

# p66<sup>Shc</sup> has a pivotal function in impaired liver regeneration in aged mice by a redox-dependent mechanism

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Liver regeneration involves complicated processes and is affected by various patho-physiological conditions. This study was designed to examine the molecular mechanisms underlying the aging-associated impairment of liver regeneration. Male C57BL/6J mice were used as young and aged mice (< 10 weeks and > 20 months old, respectively). These mice were subjected to 70% partial hepatectomy (PH). Liver regeneration and liver injury/stresses were evaluated chronologically after PH. Post-hepatectomy liver regeneration was markedly impaired in aged mice. Though the extent of hepatocyte proliferation in the regenerating liver was similar in aged and young mice, cell growth was absent in aged mice. Oxidative stress (OS) was observed immediately after hepatectomy, followed by marked apoptosis in aged mice. Signaling molecules regarding cell proliferation (mitogen-activated protein kinase, STAT3, p46/52<sup>Shc</sup>) and anti-oxidation (catalase, superoxide dismutase, Ref-1, glutathione peroxidase) were expressed/activated after hepatectomy in livers of both aged and young mice. Akt was not activated in aged-mouse liver, but its expression was similar to that in young mice. p66<sup>Shc</sup>, known as an age-/oxidant-associated protein, was strongly phosphorylated. By knocking down p66<sup>Shc</sup>, the impairment of liver regeneration was normalized. OS immediately after hepatectomy induced subsequent liver injury (apoptosis), and deletion of p66<sup>Shc</sup> suppressed both OS and hepatocyte apoptosis in the regenerating liver of aged mice. Though we need additional data in other animal models to fully understand the mechanism, p66<sup>Shc</sup> may have a pivotal function in the impairment of liver regeneration in aged mice by triggering OS and subsequent apoptosis. This data may provide a clue to understanding the mechanism underlying the association between aging and the impairment of liver regeneration.

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Liver regeneration is a series of physio-pathological phenomena resulting in quantitative recovery from loss of liver mass to compensate for decreased hepatic volume and impaired function. The liver has a unique ability to restore lost volume, which is rarely seen in other organs.<sup>1–3</sup> It is well established that normal adult hepatocytes are usually quiescent, but have the potential ability to replicate. After surgical procedures that reduce liver mass, such as partial hepatectomy (PH) or live donor-liver transplantation, rapid enlargement of the residual or grafted liver commonly takes place to restore liver mass and function. Clinically, liver

regeneration has important implications because many therapeutic strategies for surgical treatment of liver diseases, such as removal of liver tumors and liver transplantation, depend on the ability of the liver to regenerate physically and functionally. Poor or insufficient liver regeneration may be potentially fatal for these patients.<sup>4–6</sup> Therefore, better understanding of the patho-physiological features of liver regeneration could lead to clinical benefits.

In western countries, the last two decades have seen a progressive increase in the mean life expectancy of the general population, resulting in higher percentages of aged people.<sup>7,8</sup>

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Aging is an issue of growing concern in modern surgery and one which must be resolved in the near future. The influence of aging on the outcome of surgery has already been extensively analyzed and reported in some fields such as liver surgery. Hepatic resection is a common surgical treatment for a wide range of liver diseases.<sup>7,9,10</sup>

Recently, more hepatic resections have been performed in patients over the age of 60 years, with encouraging results.<sup>7,11</sup> The aged population, however, is predisposed to a variety of diseases and pathological conditions, which may contribute to a marked increase in morbidity in this subpopulation.<sup>12</sup> The incidence of liver disease increases with age, but the cellular and subcellular mechanisms that underlie this suspected predisposition to pathology remain unresolved. Several age-related changes have been documented, including reductions in liver volume, metabolism, expression of a variety of proteins, and hepatobiliary function.<sup>12,13</sup> Changes such as reduced response to oxidative stress (OS), reduced expression of growth-regulatory genes, diminished rates of DNA repair, and telomere shortening may contribute to reduced hepatic regenerative capacity, shorter post-liver transplant survival, and increased susceptibility to certain liver diseases among the aged.<sup>12</sup>

Src homology 2 domain-containing (Shc) proteins are classified into three families, designated ShcA, ShcB, and ShcC in mammals.<sup>14</sup> Although the expression of ShcB and ShcC seems to be restricted to neuronal cells, ShcA is ubiquitously expressed except for in the brain and in neurons.<sup>14,15</sup> ShcA (hereafter referred to simply as 'Shc') was identified in 1992 as an adaptor molecule coupling the activated epidermal growth factor receptor to Ras and the mitogen-activated protein kinase (MAPK) cascade.<sup>15</sup> Shc proteins have been studied mainly regarding the mechanisms of their mitogenic properties. However, p66<sup>Shc</sup> inhibits activation of the Ras/MAP kinase pathway by competing with p46<sup>Shc</sup> and p52<sup>Shc</sup> for binding to Grb2.<sup>14–18</sup> Recent studies have reported that p66<sup>Shc</sup> contributes to the regulation of cellular OS and apoptosis.<sup>19–21</sup> Fibroblasts and hepatocytes without p66<sup>Shc</sup> have increased resistance to OS and apoptosis with increased levels of Mn-superoxide dismutase (SOD), redox factor-1 (Ref-1), and Bcl-xL.<sup>19,21</sup> Furthermore, p66<sup>Shc-/-</sup> mice are protected against acute tissue damage after hind limb ischemia and after generation of reactive oxygen species caused by ischemia/reperfusion.<sup>22</sup> Although elevated levels of cellular reactive oxygen species have also been implicated in aging, p66<sup>Shc-/-</sup> mice show a greater resistance to OS and up to 30% longer lifespan compared with their wild-type counterparts.<sup>20</sup>

This study was designed to examine the function of p66<sup>Shc</sup> and associated molecules in liver regeneration in aged mice. To this end, we analyzed mechanisms of liver regeneration in the context of cell proliferation, growth, OS, and apoptosis in liver regeneration. Here, we report that p66<sup>Shc</sup> has a pivotal function in the impairment of liver regeneration by inducing OS and injury (apoptosis) in the aged-mouse PH model.

