



RESEARCH ARTICLE

Open Access

# The adaptor protein SH2B1 $\beta$ reduces hydrogen peroxide-induced cell death in PC12 cells and hippocampal neurons

Wan-Chen Lu<sup>1</sup>, Chien-Jen Chen<sup>1</sup>, Hui-Chien Hsu<sup>1</sup>, Hsin-Ling Hsu<sup>4</sup>, Linyi Chen<sup>1,2,3\*</sup>

## Abstract

**Background:** SH2B1 $\beta$  is a signaling adaptor protein that has been shown to promote neuronal differentiation in PC12 cells and is necessary for the survival of sympathetic neurons. However, the mechanism by which SH2B1 $\beta$  may influence cell survival is not known.

**Results:** In this study, we investigated the role of SH2B1 $\beta$  in oxidative stress-induced cell death. Our results suggest that overexpressing SH2B1 $\beta$  reduced H<sub>2</sub>O<sub>2</sub>-induced, caspase 3-dependent apoptosis in PC12 cells and hippocampal neurons. In response to H<sub>2</sub>O<sub>2</sub>, overexpressing SH2B1 $\beta$  enhanced PI3K (phosphatidylinositol 3-kinase)-AKT (protein kinase B) and MEK (MAPK/ERK kinase)-extracellular-signal regulated kinases 1 and 2 (ERK1/2) signaling pathways. We further demonstrated that SH2B1 $\beta$  was able to reduce H<sub>2</sub>O<sub>2</sub>-induced nuclear localization of FoxO1 and 3a transcription factors, which lie downstream of PI3K-AKT and MEK-ERK1/2 pathways. Moreover, overexpressing SH2B1 $\beta$  reduced the expression of Fas ligand (FasL), one of the target genes of FoxOs.

**Conclusions:** Overexpressing the adaptor protein SH2B1 $\beta$  enhanced H<sub>2</sub>O<sub>2</sub>-induced PI3K-AKT and MEK-ERK1/2 signaling, reduced nucleus-localized FoxOs and the expression of a pro-apoptotic gene, FasL.

## Introduction

Oxidative stress resulting from overload of toxic reactive oxygen species (ROS) is common in the etiology of human diseases. It has been implicated in various neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, and Huntington's disease [1-4]. It also contributes to acute damage resulting from hypoxic-reperfusion conditions after trauma or stroke [5,6]. The accumulation of ROS, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), leads to various forms of reversible and irreversible oxidative modification of proteins, lipids and DNA, accounting for cellular damage [7]. Depending on the extent of oxidative stress, it can induce proliferation, growth arrest, senescence, apoptosis (programmed cell death) or necrosis [8-11].

A number of signaling pathways are evolved to protect cells from ROS-induced damages, including phosphatidylinositol 3-kinase (PI3K)-AKT pathway, mitogen-

activated protein kinases (MAPKs) pathways, and phospholipase C $\gamma$  (PLC $\gamma$ ) signaling [12-20]. PI3K-AKT pathway predominantly acts to promote cell survival. The three family members of MAPKs are identified as being sensitive to oxidative stress. They are extracellular-signal regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38MAPK. Controversial reports implicating the influence of oxidative stress-induced MAPK activation on both cell survival and death are more complicated than one has anticipated [21-30]. In most cases, MEK-ERK1/2, similar to PI3K-AKT pathway, promotes cell survival in response to oxidative stress.

SH2B1 is a signaling adaptor protein that belongs to SH2B family, including SH2B1, SH2B2 (APS) and SH2B3 (Lnk) [31,32]. SH2B1 has been implicated in signaling pathways initiated by several receptor tyrosine kinases, including growth hormone, nerve growth factor (NGF), insulin, insulin-like growth factor 1, brain-derived neurotrophic factor, glial-derived neurotrophic factor, platelet-derived growth factor, and fibroblast growth factor 1 [31,33-41]. Four isoforms have been identified for SH2B1 —  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  [33]. Previous

\* Correspondence: linyic@gmail.com

<sup>1</sup>Institute of Molecular Medicine, National Tsing Hua University, Hsinchu, Taiwan

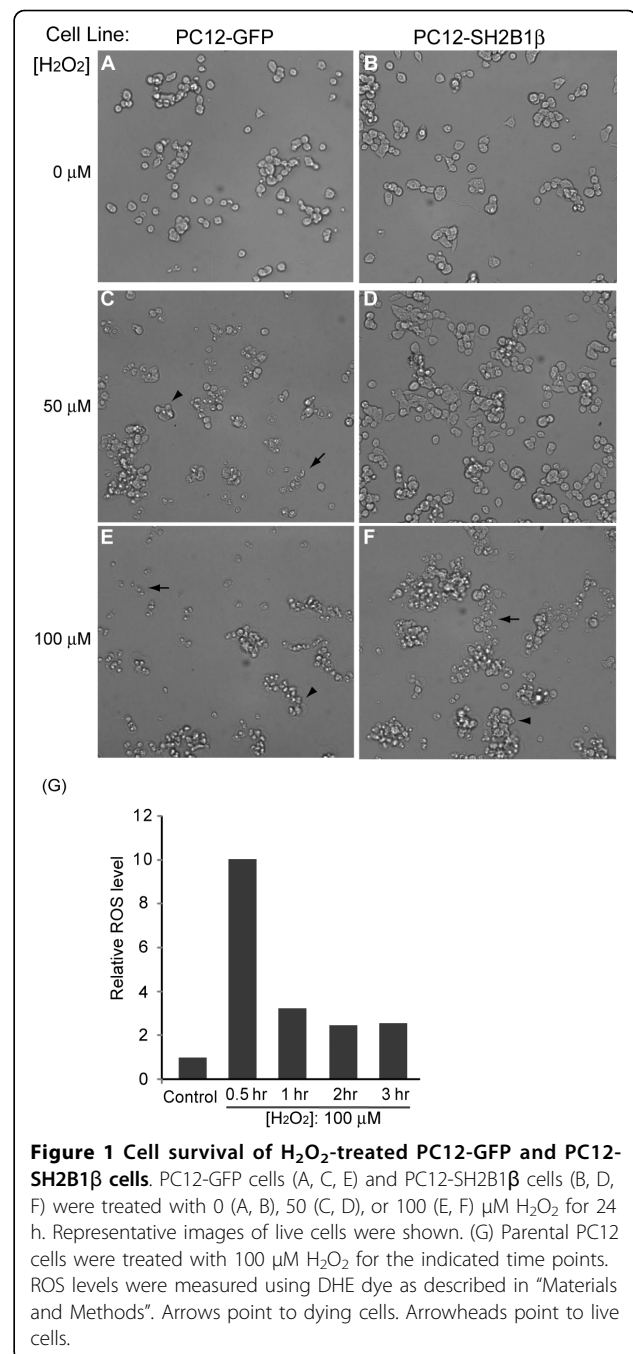
Full list of author information is available at the end of the article

studies demonstrate that SH2B1 plays an essential role in neuronal differentiation of PC12 cells, a well-established neuronal model [37,39,41,42]. SH2B1 $\beta$  also supports axonal growth of sympathetic neurons and is required for the survival of neonatal sympathetic neurons [37]. Moreover, SH2B1 $\beta$  acts as a positive mediator of NGF-mediated activation of AKT/Forkhead pathway by affecting the subcellular distribution of FoxO1 and 3a [43]. Forkhead transcription factors comprise more than 100 structurally related members that share a conserved forkhead domain (FKH) and a 100-residue DNA-binding domain. They have been named Fox (forkhead box) transcription factors [44]. Mammalian FoxO proteins (FoxO1, 3, 4 and 6) belong to O (other) class of the Fox superfamily. The nucleus-localized FoxOs are known to induce the expression of pro-apoptotic genes, such as FasL (Fas ligand) [45]. Therefore, inactivating FoxOs prevents their entry to the nucleus and triggering apoptosis. AKT is known to phosphorylate FoxOs and thus reduces their nuclear localization [46-49]. MAPKs have also been reported to phosphorylate FoxOs [50-52]. The fact that overexpressing SH2B1 $\beta$  shifts the steady-state distribution of FoxO1 in PC12 cells [43] raises a possibility that SH2B1 $\beta$  may affect cell survival through FoxO family members. To understand how SH2B1 $\beta$  may regulate cell survival/death, cells were challenged with oxidative stress and the effect of SH2B1 $\beta$  was examined. In this study, we investigated the role of SH2B1 $\beta$  in oxidative stress-induced cell death, signaling, FoxOs distribution and their target gene expression.

