., Cosentino, F., Schiavoni, M., Huang, Y., Perna, E., Camici, G.G., Lüscher, T.F., Volpe, M., 2009. p66 (Shc) protein, oxidative stress, and cardiovascular complications of diabetes: the missing link. J. Mol. Med. (Berl.) 87, 885–891.

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, P.G., 2005. Electron transfer between cytochrome c and p66Shc generates reactive oxygen species that trigger mitochondrial apoptosis. Cell 122, 221–233.

Guarente, L., Kenyon, C., 2000. Genetic pathways that regulate ageing in model organisms. Nature 408, 255–262.

Haga, S., Morita, N., Irani, K., Fujiyoshi, M., Ogino, T., Ozawa, T., Ozaki, M., 2010. p66(Shc) has a pivotal function in impaired liver regeneration in aged mice by a redox-dependent mechanism. Lab Invest. 90, 1718–1726.

Hutter, D., Yo, Y., Chen, W., Liu, P., Holbrook, N.J., Roth, G.S., Liu, Y., 2000. Age-related decline in Ras/ERK mitogen-activated protein kinase cascade is linked to a reduced association between Shc and EGF receptor. J. Gerontol. A: Biol. Sci. Med. Sci. 55, B125–B134.

Jiang, X., Edstrom, E., Altun, M., Ulfhake, B., 2003. Differential regulation of Shc adaptor proteins in skeletal muscle, spinal cord and forebrain of aged rats with sensorimotor impairment. Aging Cell 2, 47–57.

Kenyon, C., 2001. The plasticity of aging: insights from long-lived mutants. Cell 120, 449–460.

Kenyon, C., 2010. The genetics of ageing. Nature 464, 504–512.

Kojima, T., Yoshikawa, Y., Takada, S., Sato, M., Nakamura, T., Takahashi, N., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Mori, N., 2001. Genomic organization of the Shc-related phosphotyrosine adapters and characterization of the full-length Sck/ShcB: specific association of p68-Sck/ShcB with pp135. Biochem. Biophys. Res. Commun. 284, 1039–1047.

Kontani, Y., Wang, Y., Kimura, K., Inokuma, K.I., Saito, M., Suzuki-Miura, T., Wang, Z., Sato, Y., Mori, N., Yamashita, H., 2005. UCP1 deficiency increases susceptibility to diet-induced obesity with age. Aging Cell 4, 147–155.

Lai, K.M., Pawson, T., 2000. The ShcA phosphotyrosine docking protein sensitizes cardiovascular signaling in the mouse embryo. Genes Dev. 14, 1132–1145.

Liu, H.Y., Meakin, S.O., 2002. ShcB and ShcC activation by the Trk family of receptor tyrosine kinases. J. Biol. Chem. 277, 26046–26056.

Longo, V.D., Finch, C.E., 2003. Evolutionary medicine: from dwarf model systems to healthy centenarians? Science 299, 1342–1346.

Luzi, L., Confalonieri, S., Di Fiore, P.P., Pelicci, P.G., 2000. Evolution of Shc functions from nematode to human. Curr. Opin. Genet. Dev. 10, 668–674.

Migliaccio, E., Mele, S., Salcini, A.E., Pelicci, G., Lai, K.M., Superti-Furga, G., Pawson, T., Di Fiore, P.P., Lanfrancone, L., Pelicci, P.G., 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.

Migliaccio, E., Giorgio, M., Mele, S., Pelicci, G., Reboldi, P., Pandolfi, P.P., Lanfrancone, L., Pelicci, P.G., 1999. The p66shc adaptor protein controls oxidative stress response and life span in mammals. Nature 402, 309–313.

Mori, N., Mori, M., 2011. Neuronal Shc, a gene of longevity in the brain? Med. Hypoth. 77, 996–999.

Mori, N., Mizuno, T., Murai, K., Nakano, I., Yamashita, H., 2002. Effect of age on the gene expression of neural-restrictive silencing factor NRSF/REST. Neurobiol. Aging 23, 255–262.

Mori, N., Zhu, W., Sone, K., Miyamoto, Y., Kadokawa, Y., Ihara, T., Wakao, R., 2003. Roles of Shc signaling in oxidative stress response and aging. J. Clin. Biochem. Nutr. 34, 69–76.

Nakamura, T., Sanokawa, R., Sasaki, Y., Ayusawa, D., Oishi, M., Mori, N., 1996. N-Shc: a neural-specific adapter molecule that mediates signaling from neurotrophin/Trk to Ras/MAPK pathway. Oncogene 13, 1111–1121.

Nakamura, T., Muraoka, S., Sanokawa, R., Mori, N., 1998. N-Shc and Sck, two neuronally expressed Shc adapter homologs: their differential regional expression in the brain and roles in neurotrophin and Src signaling. J. Biol. Chem. 273, 6960–6967.

Nemoto, S., Finkel, T., 2002. Redox regulation of forkhead proteins through a p66shc-dependent signaling pathway. Science 295, 2450–2452.

O'Bryan, J.P., Songyang, Z., Cantley, L., Der, C.J., Pawson, T., 1996. A mammalian adaptor protein with conserved Src homology 2 and phosphotyrosine-binding domains is related to Shc and is specifically expressed in the brain. Proc. Natl. Acad. Sci. U.S.A. 93, 2729–2734.

Orsini, F., Moroni, M., Contursi, C., Yano, M., Pelicci, P., Giorgio, M., Migliaccio, E., 2006. Regulatory effects of the mitochondrial energetic status on mitochondrial p66Shc. Biol. Chem. 387, 1405–1410.

Palmer, H.J., Tuzon, C.T., Paulson, K.E., 1999. Age-dependent decline in mitogenic stimulation of hepatocytes. Reduced association between Shc and the epidermal growth factor receptor is coupled to decreased activation of Raf and extracellular signal-regulated kinases. J. Biol. Chem. 274, 11424–11430.

Pandolfi, S., Bonafè, M., Di Tella, L., Tiberi, L., Salvioli, S., Monti, D., Sorbi, S., Franceschi, C., 2005. p66(Shc) is highly expressed in fibroblasts from centenarians. Mech. Ageing Dev. 126, 839–844.

Purdom, S., Chen, Q.M., 2003. p66(Shc): at the crossroad of oxidative stress and the genetics of aging. Trends Mol. Med. 9, 206–210.

Ravichandran, K.S., 2001. Signaling via Shc family adapter proteins. Oncogene 20, 6322–6330.

Tomilov, A.A., Ramsey, J.J., Hagopian, K., Giorgio, M., Kim, K.M., Lam, A., Migliaccio, E., Lloyd, K.C., Berniakovich, I., Prolla, T.A., Pelicci, P., Cortopassi, G.A., 2011. The Shc locus regulates insulin signaling and adiposity in mammals. Aging Cell 10, 55–65.

Trinei, M., Berniakovich, I., Beltrami, E., Migliaccio, E., Fassina, A., Pelicci, P., Giorgio, M., 2009. p66Shc signals to age. Aging (Albany, NY) 1, 503–510.