## MATERIALS AND METHODS

### Animal Experiments

C57BL/6 male mice (8–10 weeks, > 13, and > 20 months old; young, middle-aged, and aged mice, respectively) were used for simple 70% PH experiments. Anesthesia was induced with an intraperitoneal injection of nembutal (pentobarbital sodium, 60 mg/100 g body weight). Mice were fasted overnight before the experiments. After laparotomy, the left and median liver lobes were surgically resected. The mice were killed for collection of liver specimens at the indicated time points before or after hepatectomy, and the liver/body weight ratios were calculated to estimate the recovery of liver mass. The surgical procedure (PH) in this study was performed by the single skillful researcher. We have preliminarily examined/confirmed the accuracy of the procedure using five mice. We calculated the resection rate by [(the resected liver)/(the whole liver)], which showed  $65.8 \pm 0.17$  (%) (mean  $\pm$  s.d.). To deliver the specific gene to mice liver, adenoviruses were administered intravenously through the tail vein 72 h before the experiments. The recombinant adenovirus Adp66<sup>Shc</sup>RNAi encodes a short hairpin loop RNA with a 19-mer N-terminal CH2 domain sequence corresponding to bases 45–63 of the cDNA of p66<sup>Shc23</sup> AdLacZ, an adenovirus vector encoding  $\beta$ -galactosidase, was used as a control.

The animals were maintained under standard conditions and treated according to the Guidelines for the Care and Use of Laboratory Animals of Hokkaido University.

### Cell Proliferation Assay

To evaluate proliferation of hepatocytes after PH, proliferating cell nuclear antigen (PCNA) positive and mitotic hepatocytes were counted. Liver tissues were removed before and 72 h post-hepatectomy (for PCNA and mitosis), fixed in 10% buffered formalin, and paraffin embedded. Hematoxylin and eosin staining and immunohistochemical staining with anti-PCNA were performed. At least 500 hepatocytes were counted for mitotic index or PCNA positivity at least three times in different sections in each group.

### Measurement of Cell Size (Hepatocytes)

The method to measure the size of hepatocytes in liver sections was described earlier.<sup>24</sup> Briefly, individual hepatocytes were outlined and cross-sectional area was determined with a computer-assisted image analyzing system (LSM Image Browser, Carl Zeiss GmBH, Jena, Germany). Cell areas of at least 500 hepatocytes were randomly selected in zone 2 and calculated in triplicate using different sections in each group.

### Western Blot Analysis

Whole liver protein extract (30  $\mu$ g) was separated by 10% SDS-PAGE and transferred to a PVDF membrane. The following antibodies were used as the primary antibodies: Shc/phospho-Shc, p44/42-MAPK, phospho-p44/42-MAPK, signal transducer and activator of transcription protein 3

(STAT3)/phospho-STAT3, Akt/phospho-Akt, caspase-3, FADD-like interleukin 1 $\beta$ -converting enzyme (FLICE) (Cell Signaling, MA, USA), FLICE-inhibitory protein (FLIP), Ref-1, Mn-SOD (BD Transduction Laboratories, NJ, USA), CuZn-SOD, Bcl-2/xL, glutathione peroxidase (GPx) (Santa Cruz, CA, USA).

### In Vivo Imaging of Mouse Liver OS and Injury (Caspase-3 Activity)

Redox-sensitive fluorescent probe of GFP (reduction/oxidation-sensitive green fluorescent protein, roGFP), which allows real-time visualization of the oxidation/reduction potentials of various cells *in vitro*, has been developed and reported recently.<sup>25,26</sup> We developed recombinant adenoviral vector coding for roGFP. Disulfide bond formation between the cysteine residues promotes protonation of the chromophore, reducing the excitation spectrum peak near 480 nm. By calculating the ratios of fluorescence intensities from excitation at 480 nm, an indication of the redox potential can be measured and hence, the extent of oxidation. Three days before each experiment, AdroGFP was administered intravenously through the tail vein in a volume of 100  $\mu$ l ( $5 \times 10^7$  pfu/body) using a 31G needle. For *in vivo* liver imaging, the organ was exposed under anesthesia to enable a CCD camera to record liver images directly.<sup>27</sup> *In vivo* imaging of the mouse liver was performed using a Photon Imager (Biospace, France). Fluorescence intensities at 530 nm from excitation at 480 nm were measured and reciprocally plotted [ $1/(\text{emission } 530 \text{ at excitation } 480 \text{ nm})$ ]. This allows the signal to increase with oxidation and to decrease with reduction, and can be used as an index of *in vivo* redox state.