## Results

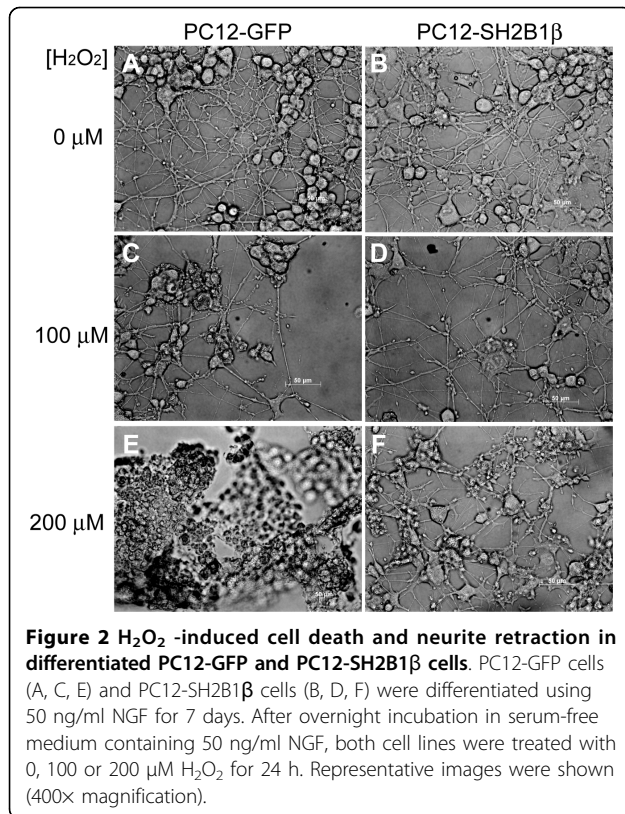
### Overexpressing SH2B1 $\beta$ reduces hydrogen peroxide-induced cell death in PC12 cells

To determine whether SH2B1 $\beta$  affects oxidative stress-induced cell death, PC12 cells stably expressing GFP (PC12-GFP cell line) or GFP-SH2B1 $\beta$  (PC12-SH2B1 $\beta$  cell line) were treated without (Figure 1A, B) or with (Figure 1C, D, E, F) H<sub>2</sub>O<sub>2</sub>. With increasing concentration of H<sub>2</sub>O<sub>2</sub>, both cell lines showed increased cell death. Notably, PC12-SH2B1 $\beta$  cells showed less cell death compared to PC12-GFP cells. To verify that H<sub>2</sub>O<sub>2</sub> treatment effectively increased cellular oxidative stress, an oxidation indicator dye, dihydroethidine (DHE), was used to monitor cellular oxidation. As shown in Figure 1G, oxidative stress was increased within 30 min of 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> treatment. The elevated ROS was reduced afterwards, likely through cellular reduction, and remained higher than basal level for at least 3 h. This dosage of H<sub>2</sub>O<sub>2</sub> also resulted in death of primary culture of hippocampal neurons (Additional file 1). The protective effect of overexpressing SH2B1 $\beta$  in H<sub>2</sub>O<sub>2</sub>-treated differentiated PC12 cells was also examined. H<sub>2</sub>O<sub>2</sub> treatment induced retraction of neurites as well as death of differentiated PC12



**Figure 1 Cell survival of H<sub>2</sub>O<sub>2</sub>-treated PC12-GFP and PC12-SH2B1 $\beta$  cells.** PC12-GFP cells (A, C, E) and PC12-SH2B1 $\beta$  cells (B, D, F) were treated with 0 (A, B), 50 (C, D), or 100 (E, F)  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 24 h. Representative images of live cells were shown. (G) Parental PC12 cells were treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for the indicated time points. ROS levels were measured using DHE dye as described in "Materials and Methods". Arrows point to dying cells. Arrowheads point to live cells.

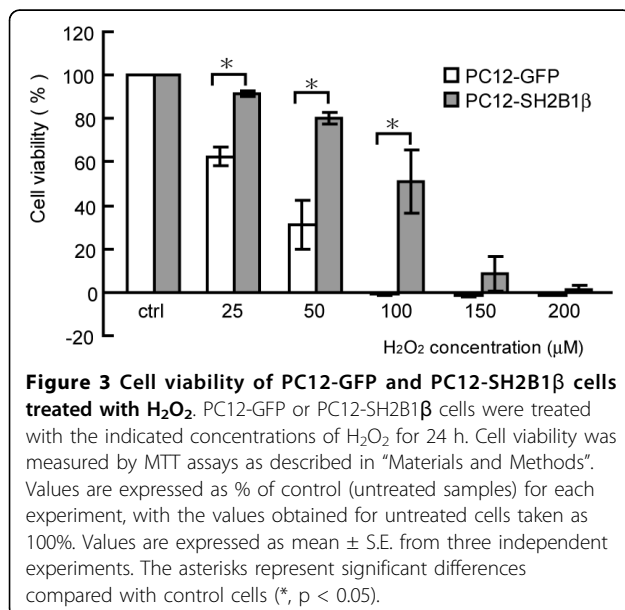
cells. Similarly, differentiated PC12-SH2B1 $\beta$  cells showed less cell death compared to differentiated PC12-GFP cells. These results suggest that overexpressing SH2B1 $\beta$  reduces H<sub>2</sub>O<sub>2</sub>-induced cell death in both undifferentiated and differentiated PC12 cells (Figure 2). To quantify cell viability, MTT assays were used to assess H<sub>2</sub>O<sub>2</sub>-induced cell death in PC12 cells. In all H<sub>2</sub>O<sub>2</sub> concentrations tested, cell survival was higher in PC12-SH2B1 $\beta$  cells compared to PC12-GFP cells (Figure 3). For instance, as most of PC12-GFP cells underwent dramatic cell death



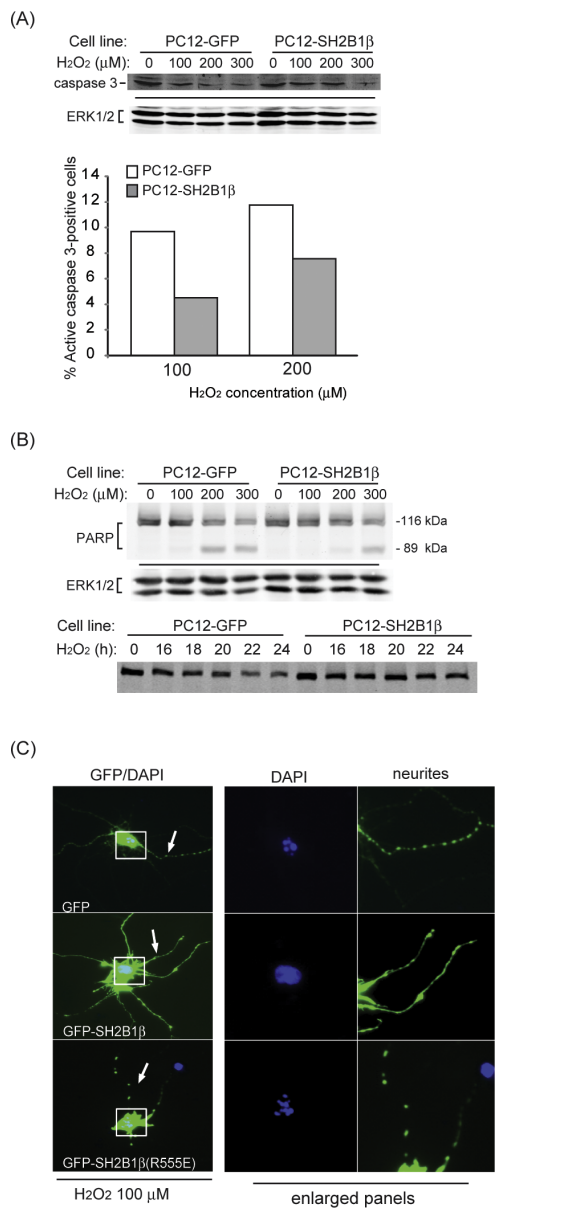
when treated with 100 μM H<sub>2</sub>O<sub>2</sub> for 24 h, PC12-SH2B1β remained nearly 50% survival rate (Figure 3).

### H<sub>2</sub>O<sub>2</sub> induces caspase 3-dependent cell death in PC12 cells

Low level of oxidative stress has been suggested to lead to apoptosis while high level of oxidative stress leads to



apoptosis and necrosis [8,9,53]. In the present study, relatively low concentrations of H<sub>2</sub>O<sub>2</sub> were used to more closely reflect the physiological stress [8]. During early apoptosis, phospholipids phosphatidylserine (PS) from the inner leaflet is translocated to the outer leaflet of the plasma membrane allowing for Annexin V binding. Thus, detecting the relative amount of Annexin V binding was measured to determine whether H<sub>2</sub>O<sub>2</sub> induces apoptosis in PC12 cells. The relative Annexin V binding was increased in response to H<sub>2</sub>O<sub>2</sub> treatment suggesting that concentrations of H<sub>2</sub>O<sub>2</sub> used in this study induced apoptosis (Additional file 2). The processes of apoptosis could be caspase-dependent or caspase-independent (e.g. necroptosis) [54-61]. To further determine whether H<sub>2</sub>O<sub>2</sub> induces caspase 3-dependent apoptosis and whether overexpressing SH2B1β affects caspase 3 activity, PC12-GFP and PC12-SH2B1β cells were treated with H<sub>2</sub>O<sub>2</sub> and the level of full length caspase 3 was determined via western blotting. In response to H<sub>2</sub>O<sub>2</sub>, full length caspase 3 was reduced, resulting from activation and cleavage of caspase 3 (Figure 4A, upper panel). The relative amount of full length caspase 3 was higher in PC12-SH2B1β cells compared to PC12-GFP cells. The population of active caspase 3-positive cells was also lower in PC12-SH2B1β cells than in PC12-GFP cells (Fig. 4A, lower panel). Along this line, the relative amount of poly (ADP-ribose) polymerase (PARP), a substrate of caspase 3, was determined in PC12-GFP and PC12-SH2B1β cells to reflect the relative activity of caspase 3. The relative level of full length PARP was higher in PC12-SH2B1β cells compared to PC12-GFP cells and the reduction of full length PARP was more dramatic after 22 h of H<sub>2</sub>O<sub>2</sub> challenge in PC12-GFP cells (Figure 4B). These data suggest that H<sub>2</sub>O<sub>2</sub> induces caspase 3-dependent apoptosis in PC12 cells and overexpressing SH2B1β reduces the activity of caspase 3 and thus PARP cleavage. Similarly, the active caspase 3 was more prominent in hippocampal neurons overexpressing GFP than those overexpressing GFP-SH2B1β. In contrast, hippocampal neurons overexpressing the dominant negative mutant of SH2B1β, GFP-SH2B1β(R555E), were more susceptible to H<sub>2</sub>O<sub>2</sub>, leading to more caspase 3 cleavage compared to control cells (Additional file 3). Another phenotype of cells undergoing apoptosis is nuclear condensation. Hippocampal neurons subjected to H<sub>2</sub>O<sub>2</sub> treatment showed obvious neurite retraction, beaded dendrites and condensation of the nucleus. As majority of neurons overexpressing GFP-SH2B1β showed intact nucleus, neurons that expressing GFP or GFP-SH2B1β(R555E) showed fragmented nucleus (Figure 4C). Together, these data demonstrate that SH2B1β reduces H<sub>2</sub>O<sub>2</sub>-induced caspase 3-dependent apoptosis in both PC12 cells and hippocampal neurons.



**Figure 4 H<sub>2</sub>O<sub>2</sub> induces caspase 3-dependent apoptosis in PC12-GFP and PC12-SH2B1 $\beta$  cells.** PC12-GFP and PC12-SH2B1 $\beta$  cells were treated with 0, 100, 200, 300  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 18 h. Equal amounts of proteins from the lysates were resolved with SDS-PAGE and immunoblotted with (A) anti-caspase 3 (upper panel), or (B) anti-PARP antibody. ERK levels were used as loading controls. For % cells with anti-active caspase 3 staining, cells were treated with 100 or 200  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 18 h and then subjected to immunofluorescence staining using anti-active caspase 3 antibody (A, lower panel). Percentages of active caspase 3-positive cells were counted from 145-211 cells/condition. (C) Hippocampal neurons from E18 embryos were transiently transfected with GFP, GFP-SH2B1 $\beta$  or GFP-SH2B1 $\beta$ (R555E) and then treated with H<sub>2</sub>O<sub>2</sub> for 18 h. Cells were then subjected to immunofluorescence staining with DAPI (shown in blue) to mark the nucleus. Green fluorescence (GFP) showed the transfected cells. Boxes mark the nucleus and arrows point to the neurites. Enlarged images of the nucleus and neurites are shown on the right panels.

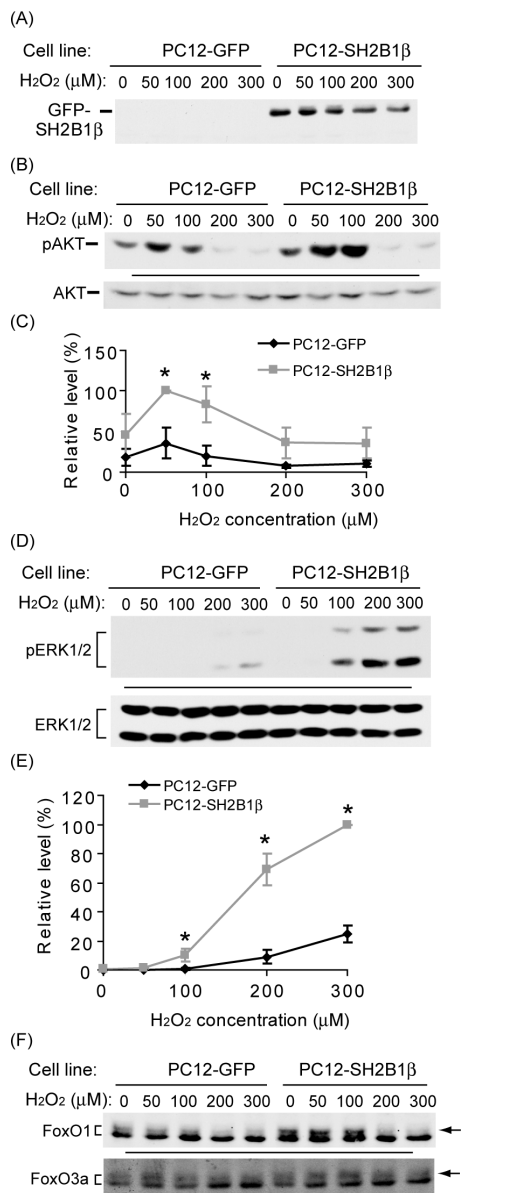
### Overexpressing SH2B1 $\beta$ enhances H<sub>2</sub>O<sub>2</sub>-induced phosphorylation of AKT and ERK1/2

To investigate the mechanisms by which SH2B1 $\beta$  protects cells from oxidative stress, the effect of overexpressing SH2B1 $\beta$  on H<sub>2</sub>O<sub>2</sub>-induced cellular signaling was examined. Figure 5A showed that GFP-SH2B1 $\beta$  was overexpressed in PC12-SH2B1 $\beta$  cells but not in PC12-GFP cells. In PC12-GFP cells, phosphorylation of AKT (pAKT) was induced in response to 50  $\mu$ M H<sub>2</sub>O<sub>2</sub>. On the other hand, overexpressing SH2B1 $\beta$  significantly enhanced the levels of pAKT in response to 50 and 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> and, as H<sub>2</sub>O<sub>2</sub> concentration increased, pAKT decreased (Figure 5B, C). Overall, the levels of pAKT were higher in PC12-SH2B1 $\beta$  than in PC12-GFP cells. Different from pAKT signal, phosphorylation of ERK1/2 (pERK1/2) was induced by H<sub>2</sub>O<sub>2</sub> concentration higher than 200  $\mu$ M in PC12-GFP cells and 100  $\mu$ M in PC12-SH2B1 $\beta$  cells. H<sub>2</sub>O<sub>2</sub>-induced pERK1/2 was much more enhanced in PC12-SH2B1 $\beta$  cells compared to PC12-GFP cells (Figure 5D). The quantified results are shown in Figure 5E. Together, these results suggest that SH2B1 $\beta$  enhances H<sub>2</sub>O<sub>2</sub>-induced PI3K-AKT and MEK-ERK1/2 signaling.

### SH2B1 $\beta$ enhances phosphorylation of FoxOs, reduces their nuclear localization and target gene expression

FoxO transcription factors are known downstream effectors of AKT [46,51,62,63]. They have also been reported to be substrates of pERK1/2, p38MAPK and pJNK [23,50,64]. Since their subcellular distribution is controlled by phosphorylation, the downstream gene expression is likely affected by their phosphorylation status. As SH2B1 $\beta$  enhanced both pAKT and pERK1/2 levels, the phosphorylations of FoxO1 and 3a were examined. As in Figure 5F, phosphorylated FoxO1 and 3a were slightly increased in response to 50  $\mu$ M H<sub>2</sub>O<sub>2</sub> and then decreased when treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> and above. The extents of FoxO1 and 3a phosphorylation were more prominent in PC12-SH2B1 $\beta$  cells than those in PC12-GFP cells.

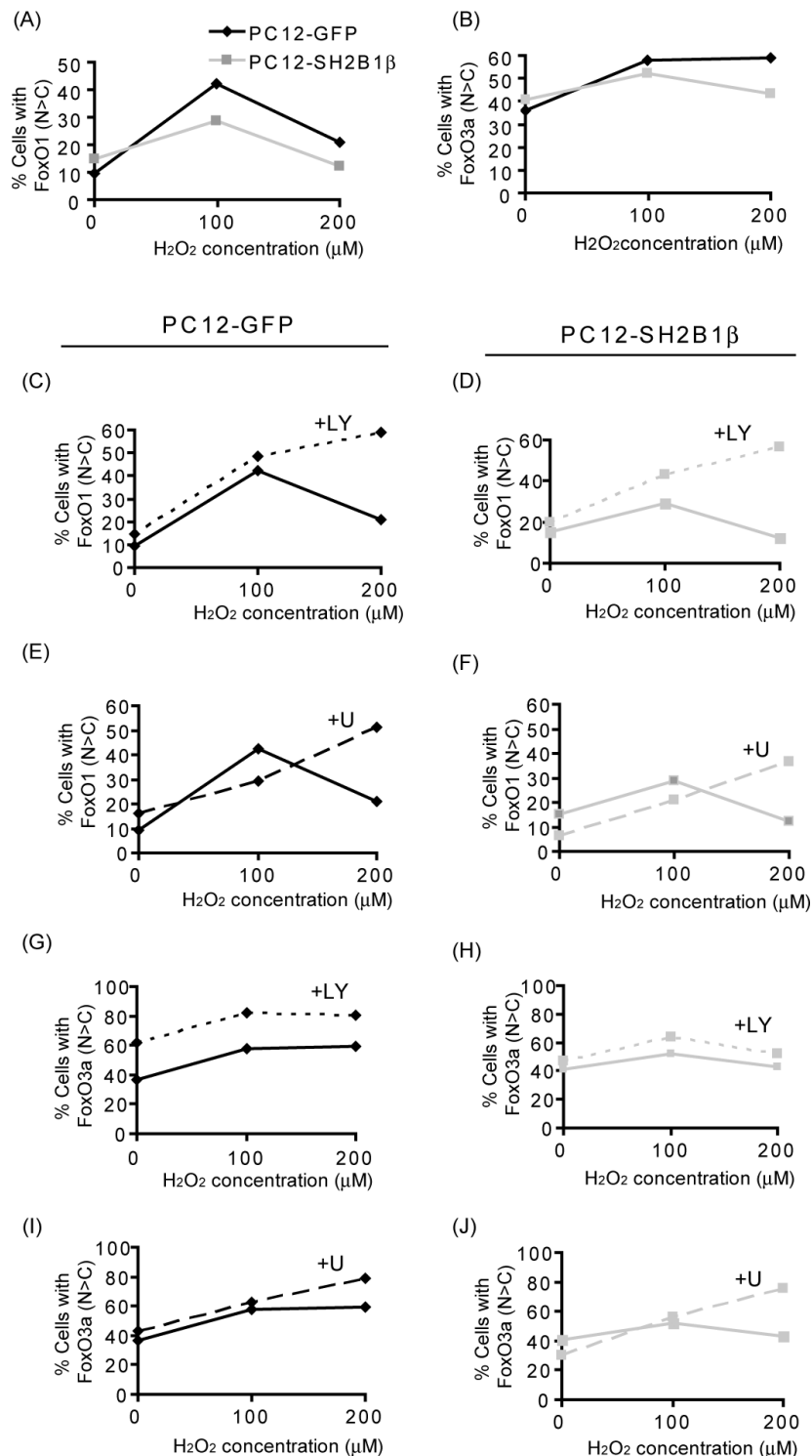
To examine the effect of SH2B1 $\beta$  on the distribution of FoxOs, PC12-GFP and PC12-SH2B1 $\beta$  cells were treated with H<sub>2</sub>O<sub>2</sub> and the localization of FoxO1 and 3a were determined via immunofluorescence staining. The percentage of cells with FoxO1 fluorescence intensity in the nucleus higher than that in the cytoplasm was quantified and compared between the two stable cell lines. As expected, H<sub>2</sub>O<sub>2</sub> increased nuclear localization of FoxO1 in both cell lines. Overexpressing SH2B1 $\beta$  reduced nuclear localization of FoxO1 by 15% and 8% in response to 100 and 200  $\mu$ M H<sub>2</sub>O<sub>2</sub> respectively (Figure 6A). In contrast, SH2B1 $\beta$  reduced nuclear localization of FoxO3a by 6% and 16% in response to 100 and 200  $\mu$ M H<sub>2</sub>O<sub>2</sub> (Figure 6B). Because pAKT and pERK1/2