The optic probe, termed pcFluc-DEVD, sensing real-time caspase-3 activity has been developed.<sup>28</sup> Split N- and C-terminal ends of firefly Luciferase (Fluc) are connected with the substrate sequence for caspase-3, DEVD, then pcFluc-DEVD makes cyclic Fluc indicative of the inactive form. Once caspase-3 is activated in the cells, Fluc changes into an active form and luminescence activity is restored. We developed recombinant adenoviral vector coding for pcFluc-DEVD, and infected it to mouse ( $5 \times 10^7$  pfu/body); D-luciferin, a luciferase substrate, was injected intraperitoneally at a dose of 3 mg/100  $\mu$ l in PBS. *In vivo* imaging of the mouse liver was performed using an *in vivo* imager for 5 min, from 5 to 10 min after injection.

### Biochemical Measurement of OS and Apoptosis in Liver Tissue

For evaluation of apoptotic cell death in liver tissue, an ELISA kit (Cell Death Detection ELISA<sup>PLUS</sup> Roche, Basel, Switzerland) was used according to the manufacturer's instructions. Biochemical analyses, such as tissue 4-hydroxy-2-nonenal (4-HNE) as a peroxidation index and serum GOT/GPT/LDH levels as liver damage indices, were performed at the time points indicated before and after reperfusion. For

western blot analysis, anti-4-HNE was purchased from Japan Institute for the Control of Aging (JaICA, Shizuoka, Japan).

### Statistical Analysis

The results are expressed as mean  $\pm$  s.e.m. Statistical analyses were performed with Fishers' test and *P*-values <0.05 were considered significant.

## RESULTS

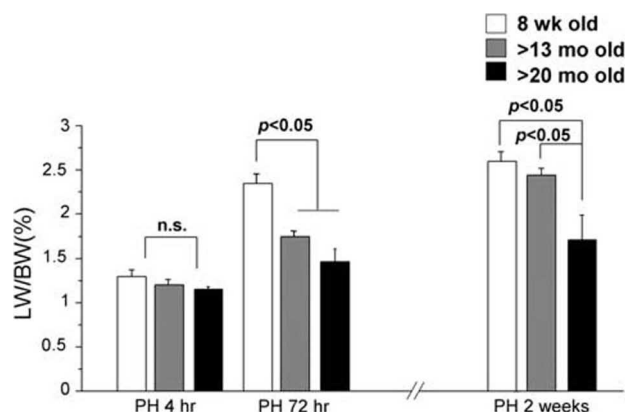
### Liver Regeneration Was Impaired After PH in Aged Mice

We first examined liver recovery after 70% PH in the young, middle-aged, and aged mice (8–10 weeks, >13, and >20 months old, respectively) (Figure 1). In the young mice, liver recovery occurred immediately after PH and continued until at least 2 weeks post-PH. In the middle-aged mice, liver recovery was insufficient at 72 h post-PH, but there was partial recovery by day 14 post-PH. In the aged mice (>20 months old), liver recovery was markedly impaired until 14 days post-PH, though recovery was somewhat observed. Liver mass recovery was impaired according to age.

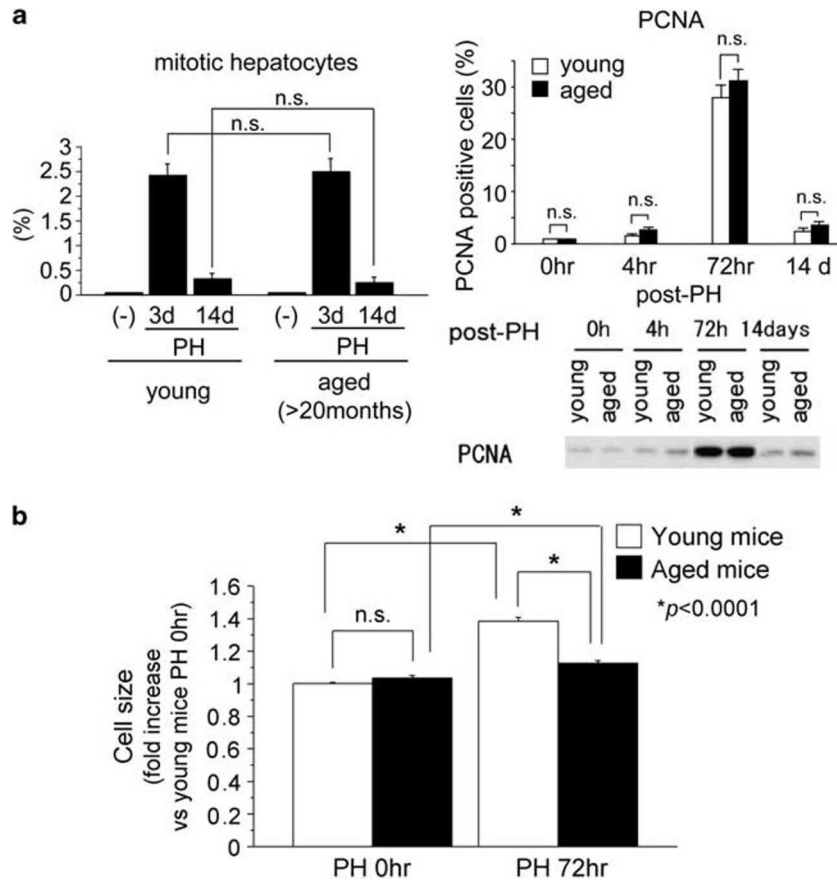
To examine the mechanism of impaired liver regeneration associated with aging, we compared young and aged mice in further studies.