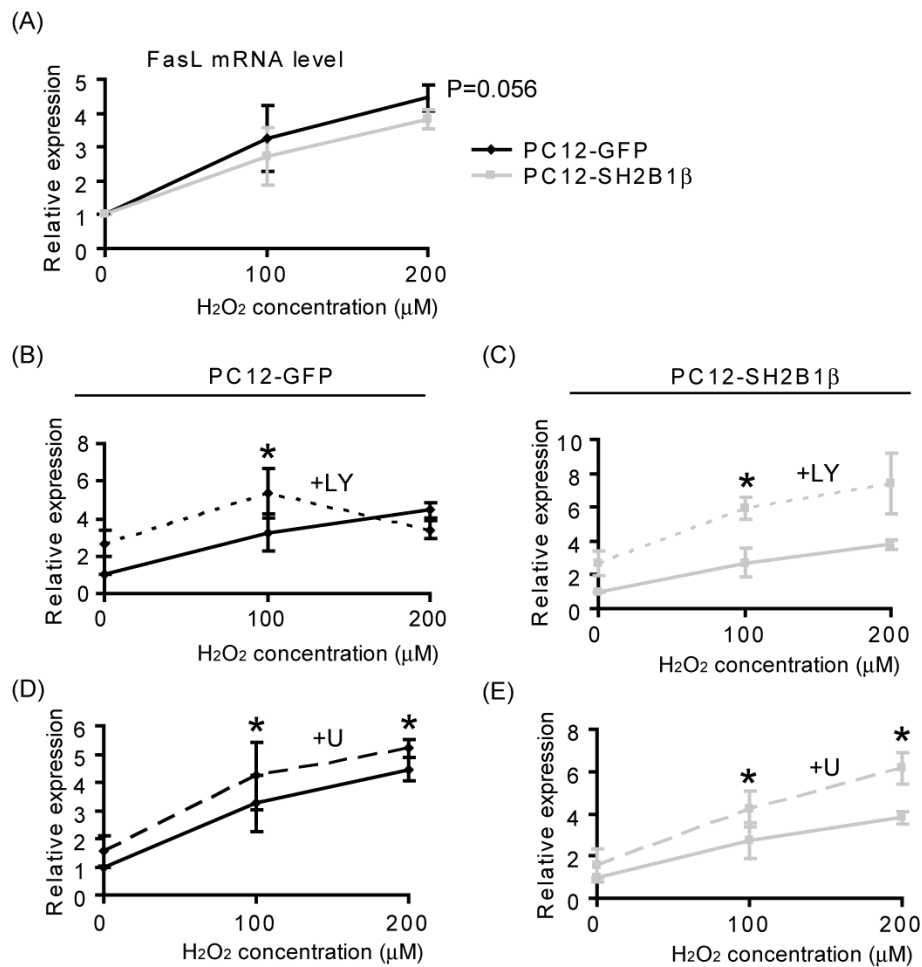
**Figure 5 SH2B1β enhances the phosphorylation levels of AKT, ERK1/2 and FoxOs.** PC12-GFP and PC12-SH2B1β cells were incubated in serum-free medium overnight followed by H<sub>2</sub>O<sub>2</sub> stimulation with the indicated concentrations for 10 min. Equal amounts of proteins from the lysates were resolved via SDS-PAGE and immunoblotted with (A) anti-GFP; (B) anti-pAKT(Ser473) and anti-AKT; (D) anti-pERK1/2 and anti-ERK1/2; (F) anti-FoxO1 and anti-FoxO3a antibodies. Representative blots were shown. (C) The quantified results of pAKT in PC12-GFP and PC12-SH2B1β cells were shown. The levels of pAKT were normalized to levels of AKT at each condition. The relative level of pAKT at 50 μM of H<sub>2</sub>O<sub>2</sub> in PC12-SH2B1β cells was defined as 100% in each experiment and others were normalized to this value. (E) The levels of pERK1/2 were normalized to total ERK1/2 levels, the relative level of pERK1/2 at 300 μM in PC12-SH2B1β cells was defined as 100% for each experiment and others were normalized to this value. Data are expressed as mean ± S.E. from three (for AKT) or five (for ERK1/2) independent experiments. Arrows point to the phospho-FoxO1 and 3a.

were induced by different concentration of H<sub>2</sub>O<sub>2</sub>, the contribution of these signaling pathways to FoxO distribution was determined through inhibitor assays. In PC12-GFP cells, H<sub>2</sub>O<sub>2</sub>-induced nuclear distribution of FoxO1 was increased in the presence of PI3K and MEK inhibitors (LY294002 and U0126 respectively), suggesting the involvement of pAKT and pERK1/2 in cellular distribution of FoxO1 (Figure 6C, E). In PC12-SH2B1β cells, inhibiting PI3K increased nuclear localization of FoxO1 when treated with 100 and 200 μM H<sub>2</sub>O<sub>2</sub>, while inhibiting MEK increased the nuclear localization of FoxO1 at 200 μM H<sub>2</sub>O<sub>2</sub> (Figure 6D, F). The effect of PI3K inhibitor on FoxO1 localization in PC12-SH2B1β cells was much more significant than that in PC12-GFP cells suggesting that SH2B1β promotes the cytoplasmic distribution of FoxO1 largely through PI3K-AKT pathway.

For FoxO3a distribution, inhibiting PI3K increased its nuclear localization for both cell lines whereas inhibiting MEK increased its nuclear localization when treated with 200 μM H<sub>2</sub>O<sub>2</sub> (Figure 6G, H, I, J). The effect of MEK inhibitor on the nuclear localization of FoxO3a was more prominent in PC12-SH2B1β cells than that in PC12-GFP cells suggesting that SH2B1β may increase pERK1/2 to regulate the distribution of FoxO3a in response to 200 μM H<sub>2</sub>O<sub>2</sub>. To determine whether SH2B1β regulates the transcriptional activity of FoxOs, the expressions of FasL were assessed via semi-quantitative real time polymerase chain reaction (Q-PCR). As in Figure 7A, the expression of FasL was induced in response to H<sub>2</sub>O<sub>2</sub> treatment and the induction was reduced when SH2B1β was overexpressed. Inhibiting PI3K using LY294002 significantly increased the expression of FasL for both cell lines in response to 100 μM H<sub>2</sub>O<sub>2</sub> treatment (Figure 7B, C). The extent of increase was more pronounced in PC12-SH2B1β cells than in PC12-GFP cells. Inhibiting MEK using U0126 significantly increased the expression of FasL for both cell lines in response to 100 as well as 200 μM H<sub>2</sub>O<sub>2</sub> stimulation (Figure 7D, E). Similarly, the increase of FasL expression was more in PC12-SH2B1β cells than that in PC12-GFP cells. These results suggest that overexpressing SH2B1β enhances H<sub>2</sub>O<sub>2</sub>-induced PI3K-AKT and MEK-ERK1/2 signaling, leading to reduced nuclear localization of FoxO3a, and thus the reduction of FasL expression. To examine the contribution of PI3K-AKT and MEK-ERK1/2 signaling to SH2B1β-mediated cell survival, MTT assays were performed. As in Figure 8, inhibiting PI3K or MEK reduced cell viability by 5-10% in PC12-GFP cells and by 10-15% in PC12-SH2B1β cells for each inhibitor. These results suggest that both PI3K-AKT and MEK-ERK1/2 signaling contributes to SH2B1β-mediated cell survival.



**Figure 6 SH2B1β reduces H<sub>2</sub>O<sub>2</sub>-induced nuclear distributions of FoxO1 and 3a.** PC12-GFP and PC12-SH2B1β cells were incubated in serum-free medium overnight before 0, 100, or 200 μM H<sub>2</sub>O<sub>2</sub> treatment for 10 min, without (A-B) or with 20 μM LY294002 (+LY) (C-D, G-H) or 20 μM U0126 (+U) (E-F, I-J) pretreatment for 30 min. The localization of FoxOs was determined via immunofluorescence staining using anti-FoxO1 (A, C, E, G, I) or anti-FoxO3a (B, D, F, H, J) antibody followed by Alexa Fluor 555-conjugated secondary antibody. Images were taken using inverted Zeiss Axiovert 135 fluorescence microscope. Percentage of cells with fluorescence intensity of FoxO1 or FoxO3a in the nucleus higher than in the cytoplasm (N > C) was quantified. A total of 90-110 cells were counted for each condition. For inhibitor assays, results from PC12-GFP cells are shown on the left panels and those from PC12-SH2B1β cells are shown on the right.



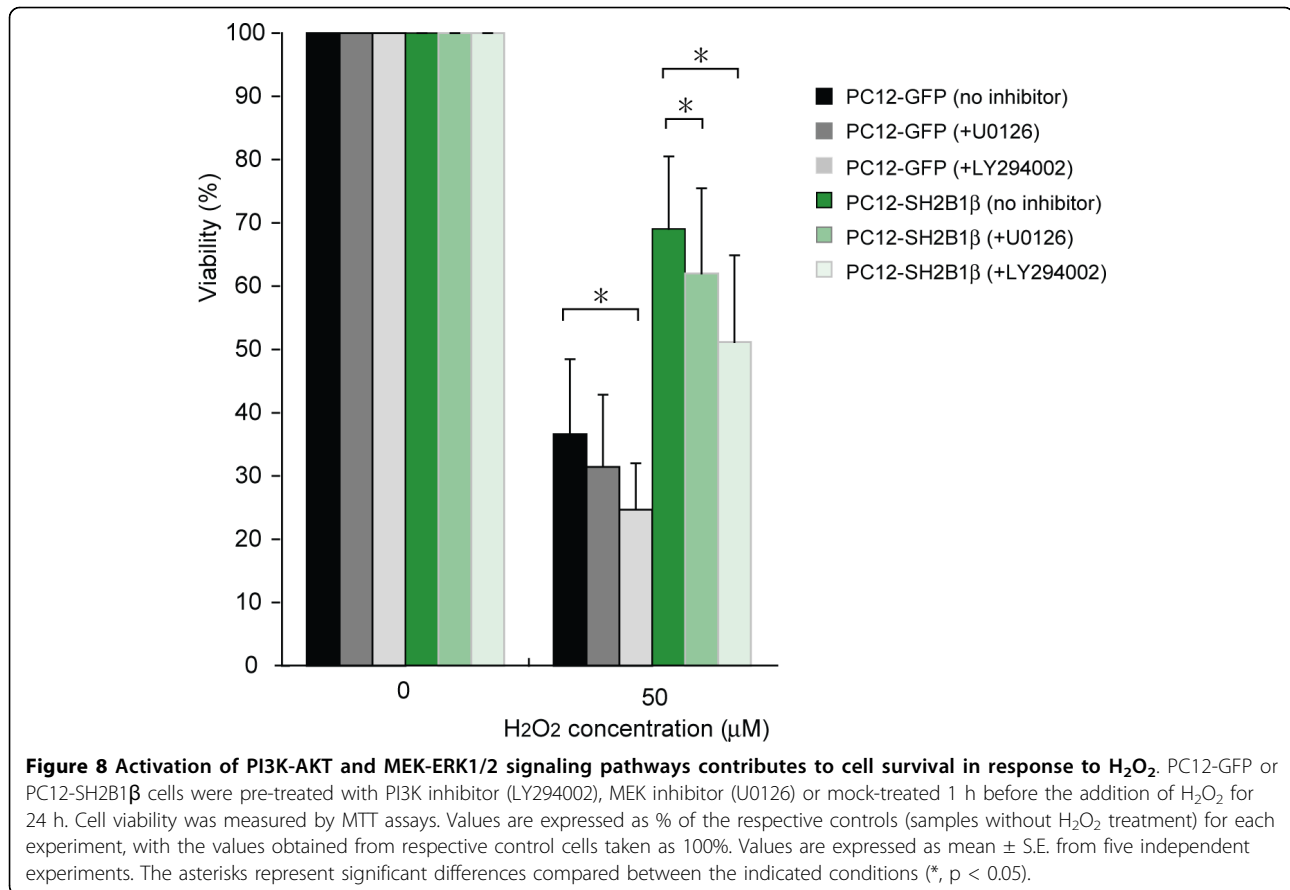
**Figure 7 SH2B1β reduces H<sub>2</sub>O<sub>2</sub>-induced expression of FasL through PI3K-AKT and MEK-ERK1/2.** PC12-GFP and PC12-SH2B1β cells were incubated in serum-free medium overnight, before H<sub>2</sub>O<sub>2</sub> (0, 100, or 200 μM) treatment for 4 h without (A) or with 20 μM LY294002 (+LY) (B, C) or 20 μM U0126 (+U) (D, E) pre-treatment for 30 min. The expression of FasL was determined by Q-PCR. The relative levels of FasL were normalized to the expression of GAPDH. The relative expression levels of H<sub>2</sub>O<sub>2</sub>-treated samples were normalized to untreated samples in each cell line for each experiment. Values are expressed as mean ± S.E. from three independent experiments and statistically compared using student's *t*-test (\*, *P* < 0.05). For inhibitor assays, results from PC12-GFP cells are shown on the left panels and those from PC12-SH2B1β cells are shown on the right.

Taken together, results from this study suggest that the adaptor protein SH2B1β reduces H<sub>2</sub>O<sub>2</sub>-induced apoptosis in PC12 cells and hippocampal neurons. SH2B1β protects cells in part through enhancing H<sub>2</sub>O<sub>2</sub>-induced phosphorylation of AKT and ERK1/2, reducing the nuclear localization of FoxOs and thus reducing the expression of a pro-apoptotic gene, FasL. This is the first demonstration that the adaptor protein SH2B1β reduces H<sub>2</sub>O<sub>2</sub>-induced and caspase 3-dependent apoptosis.

## Discussion

SH2B1 has been implicated in neuronal differentiation, cell growth, metabolism, obesity and diabetes [39,41,65-68]. Its ability to modulate cellular signaling

confers its ability to regulate diverse functions. The only evidence so far that directly demonstrates its importance in cell survival is a study by Qian *et al* [67]. Injecting anti-SH2B1 antibody to sympathetic neurons leads to cell death suggesting that SH2B1 is required for neuronal survival [67]. However, it is not known how SH2B1 may influence live and death decision of cells. In the present study, we demonstrated that overexpressing SH2B1β reduced H<sub>2</sub>O<sub>2</sub>-induced cell death in PC12 cells and hippocampal neurons. In addition, overexpressing SH2B1β enhanced PI3K-AKT and MEK-ERK1/2 survival pathways in response to H<sub>2</sub>O<sub>2</sub>. Consistent with what Davila *D et al* have shown, phosphorylation of AKT was reduced as the concentration of H<sub>2</sub>O<sub>2</sub> increased [22]. This reduction of pAKT may result from oxidation of



**Figure 8 Activation of PI3K-AKT and MEK-ERK1/2 signaling pathways contributes to cell survival in response to H<sub>2</sub>O<sub>2</sub>.** PC12-GFP or PC12-SH2B1β cells were pre-treated with PI3K inhibitor (LY294002), MEK inhibitor (U0126) or mock-treated 1 h before the addition of H<sub>2</sub>O<sub>2</sub> for 24 h. Cell viability was measured by MTT assays. Values are expressed as % of the respective controls (samples without H<sub>2</sub>O<sub>2</sub> treatment) for each experiment, with the values obtained from respective control cells taken as 100%. Values are expressed as mean ± S.E. from five independent experiments. The asterisks represent significant differences compared between the indicated conditions (\*, p < 0.05).

plasma membrane and inactivation of surface receptors [69]. As oxidative stress increases, intracellular phosphatase, such as PP2A, is inhibited leading to the increase of pERK1/2 [21].

Overexpressing SH2B1β enhanced the phosphorylation of AKT and ERK1/2 which reduced the nuclear localization of FoxOs and FasL expression. Along this line, various reports also suggest the involvement of PI3K-AKT in promoting cell survival in hippocampal neurons [70-73] and our data suggest that SH2B1β-overexpressing neurons were not able to protect cells in the presence of PI3K inhibitor (data not shown). These results strongly implicate that SH2B1β protects neurons in part through PI3K-AKT pathway. In contrast, H<sub>2</sub>O<sub>2</sub> slightly induced the expression of another FoxO-responsive gene — MnSOD (Manganese Superoxide Dismutase) in PC12-GFP cells but the induction was much higher in PC12-SH2B1β cells (Additional file 4A). Furthermore, the expression of MnSOD was not significantly affected by either PI3K or MEK inhibitor (data not shown). Thus, SH2B1β may utilize PI3K-AKT- and MEK-ERK1/2-independent mechanisms to regulate the expression of MnSOD.

A report suggests that protein kinase D (PKD) triggers the activation of NFκB to increase MnSOD expression in response to oxidative stress [74]. However, we have not been able to detect H<sub>2</sub>O<sub>2</sub>-induced activation of NFκB. Accumulating evidence have demonstrated that the Janus tyrosine kinase (JAK)-Signal transduction and activators of transcription (STAT) signaling pathway plays an important role in the expression of stress-responsive genes as well as in cytoprotection in response to H<sub>2</sub>O<sub>2</sub> [75,76]. A study also points to the involvement of STAT3 in MnSOD expression in response to hypoxia/reperfusion-induced injury and during liver regeneration [77,78]. Along the line, Stephanou *et al.* have shown that the JAK-STAT pathway participates in the modulation of expression of pro-survival Bcl2 proteins [79]. Interestingly, mRNA level of Bcl2 was found higher in PC12-SH2B1β cells compared to control cells (Additional file 4B). These findings suggest that SH2B1β may enhance the expression of survival genes through STAT3. The results from this study raise an intriguing possibility that the adaptor protein SH2B1β may utilize more than one mechanism to protect cells against stress and could act as a survival factor in general.