### Post-PH Mitotic Response Occurred Equally in the Young and Aged Mice

Histological examination revealed that post-PH proliferation of hepatocytes occurred equally in the young and aged mice. The marked mitotic response terminated by day 14 post-PH in both groups. The mitotic hepatocytes were equally observed and peaked at 3 days post-PH in the young and aged mice (Figure 2a). Western blot and immunohistochemical analyses for PCNA also showed a peak at 72 h post-PH and a decrease thereafter (Figure 2a).



**Figure 1** Liver regeneration was impaired after partial hepatectomy (PH) in aged mice. Liver regeneration was evaluated by the recovery of liver mass (wet weight) until 14 days post-PH in young (<10 weeks), middle-aged (>12 months), and aged (>20 months) mice. At least five mice were used for each experiment. Data are expressed as mean  $\pm$  s.e.m.



**Figure 2** Post-PH mitotic response occurred equally in young and aged mice. **(a)** Histological examination (hematoxylin and eosin stain) revealed similar mitotic responses after PH in the liver of the young and aged mice. Mitotic cells were counted 72 h and 14 days post-PH. Counts of PCNA-positive hepatocytes in the regenerating liver showed equivalent mitotic responses in young and aged liver. Western blot and immunohistological analyses of PCNA confirmed the mitotic response in the liver of aged mice. Data are expressed as mean  $\pm$  s.e.m. **(b)** Hepatocytes slightly increased in size in response to PH even in aged mice, although they increased in size more in the young mice.

It has been reported that responsive cell growth (increase of cell size) mediated by phosphoinositide-dependent protein kinase 1 (PDK1)-Akt is critical for good liver regeneration in some physio-pathological situations.<sup>24,29</sup> Therefore, we compared the cell size of the hepatocytes in young and aged mice before and after PH. Hepatocytes were equal in size before PH both in the young and aged mice. Hepatocytes became larger in size in response to PH in the young mice, but not in the aged mice (Figure 2b). The defective cell growth of hepatocytes in the aged mice may partly account for the impaired liver regeneration.

### Liver Was Damaged Immediately After PH in Aged Mice

In the aged mice, liver injury occurred immediately after PH. Apoptotic cell death in the liver occurred 4 h post-PH in the aged mice (Figure 3a). Serum levels of GOT and GPT were also elevated at 4 and 72 h post-PH, and bilirubin at 72 h post-PH. These parameters recovered to baseline levels by day 14 post-PH (Figure 3b and c). Interestingly, mild apoptosis

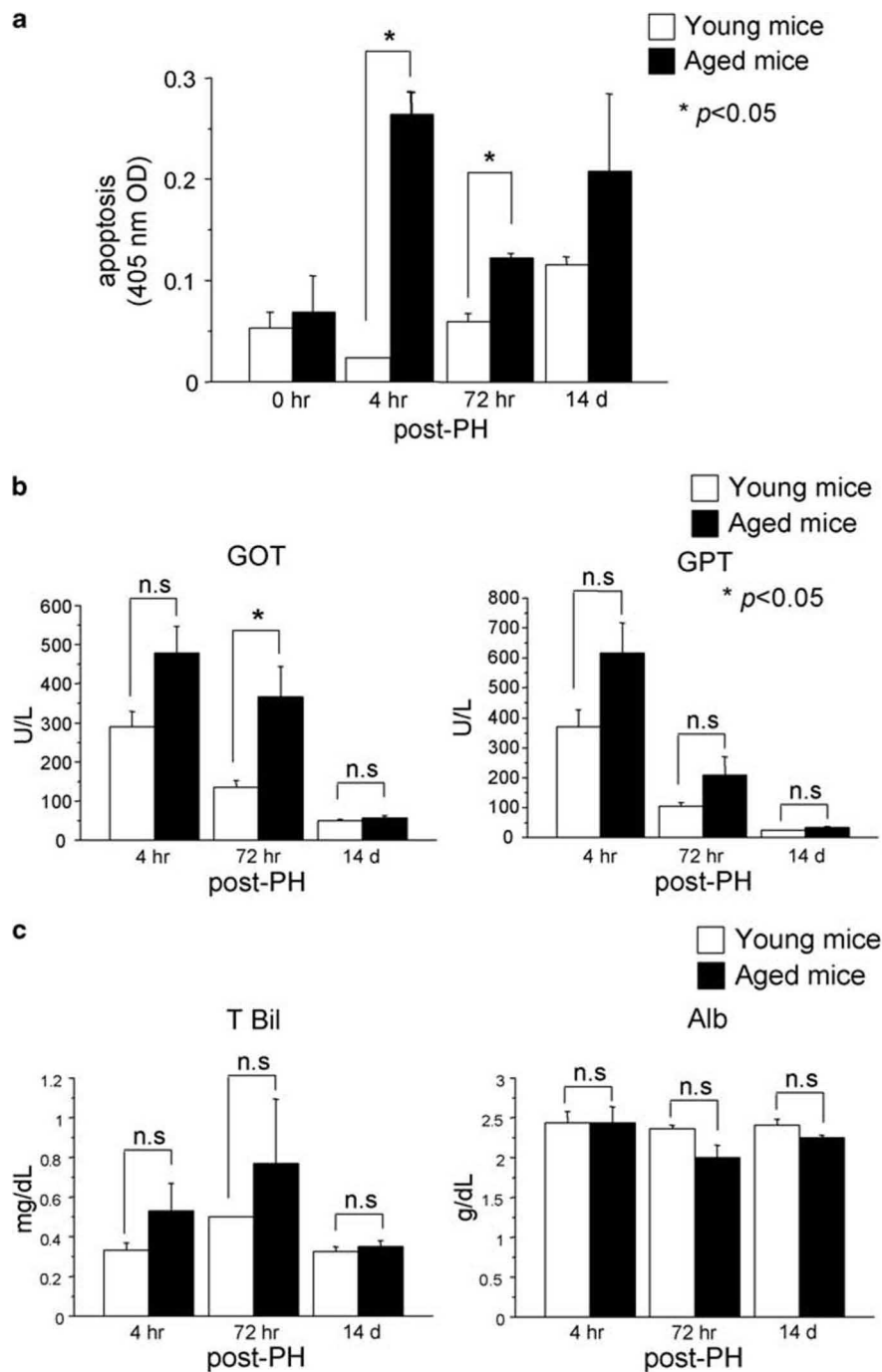
occurred 14 days post-PH in the aged mice, but did not affect serum markers of liver injury.