## Materials and methods

### Antibodies and reagents

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was purchased from USB Corporation (Cleveland, OH). Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), U0126 and LY294002 were from Calbiochem (San Diego, CA). Polyclonal antibody to rat SH2B1 $\beta$  was raised against a glutathione S-transferase fusion protein containing amino acids 527-670 of SH2B1 $\beta$  as described previously [32]. Whole antiserum against ERK1/2 was purchased from Sigma (St. Louis, MO). Mouse monoclonal antibodies to phospho-ERK1/2, phospho-S473 of AKT, rabbit polyclonal antibodies against AKT, phospho-FoxO1 (Ser256), FoxO1, FoxO3a and PARP were from Cell Signaling (Danvers, MA). Rabbit polyclonal antibody against phospho-FoxO3a/FKHRL1 (Thr32) was from Upstate (Temecula, CA). Anti- $\beta$ III tubulin (TUJ1) antibody was from Covance (Princeton, NJ). NGF, rat-tail collagen I, and growth factor-reduced Matrigel were purchased from BD Bioscience (Bradford, MA). Protein Assay Kit was purchased from Strong Biotech Corporation, Taiwan.

### Cell culture and microscopy

The stock of PC12 cells was purchased from American Type Culture Collection. PC12 cells were maintained on the collagen-coated plates (0.1 mg/ml) in complete media (DMEM supplemented with 10% heat-inactivated horse serum, 5% fetal bovine serum, 1 mM L-glutamine and 1 mM antibiotic-antimycotic). PC12 cells stably overexpressing GFP (PC12-GFP cells) or GFP-SH2B1 $\beta$  (PC12-SH2B1 $\beta$  cells) were made and cultured as described in Chen *et al* [65]. Pooled population was used to avoid clonal variation. The serum-free medium used was DMEM supplemented with 1% BSA, 1 mM L-glutamine and 1 mM antibiotic-antimycotic. For immunofluorescence staining, PC12-GFP and PC12-SH2B1 $\beta$  cells were treated with H<sub>2</sub>O<sub>2</sub> for 10 min, then fixed, permeabilized and incubated with the indicated antibodies. Fluorescent images were taken using inverted Zeiss Axiovert 135 fluorescence microscope (400 $\times$  magnification). For anti-active caspase 3 staining, digital images were captured using upright Fluorescent Microscope Zeiss/Axioskop 2 mot plus. The fluorescent pixel spatial orientation and pixel intensity were measured by AxioVision 4.8 software. Signal of active caspase-3 fluorescence was localized mostly to cell nucleus and its fluorescent intensity in the nucleus was quantified using AxioVision 4.8.

### MTT and inhibitor assays

Cells were plated at a density of  $3 \times 10^4$  cells/well in the Matrigel-coated 96-well plates. After overnight incubation, cells were treated with freshly prepared H<sub>2</sub>O<sub>2</sub>. Cell viability was assayed by the reduction of MTT following the

manufacturer's instruction. Results are presented as percentage of the control using the absorbance of the control cells is 100%. For inhibitor assay, cells were pretreated with inhibitors (20  $\mu$ M U0126 or 20  $\mu$ M LY294002) for 1 h (for MTT assays) or 30 min prior to H<sub>2</sub>O<sub>2</sub> treatment.

### H<sub>2</sub>O<sub>2</sub> treatment and immunoblotting

Cells were incubated in serum-free medium overnight before H<sub>2</sub>O<sub>2</sub> treatment. Cells were lysed using lysis buffer (RIPA) containing freshly added 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM phenylmethanesulphonylfluoride (PMSF), 10 ng/ml aprotinin and 10 ng/ml leupeptin. Protein concentration of each sample was determined by protein assay kit. Samples with equal amount of proteins were resolved using 8% SDS-PAGE followed by Western blotting with specific primary antibodies. The immunoblots were detected using either IRDye 700- or IRDye 800CW-conjugated IgG and an Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE) or horseradish peroxidase-conjugated IgG and the ECL (enhanced chemiluminescence) system. Western blots results were quantified using NIH Image J software.

### Measurement of intracellular ROS levels

Dihydroethidium (DHE) was purchased from Invitrogen (Carlsband, CA), and used to measure the production of intracellular ROS. DHE shows a blue fluorescence in cell cytoplasm until oxidization to form red fluorescent-ethidium which is trapped in the nucleus by intercalating into DNA. ROS levels were analyzed in FACSCalibur flow cytometer (Becton Dickinson, CA). Fluorescence was detected by filter FL-3 (670 nm). Histograms of 10,000 events were analyzed and DHE fluorescence was evaluated by using the CellQuest software (Becton Dickinson).

### Preparation of rat hippocampal neurons and transient transfection

Primary hippocampal neuron cultures were prepared from Sprague-Dawley rats as described previously [80,81]. Briefly, cells were dissociated from hippocampus dissected from embryonic day 18 (E18) rat embryos by treatment with papain (10 U/ml). Dissociated cells were washed and suspended in MEM supplemented with 5% horse serum and 5% fetal calf serum. Neurons were then plated onto coverslips coated with poly-L-lysine, and cultured in neurobasal medium with B27 (containing additional 0.025 mM glutamate) on DIV (day *in vitro*) 1. On DIV 3, the cells were treated with 5  $\mu$ M cytosine 1- $\beta$ -D-arabinofuranoside (ARC) for 1 day to inhibit the growth of glial cells. Medium was replaced by half of the fresh neurobasal/B27 medium on DIV4 and twice a week thereafter. GFP, GFP-SH2B1 $\beta$  or GFP-SH2B1 $\beta$ (R555E) was transfected to neurons on DIV3 using the CaCl<sub>2</sub> transfection kits from

Promega (Madison, WI). Two days after transfection, neurons were treated with H<sub>2</sub>O<sub>2</sub> as indicated.

#### RNA preparation and semi-quantitative real-time PCR

TRIzol reagent was used to isolate total RNA from PC12 cells with or without treatment at the indicated time. Concentrations and A<sub>260/280</sub> ratios of RNAs were measured using spectrophotometer (NanoDrop 1000, Thermo). Total RNA of each sample was reverse transcribed into cDNA and the relative gene expressions of FasL and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were determined via semi-quantitative PCR (Q-PCR) assay using SYBR green master mix and the ABI7500 system. Primer sequences for each gene were designed using PrimerExpress software. Amplicons generated from each primer pair were between 50 to 100 bp. Loading of each sample was normalized with ROX dye. All readings were normalized to the expression of GAPDH. The forward primer for FasL is 5' CTGGTGGCTCTGGTTGGAAT3' and the reverse primer is 5' CTCACGGAGTTCTGCCAGTTC 3'. The forward primer for GAPDH is 5' ATGACTCTACCACGGCAAGTT3' and the reverse primer is 5' TCCATTCTCAGCCTTGACTGT 3'.

#### Statistical analysis

Data were expressed as mean ± S.E., and significant differences were analyzed by Student's *t*-test. The results are considered significant when *P* < 0.05.

#### Additional material

**Additional file 1: Hydrogen peroxide induces death of hippocampal neurons.** Hippocampal neurons from E18 embryos were isolated as described in the Materials and Methods. Neurons were treated with the indicated concentrations of H<sub>2</sub>O<sub>2</sub> for 18 h, then fixed for immunofluorescence staining using anti-βIII tubulin (neuronal tubulin) antibody (shown in green) and DAPI (shown in blue).

**Additional file 2: Overexpressing SH2B1β reduces H<sub>2</sub>O<sub>2</sub>-induced levels of Annexin V.** PC12-GFP and PC12-SH2B1β cells were treated with 0, 100 or 200 μM H<sub>2</sub>O<sub>2</sub> for 18 h. The levels of Annexin V were quantified through flow cytometry.

**Additional file 3: SH2B1β reduces and SH2B1β(R555E) increases H<sub>2</sub>O<sub>2</sub>-induced levels of active caspase 3 in hippocampal neurons.** Hippocampal neurons from E18 embryos were transiently transfected with GFP, GFP-SH2B1β or GFP-SH2B1β(R555E) on DIV 3 and then treated with H<sub>2</sub>O<sub>2</sub> on DIV 5 for 18 h. Cells were fixed and subjected to immunofluorescence staining using anti-active caspase 3 antibody (shown in red) and DAPI (shown in blue). Fluorescence intensity was quantified using AxioVision 4.8 (Zeiss) and shown in the bottom panel.

**Additional file 4: Overexpressing SH2B1β increases the gene expressions of MnSOD and Bcl2.** PC12-GFP and PC12-SH2B1β cells were treated with 0, 100 or 200 μM H<sub>2</sub>O<sub>2</sub> for 4 h. Total RNAs were extracted and subjected to Q-PCR analysis. (A) Primers for MnSOD: forward 5' ATTAACGCGCAGATCATGCAG 3'; reverse 5' TTTTCAGATAGTCAGGTCTGACGTT 3'. (B) Primers for Bcl2: forward 5' TGGGATGCCTTGTGGAAC 3'; reverse 5' CAGCCAGGAGAAATCAAACAGA 3'. Data were normalized to GAPDH and untreated PC12-GFP samples.

#### Abbreviations

ERK: extracellular signal-regulated kinase; MAPK: mitogen-activated protein kinases; MnSOD: Manganese superoxide dismutase; MEK: MAPK/ERK kinase; PI3K: phosphatidylinositol 3-kinase; AKT: protein kinase B. FoxO: forkhead box O, forkhead members of the O class; PARP: poly (ADP-ribose) polymerase; FasL: Fas ligand.

#### Acknowledgements

This study was supported by grants from the National Health Research Institute (NHRI-EX98-9719NC and NHRI-EX99-9719NC), the National Science Council (NSC97-2311-B-007-007-MY3), and the National Tsing Hua University (97N2504E1).

#### Author details

<sup>1</sup>Institute of Molecular Medicine, National Tsing Hua University, Hsinchu, Taiwan. <sup>2</sup>Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan. <sup>3</sup>Brain Research Center, National Tsing Hua University, Hsinchu, Taiwan. <sup>4</sup>Division of Molecular and Genomic Medicine, National Health Research Institutes, Miaoli County, Taiwan.