### Hepatic Signal Transduction Associated with Cell Proliferation/Survival, Apoptosis, and OS

To elucidate the mechanisms of apoptotic liver injury immediately after PH and the impaired liver regeneration in aged mice, we examined the signaling molecules involved in proliferation, survival, apoptosis, and anti-oxidation.

p42/44-MAPK, STAT3, and p46/52<sup>Shc</sup>, which are associated with cell proliferation, were equally expressed, but activated more in the liver of aged mice immediately after PH (Figure 4a and e). Akt was also expressed in the liver of aged mice, but was not activated, in contrast with liver from young mice.

FLICE and FLIP were expressed equally in liver from young and aged mice (Figure 4b). Bcl-2 was markedly expressed in aged mice even before PH, whereas Bcl-xL expression was slightly suppressed. Caspase-3 was activated 4 h and 14 days post-PH in aged-mouse liver, but only at 14 days post-PH in young-mouse liver.



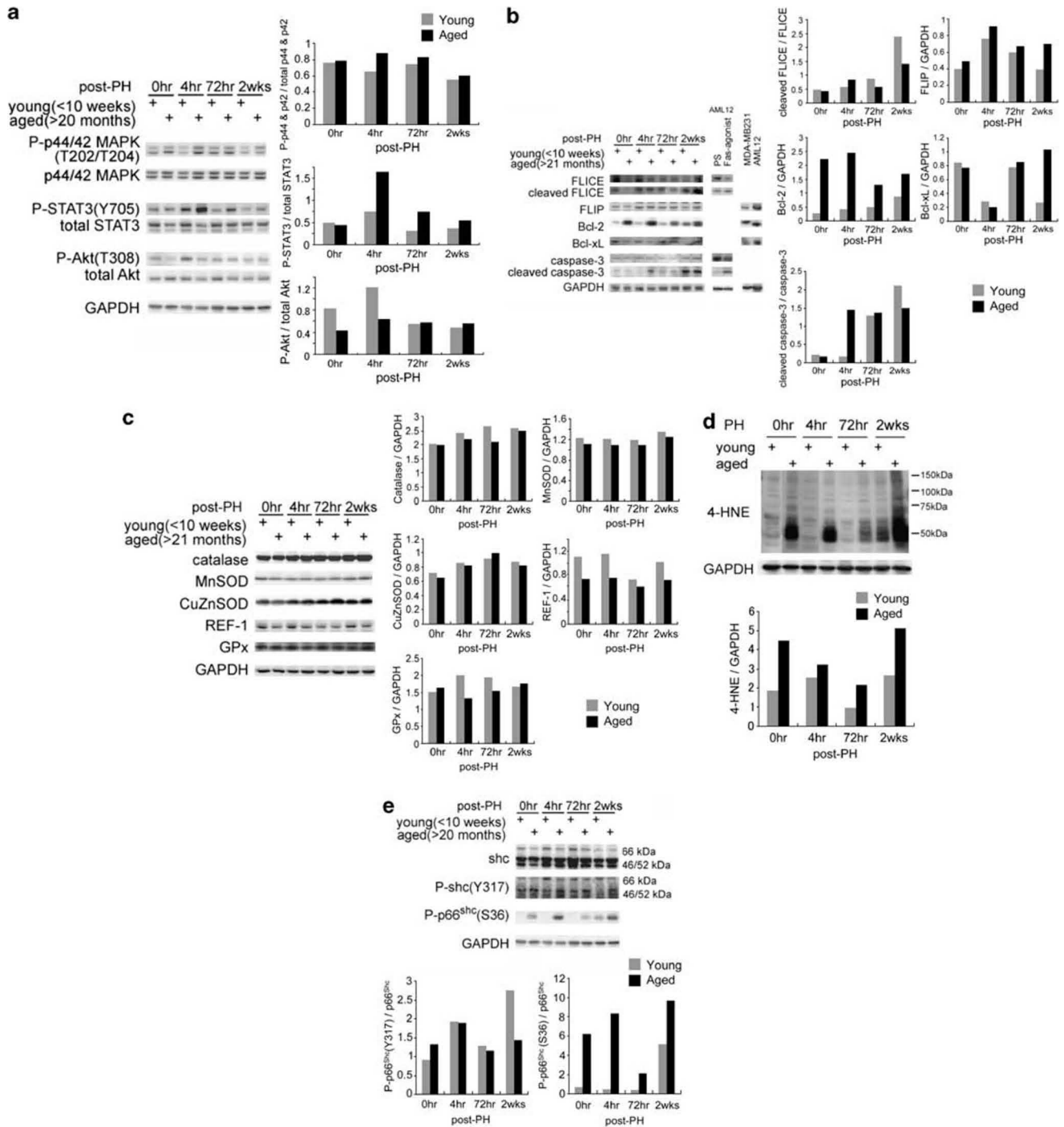
**Figure 3** Liver was damaged immediately after PH in aged mice. (a) Apoptotic cell death was observed markedly 4 h post-PH only in aged mice, and mildly 14 days post-PH both in both young and aged mice. Blood biochemistry showed immediate liver injury (b) and function (c) after PH in aged mice (GOT/GPT and bilirubin/albumin, respectively).

Anti-oxidant proteins, catalase, Mn-/CuZn-SOD, Ref-1, and GPx were equally expressed in the young- and aged-mouse liver (Figure 4c). Elevated expression of 4-HNE, a byproduct of lipid peroxidation, showed strong OS in the aged mice especially post-PH (Figure 4d). Shc protein was expressed slightly more in the liver of young mice. p66<sup>Shc</sup>, an age/redox-associated protein, was markedly phosphorylated

at serine 36 immediately after PH (Figure 4e), which has been reported to be associated with Akt activity and OS.<sup>21</sup>

#### Post-PH Liver Regeneration Was Recovered by Ablating Hepatic p66<sup>Shc</sup> in the Aged Mice

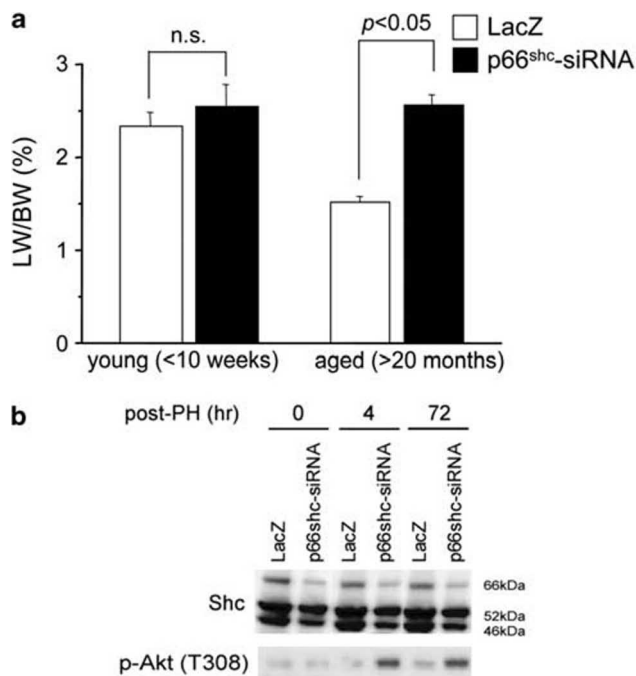
After PH in aged mice, liver injury markers (apoptosis, sGOT/GPT, caspase-3 activity, and OS) were all elevated in



**Figure 4** Signal transduction of hepatocytes associated with cell proliferation/survival, apoptosis, and oxidative stress. Expression and phosphorylation (activation) of signaling molecules were analyzed by western blotting (cell proliferation-associated molecules (a), apoptosis-associated molecules (b), anti-oxidant-associated molecules (c), oxidized product (d), and p66<sup>Shc</sup>, an age-associated molecule (e)). Whole cell extract from Fas-L (Jo2)-treated AML12 cells (1.25 μg/ml) were used as positive controls for FLICE/caspase-3 activation (b). And the whole cell extracts of MDA-MB231 breast cancer cells and AML12 liver cells were used as positive controls for FLIP and Bcl-2/-xL expressions (b).

parallel with age-associated p66<sup>Shc</sup> activation. These observations prompted us to study the function of p66<sup>Shc</sup> in the liver injury and impaired regeneration after PH in aged mice.

Ablation of hepatic p66<sup>Shc</sup> did not affect post-PH liver regeneration in young mice, but remarkably improved liver regeneration in aged mice (Figure 5a). By ablating hepatic p66<sup>Shc</sup>, the liver in aged mice recovered post-PH equally to



**Figure 5** Post-PH liver regeneration recovered as a result of ablating hepatic p66<sup>Shc</sup> in aged mice. **(a)** Adenoviral transfection of p66<sup>Shc</sup>-siRNA improved liver regeneration in aged mice. Note: post-PH liver regeneration was not affected in young mice. **(b)** By ablation of p66<sup>Shc</sup>, phosphorylation/activation of Akt was induced in the liver of aged mice. Note: ablation of p66<sup>Shc</sup> by itself did not phosphorylate/activate Akt.

that in young mice. This suggests that p66<sup>Shc</sup> and its activation have a pivotal function in the impairment of regeneration in the aged liver, possibly by inducing OS and injury (apoptosis). As expected, Akt, a pro-survival/anti-apoptotic/anti-oxidant protein, was also activated by ablating p66<sup>Shc</sup> in the liver post-PH in aged mice (Figure 5b).

### Ablation of p66<sup>Shc</sup> Protected Post-PH Liver from OS and Injury in the Aged Mice

To examine the function of p66<sup>Shc</sup> in liver regeneration in aged mice, we evaluated OS and injury (apoptosis) after PH. By transfecting roGFP and the functional bioluminescent probe for caspase-3 to liver, we measured hepatic OS and apoptosis non-invasively and chronologically in live mice.