#### Authors' contributions

WCL performed signaling experiments, caspase 3 and PARP western blot analysis, immunofluorescence staining and quantification of FoxOs, and gene expression. CJC performed hippocampal neuron isolation, transient transfection, active caspase 3 staining and quantification, ROS detection, and Annexin V staining. HCH contributed to signaling experiments and MTT assays. HLH performed MTT assays. LC is the corresponding author that designed, coordinated all experiments, wrote the manuscript and formatted figures. All authors read and approved the final manuscript.

#### Competing interests

The authors declare that they have no competing interests.

Received: 21 July 2010 Accepted: 27 September 2010

Published: 27 September 2010

#### References

1. Barnham KJ, Masters CL, Bush AI: **Neurodegenerative diseases and oxidative stress.** *Nat Rev Drug Discov* 2004, **3**(3):205-214.
2. Everse J, Coates PW: **Neurodegeneration and peroxidases.** *Neurobiol Aging* 2009, **30**(7):1011-1025.
3. Simonian NA, Coyle JT: **Oxidative stress in neurodegenerative diseases.** *Annu Rev Pharmacol Toxicol* 1996, **36**:83-106.
4. Jenner P: **Oxidative stress in Parkinson's disease.** *Ann Neurol* 2003, **53**(Suppl 3):S26-36, discussion S36-28.
5. Chan PH: **Reactive oxygen radicals in signaling and damage in the ischemic brain.** *J Cereb Blood Flow Metab* 2001, **21**(1):2-14.
6. Allen CL, Bayraktutan U: **Oxidative stress and its role in the pathogenesis of ischaemic stroke.** *Int J Stroke* 2009, **4**(6):461-470.
7. Giorgio M, Trinei M, Migliaccio E, Pellicci PG: **Hydrogen peroxide: a metabolic by-product or a common mediator of ageing signals?** *Nat Rev Mol Cell Biol* 2007, **8**(9):722-728.
8. Burdon RH, Alliangana D, Gill V: **Hydrogen peroxide and the proliferation of BHK-21 cells.** *Free Radic Res* 1995, **23**(5):471-486.
9. Burdon RH, Gill V, Alliangana D: **Hydrogen peroxide in relation to proliferation and apoptosis in BHK-21 hamster fibroblasts.** *Free Radic Res* 1996, **24**(2):81-93.
10. Davies KJ: **The broad spectrum of responses to oxidants in proliferating cells: a new paradigm for oxidative stress.** *IUBMB Life* 1999, **48**(1):41-47.
11. Kim DK, Cho ES, Seong JK, Um HD: **Adaptive concentrations of hydrogen peroxide suppress cell death by blocking the activation of SAPK/JNK pathway.** *J Cell Sci* 2001, **114**(Pt 23):4329-4334.
12. Madamanchi NR, Li S, Patterson C, Runge MS: **Reactive oxygen species regulate heat-shock protein 70 via the JAK/STAT pathway.** *Arterioscler Thromb Vasc Biol* 2001, **21**(3):321-326.
13. Wang XT, McCullough KD, Wang XJ, Carpenter G, Holbrook NJ: **Oxidative stress-induced phospholipase C-gamma 1 activation enhances cell survival.** *J Biol Chem* 2001, **276**(30):28364-28371.

14. Wang X, Martindale JL, Liu Y, Holbrook NJ: The cellular response to oxidative stress: influences of mitogen-activated protein kinase signalling pathways on cell survival. *Biochem J* 1998, **333**(Pt 2):291-300.
15. Ryter SW, Kim HP, Hoetzel A, Park JW, Nakahira K, Wang X, Choi AM: Mechanisms of cell death in oxidative stress. *Antioxid Redox Signal* 2007, **9**(1):49-89.
16. Goldshmit Y, Erlich S, Pinkas-Kramarski R: Neuregulin rescues PC12-ErbB4 cells from cell death induced by H<sub>2</sub>O<sub>2</sub>. Regulation of reactive oxygen species levels by phosphatidylinositol 3-kinase. *J Biol Chem* 2001, **276**(49):46379-46385.
17. Yoshizumi M, Kogame T, Suzuki Y, Fujita Y, Kyaw M, Kirima K, Ishizawa K, Tsuchiya K, Kagami S, Tamaki T: Ebselen attenuates oxidative stress-induced apoptosis via the inhibition of the c-Jun N-terminal kinase and activator protein-1 signalling pathway in PC12 cells. *Br J Pharmacol* 2002, **136**(7):1023-1032.
18. Nair VD, Yuen T, Olanow CW, Sealfon SC: Early single cell bifurcation of pro- and antiapoptotic states during oxidative stress. *J Biol Chem* 2004, **279**(26):27494-27501.
19. Sen P, Chakraborty PK, Raha S: Activation of p38MAPK by repetitive low-grade oxidative stress leads to pro-survival effects. *Biochim Biophys Acta* 2007, **1773**(3):367-374.
20. Blanc A, Pandey NR, Srivastava AK: Synchronous activation of ERK 1/2, p38mapk and PKB/Akt signaling by H<sub>2</sub>O<sub>2</sub> in vascular smooth muscle cells: potential involvement in vascular disease (review). *Int J Mol Med* 2003, **11**(2):229-234.
21. Chen L, Liu L, Yin J, Luo Y, Huang S: Hydrogen peroxide-induced neuronal apoptosis is associated with inhibition of protein phosphatase 2A and 5, leading to activation of MAPK pathway. *Int J Biochem Cell Biol* 2009, **41**(6):1284-1295.
22. Davila D, Torres-Aleman I: Neuronal death by oxidative stress involves activation of FOXO3 through a two-arm pathway that activates stress kinases and attenuates insulin-like growth factor I signaling. *Mol Biol Cell* 2008, **19**(5):2014-2025.
23. Essers MA, Weijnen S, de Vries-Smits AM, Saarloos I, de Ruiter ND, Bos JL, Burgering BM: FOXO transcription factor activation by oxidative stress mediated by the small GTPase Ral and JNK. *EMBO J* 2004, **23**(24):4802-4812.
24. Mehdi MZ, Azar ZM, Srivastava AK: Role of receptor and nonreceptor protein tyrosine kinases in H<sub>2</sub>O<sub>2</sub>-induced PKB and ERK1/2 signaling. *Cell Biochem Biophys* 2007, **47**(1):1-10.
25. Ruffels J, Griffin M, Dickenson JM: Activation of ERK1/2, JNK and PKB by hydrogen peroxide in human SH-SY5Y neuroblastoma cells: role of ERK1/2 in H<sub>2</sub>O<sub>2</sub>-induced cell death. *Eur J Pharmacol* 2004, **483**(2-3):163-173.
26. Jiang H, Zhang L, Koubi D, Kuo J, Groc L, Rodriguez AI, Hunter TJ, Tang S, Lazarovici P, Gautam SC, et al: Roles of Ras-Erk in apoptosis of PC12 cells induced by trophic factor withdrawal or oxidative stress. *J Mol Neurosci* 2005, **25**(2):133-140.
27. Tuo QH, Wang C, Yan FX, Liao DF: MAPK pathway mediates the protective effects of onychin on oxidative stress-induced apoptosis in ECV304 endothelial cells. *Life Sci* 2004, **76**(5):487-497.
28. Vauzour D, Vafeiadou K, Rice-Evans C, Williams RJ, Spencer JP: Activation of pro-survival Akt and ERK1/2 signalling pathways underlie the anti-apoptotic effects of flavanones in cortical neurons. *J Neurochem* 2007, **103**(4):1355-1367.
29. Cuda G, Paterno R, Ceravolo R, Candigliota M, Perrotti N, Perticone F, Faniello MC, Schepis F, Ruocco A, Mele E, et al: Protection of human endothelial cells from oxidative stress: role of Ras-ERK1/2 signaling. *Circulation* 2002, **105**(8):968-974.
30. Guyton KZ, Liu Y, Gorospe M, Xu Q, Holbrook NJ: Activation of mitogen-activated protein kinase by H<sub>2</sub>O<sub>2</sub>. Role in cell survival following oxidant injury. *J Biol Chem* 1996, **271**(8):4138-4142.
31. Riedel H, Wang J, Hansen H, Yousaf N: PSM, an insulin-dependent, pro-rich, PH, SH2 domain containing partner of the insulin receptor. *J Biochem* 1997, **122**(6):1105-1113.
32. Rui L, Mathews LS, Hotta K, Gustafson TA, Carter-Su C: Identification of SH2-B $\beta$  as a substrate of the tyrosine kinase JAK2 involved in growth hormone signaling. *Mol Cell Biol* 1997, **17**(11):6633-6644.
33. Yousaf N, Deng Y, Kang Y, Riedel H: Four PSM/SH2-B alternative splice variants and their differential roles in mitogenesis. *J Biol Chem* 2001, **276**(44):40940-40948.
34. Rui L, Carter-Su C: Platelet-derived growth factor (PDGF) stimulates the association of SH2-B $\beta$  with PDGF receptor and phosphorylation of SH2-B $\beta$ . *J Biol Chem* 1998, **273**(33):21239-21245.
35. Riedel H, Yousaf N, Zhao Y, Dai H, Deng Y, Wang J: PSM, a mediator of PDGF-BB-, IGF-I-, and insulin-stimulated mitogenesis. *Oncogene* 2000, **19**:39-50.
36. Wang J, Riedel H: Insulin-like growth factor-I receptor and insulin receptor association with a Src homology-2 domain-containing putative adapter. *J Biol Chem* 1998, **273**(6):3136-3139.
37. Qian X, Riccio A, Zhang Y, Ginty DD: Identification and characterization of novel substrates of Trk receptors in developing neurons. *Neuron* 1998, **21**(5):1017-1029.
38. Kotani K, Wilden P, Pillay TS: SH2-B $\alpha$  is an insulin-receptor adapter protein and substrate that interacts with the activation loop of the insulin-receptor kinase. *Biochem J* 1998, **335**(Pt 1):103-109.
39. Lin WF, Chen CJ, Chang YJ, Chen SL, Chiu IM, Chen L: SH2B1beta enhances fibroblast growth factor 1 (FGF1)-induced neurite outgrowth through MEK-ERK1/2-STAT3-Egr1 pathway. *Cell Signal* 2009, **21**(7):1060-1072.
40. Kong M, Wang CS, Donoghue DJ: Interaction of fibroblast growth factor receptor 3 and the adapter protein SH2-B. A role in STAT5 activation. *J Biol Chem* 2002, **277**(18):15962-15970.
41. Rui L, Herrington J, Carter-Su C: SH2-B is required for nerve growth factor-induced neuronal differentiation. *J Biol Chem* 1999, **274**(15):10590-10594.
42. Donatello S, Fiorino A, Degl'Innocenti D, Alberti L, Miranda C, Gorla L, Bongarzone I, Rizzetti MG, Pierotti MA, Borrello MG: SH2B1beta adaptor is a key enhancer of RET tyrosine kinase signaling. *Oncogene* 2007, **26**(45):6546-6559.
43. Wang X, Chen L, Maures TJ, Herrington J, Carter-Su C: SH2-B is a positive regulator of nerve growth factor-mediated activation of the Akt/Forkhead pathway in PC12 cells. *J Biol Chem* 2004, **279**(1):133-141.
44. de Melker AA, Desban N, Duband JL: Cellular localization and signaling activity of beta-catenin in migrating neural crest cells. *Dev Dyn* 2004, **230**(4):708-726.
45. Ciechomska I, Pyrzynska B, Kazmierczak P, Kaminska B: Inhibition of Akt kinase signalling and activation of Forkhead are indispensable for upregulation of FasL expression in apoptosis of glioma cells. *Oncogene* 2003, **22**(48):7617-7627.
46. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME: Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell* 1999, **96**(6):857-868.
47. Biggs III WH, Meisenhelder J, Hunter T, Cavenee WK, Arden KC: Protein kinase B/Akt-mediated phosphorylation promotes nuclear exclusion of the winged helix transcription factor FKHR1. *Proc Natl Acad Sci USA* 1999, **96**(13):7421-7426.
48. Tang ED, Nunez G, Barr FG, Guan KL: Negative regulation of the forkhead transcription factor FKHR by Akt. *J Biol Chem* 1999, **274**(24):16741-16746.
49. Gilley J, Coffey PJ, Ham J: FOXO transcription factors directly activate *bim* gene expression and promote apoptosis in sympathetic neurons. *J Cell Biol* 2003, **162**(4):613-622.
50. Asada S, Daitoku H, Matsuzaki H, Saito T, Sudo T, Mukai H, Iwashita S, Kako K, Kishi T, Kasuya Y, et al: Mitogen-activated protein kinases, Erk and p38, phosphorylate and regulate Foxo1. *Cell Signal* 2007, **19**(3):519-527.
51. Huang H, Tindall DJ: Dynamic FoxO transcription factors. *J Cell Sci* 2007, **120**(Pt 15):2479-2487.
52. Yang W, Dolloff NG, El-Deiry WS: ERK and MDM2 prey on FOXO3a. *Nat Cell Biol* 2008, **10**(2):125-126.
53. Fiers W, Beyaert R, Declercq W, Vandenaabeele P: More than one way to die: apoptosis, necrosis and reactive oxygen damage. *Oncogene* 1999, **18**(54):7719-7730.
54. Degterev A, Boyce M, Yuan J: A decade of caspases. *Oncogene* 2003, **22**(53):8543-8567.
55. Degterev A, Yuan J: Expansion and evolution of cell death programmes. *Nat Rev Mol Cell Biol* 2008, **9**(5):378-390.
56. Yuan J, Lipinski M, Degterev A: Diversity in the mechanisms of neuronal cell death. *Neuron* 2003, **40**(2):401-413.
57. Rosenbaum DM, Degterev A, David J, Rosenbaum PS, Roth S, Grotta JC, Cuny GD, Yuan J, Savitz SI: Necroptosis, a novel form of caspase-independent cell death, contributes to neuronal damage in a retinal ischemia-reperfusion injury model. *J Neurosci Res* 88(7):1569-1576.