In aged mice, marked hepatic OS was observed within 1 h post-PH and OS returned to baseline level by 4 h post-PH. In contrast, OS post-PH was not observed until at least 4 h post-PH in young mice. Ablation of p66<sup>Shc</sup> suppressed post-PH OS to pre-PH baseline level in aged mice (Figure 6a). This may indicate that p66<sup>Shc</sup> is conclusively and specifically involved in the generation of reactive oxygen species in the post-PH liver.

*In vivo* imaging of hepatic caspase-3 activity in the post-PH liver revealed that hepatic damage was initiated and peaked 2 and 8 h post-PH, respectively, in aged mice, whereas the liver was not damaged at all in young mice. Knockdown

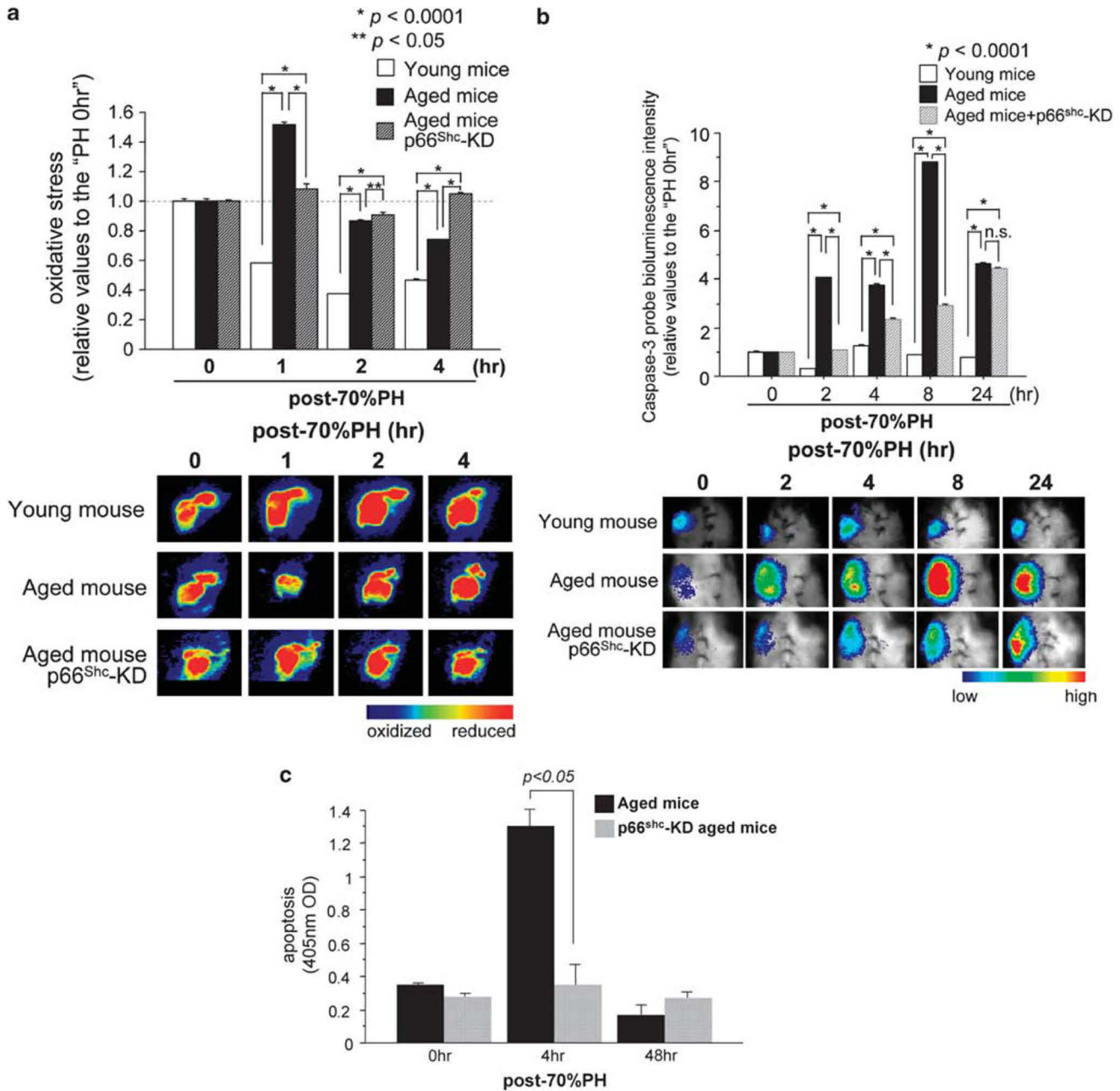
of p66<sup>Shc</sup> reduced damage in the post-PH liver in aged mice, though gradual liver damage occurred until 24 h post-PH (Figure 6b). This was also confirmed by apoptosis assay of post-PH liver (Figure 6c). Ablation of hepatic p66<sup>Shc</sup> did not affect the size of hepatocytes post-PH (data not shown).

### DISCUSSION

In this study, we showed that p66<sup>Shc</sup> primarily has a pivotal function in impaired liver regeneration in aged mice by inducing OS and damage (apoptosis) immediately after hepatectomy. Hepatocytes proliferated in response to PH equally in young and aged mice. Hepatocyte proliferation assessed by mitotic index and PCNA positivity in liver tissue did not show any difference between young and aged mice. Acute liver injury after PH occurred only in the aged mice and was the main cause for the insufficient liver regeneration. Immediate post-PH, injury was caused mainly by OS-mediated apoptosis, which was regulated by age-associated p66<sup>Shc</sup>. It also has been reported that responsive cell growth (increase of cell size) after PH is critical for normal liver regeneration in some patho-physiological situations and is regulated by the PDK1-Akt pathway.<sup>24,29</sup> These findings support our present data that hepatocytes lacking Akt activity failed to increase their size after PH in the aged mice. Taken together, acute liver injury and lack of cell growth are the most conceivable causes for the impaired liver regeneration in aged mice. p66<sup>Shc</sup>, an age/redox-associated molecule, had a pivotal function in the impairment of liver regeneration by up-regulating post-PH OS/injury and reducing Akt activity/cell size.

Analyses of signals in the regenerating liver revealed that cell proliferation-associated molecules (MAPK, STAT3, p46/52<sup>Shc</sup>) were all activated in response to PH even in aged mice and actually induced hepatocyte proliferation to a degree comparable with that of young mice. There were no differences in the expression of the apoptosis-associated proteins such as FLICE and FLIP. Although Bcl-XL expression was slightly reduced, Bcl-2 expression was markedly increased in aged-mouse liver. It is not clear why these two proteins were differentially expressed in aged mice. Strong expression of Bcl-2 may have occurred in response to chronic OS in the liver of aged mice (Figure 4d), though aged mice liver expressed anti-oxidant proteins such as Catalase, Mn-/CuZn-SOD, Ref-1, GPx to a degree comparable with those of young mice liver.

p66<sup>Shc</sup>, an aging- and OS-associated molecule, was markedly phosphorylated at serine 36 in the liver of aged mice liver, although phosphorylation at tyrosine 317 was weak. Phosphorylation of p66<sup>Shc</sup> at serine 36 has been reported to induce cellular OS by regulating (suppressing) Akt activity and catalase expression,<sup>21</sup> which can be a mechanism underlying post-PH liver damage in aged mice. In young mice, PH caused liver damage as a result of surgical stress, but did not induce OS and apoptosis immediately post-PH (Figure 3) and did not activate p66<sup>Shc</sup>.



**Figure 6** Ablation of p66<sup>Shc</sup> protected post-PH liver from oxidative stress and injury in aged mice. (a) Bioimaging of liver OS after PH. UV emission from roGFP from remnant right liver lobe was directly measured from the exposed liver surface and imaged 0, 1, 2, and 4 h after PH and quantified. Each experiment was performed at least three times in each group and the photos are representative of at least three independent experiments. Data in the graph are expressed as mean  $\pm$  s.e.m., and were expressed relative to the pre-PH control in each group. Note: signals (red color area) in aged mice were transiently reduced 1 h after PH, indicating liver OS. Ablation of p66<sup>Shc</sup> reduced OS in the post-PH liver. (b) Bioimaging of liver caspase-3 activity after PH. Luciferase/luciferin-induced bioluminescent signals of remnant right liver lobe were measured from the body surface for 5 min (5–10 min after intraperitoneal injection of luciferin) and imaged 0, 2, 4, 8, and 24 h after PH. Each experiment was performed at least three times in each group and the photos are the representative of at least three independent experiments. Data are expressed as mean  $\pm$  s.e.m. relative to the pre-PH control in each group. (c) Apoptotic cell death was markedly suppressed 4 h post-PH in p66<sup>Shc</sup>-deleted aged mice. The results are expressed as mean  $\pm$  s.e.m. of three independent experiments. A  $P$ -value  $< 0.05$  was considered significant.

In this study, phosphorylation of p66<sup>Shc</sup> at serine 36 occurred in response to PH only in aged mice, although the expression of major anti-oxidant molecules examined was not changed. Knocking down of liver p66<sup>Shc</sup> led to marked

recovery of liver regeneration after PH in aged mice, but did not affect regeneration in young mice. In addition, deletion of p66<sup>Shc</sup> reduced OS and caspase-3 activity and improved liver injury and regeneration post-PH in the liver of aged



mice. These facts suggest that p66<sup>Shc</sup> has a pivotal function in the OS-mediated injury post-PH and the impaired liver regeneration in aged mice.

Akt was activated immediately after PH in the liver of young but not aged mice. By ablating p66<sup>Shc</sup>, Akt was phosphorylated/activated in the post-PH liver. Ablation of p66<sup>Shc</sup> has earlier been reported to phosphorylate Akt in some types of cells.<sup>19,21,30</sup> In this study, knockdown of p66<sup>Shc</sup> phosphorylated/activated Akt in the post-PH liver, similarly to the young mice. As Akt is known as an anti-oxidant/pro-survival molecule,<sup>31–33</sup> Akt activation immediately after PH resulting from p66<sup>Shc</sup> knockdown may have a major function in suppressing post-PH OS/apoptosis and injury in the post-PH liver of aged mice.

In this study, we elucidated the age-specific mechanisms of impaired liver regeneration after PH by identifying the function of p66<sup>Shc</sup> in post-PH OS and apoptosis. By regulating hepatic OS, p66<sup>Shc</sup> may have a pivotal function in liver regeneration in aged mice. Further studies using other animal models are needed to more fully elucidate and conclude the mechanisms by which aging leads to the impairment of liver regeneration. This study, however, provides some potentially important clues to understand the relationship between aging and liver regeneration.

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#### DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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