58. Kitanaka C, Kuchino Y: **Caspase-independent programmed cell death with necrotic morphology.** *Cell Death Differ* 1999, **6**(6):508-515.
59. Kim R, Emi M, Tanabe K, Murakami S, Uchida Y, Arihiro K: **Regulation and interplay of apoptotic and non-apoptotic cell death.** *J Pathol* 2006, **208**(3):319-326.
60. Stefanis L: **Caspase-dependent and -independent neuronal death: two distinct pathways to neuronal injury.** *Neuroscientist* 2005, **11**(1):50-62.
61. Yakovlev AG, Faden AI: **Caspase-dependent apoptotic pathways in CNS injury.** *Mol Neurobiol* 2001, **24**(1-3):131-144.
62. Huang H, Tindall DJ: **FOXO factors: a matter of life and death.** *Future Oncol* 2006, **2**(1):83-89.
63. Tang ED, Nunez G, Barr FG, Guan KL: **Negative regulation of the forkhead transcription factor FKHR by Akt.** *J Biol Chem* 1999, **274**(24):16741-16746.
64. Barthelemy C, Henderson CE, Pettmann B: **Foxo3a induces motoneuron death through the Fas pathway in cooperation with JNK.** *BMC Neurosci* 2004, **5**:48.
65. Chen L, Maures TJ, Jin H, Huo JS, Rabbani SA, Schwartz J, Carter-Su C: **SH2B1beta (SH2-Bbeta) enhances expression of a subset of nerve growth factor-regulated genes important for neuronal differentiation including genes encoding urokinase plasminogen activator receptor and matrix metalloproteinase 3/10.** *Mol Endocrinol* 2008, **22**(2):454-476.
66. Duan C, Yang H, White MF, Rui L: **Disruption of SH2-B causes age-dependent insulin resistance and glucose intolerance.** *Mol Cell Biol* 2004, **24**(17):743507443.
67. Qian X, Riccio A, Zhang Y, Ginty DD: **Identification and characterization of novel substrates of Trk receptors in developing neurons.** *Neuron* 1998, **21**(5):1017-1029.
68. Ren D, Li M, Duan C, Rui L: **Identification of SH2-B as a key regulator of leptin sensitivity, energy balance and body weight in mice.** *Cell Metab* 2005, **2**.
69. Fatokun AA, Stone TW, Smith RA: **Oxidative stress in neurodegeneration and available means of protection.** *Front Biosci* 2008, **13**:3288-3311.
70. Zheng WH, Kar S, Quirion R: **Insulin-like growth factor-1-induced phosphorylation of transcription factor FKHL1 is mediated by phosphatidylinositol 3-kinase/Akt kinase and role of this pathway in insulin-like growth factor-1-induced survival of cultured hippocampal neurons.** *Mol Pharmacol* 2002, **62**(2):225-233.
71. Jover-Mengual T, Miyawaki T, Latuszek A, Alborch E, Zukin RS, Etgen AM: **Acute estradiol protects CA1 neurons from ischemia-induced apoptotic cell death via the PI3K/Akt pathway.** *Brain Res* 1321:1-12.
72. Wine RN, McPherson CA, Harry GJ: **IGF-1 and pAKT signaling promote hippocampal CA1 neuronal survival following injury to dentate granule cells.** *Neurotox Res* 2009, **16**(3):280-292.
73. Peltier J, O'Neill A, Schaffer DV: **PI3K/Akt and CREB regulate adult neural hippocampal progenitor proliferation and differentiation.** *Dev Neurobiol* 2007, **67**(10):1348-1361.
74. Storz P, Doppler H, Tokar A: **Protein kinase D mediates mitochondrion-to-nucleus signaling and detoxification from mitochondrial reactive oxygen species.** *Mol Cell Biol* 2005, **25**(19):8520-8530.
75. Imada K, Leonard WJ: **The Jak-STAT pathway.** *Mol Immunol* 2000, **37**(1-2):1-11.
76. Yu HM, Zhi JL, Cui Y, Tang EH, Sun SN, Feng JQ, Chen PX: **Role of the JAK-STAT pathway in protection of hydrogen peroxide preconditioning against apoptosis induced by oxidative stress in PC12 cells.** *Apoptosis* 2006, **11**(6):931-941.
77. Negoro S, Kunisada K, Fujio Y, Funamoto M, Darville MI, Eizirik DL, Osugi T, Izumi M, Oshima Y, Nakaoka Y, et al: **Activation of signal transducer and activator of transcription 3 protects cardiomyocytes from hypoxia/reoxygenation-induced oxidative stress through the upregulation of manganese superoxide dismutase.** *Circulation* 2001, **104**(9):979-981.
78. Fujiyoshi M, Ozaki M: **Molecular mechanisms of liver regeneration and protection for treatment of liver dysfunction and diseases.** *J Hepatobiliary Pancreat Sci* 2010.
79. Stephanou A, Brar BK, Knight RA, Latchman DS: **Opposing actions of STAT-1 and STAT-3 on the Bcl-2 and Bcl-x promoters.** *Cell Death Differ* 2000, **7**(3):329-330.
80. Roback JD, Large TH, Otten U, Wainer BH: **Nerve growth factor expression in the developing hippocampus isolated in vitro.** *Dev Biol* 1990, **137**(2):451-455.
81. Brewer GJ: **Isolation and culture of adult rat hippocampal neurons.** *J Neurosci Methods* 1997, **71**(2):143-155.

doi:10.1186/1750-2187-5-17

**Cite this article as:** Lu et al: The adaptor protein SH2B1 $\beta$  reduces hydrogen peroxide-induced cell death in PC12 cells and hippocampal neurons. *Journal of Molecular Signaling* 2010 5:17.

**Submit your next manuscript to BioMed Central and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
www.biomedcentral.com/submit

