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Subcellular distribution of prohibitin 1 in rat liver during liver regeneration and its cellular implication

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Abstract

BACKGROUND

The function of prohibitin 1 (Phb1) during liver regeneration (LR) remains relatively unexplored. Our previous research identified downregulation of Phb1 in rat liver mitochondria 24 h after 70% partial hepatectomy (PHx), as determined by subcellular proteomic analysis.

AIM

To investigate the potential role of Phb1 during LR.

METHODS

We examined changes in Phb1 mRNA and protein levels, subcellular distribution, and abundance in rat liver during LR following 70% PHx. We also evaluated mitochondrial changes and apoptosis using electron microscopy and flow cytometry. RNA-interference-mediated knockdown of Phb1 (PHBi) was performed in BRL-3A cells.

RESULTS

Compared with sham-operation control groups, Phb1 mRNA and protein levels in 70% PHx test groups were downregulated at 24 h, then upregulated at 72 and 168 h. Phb1 was mainly located in mitochondria, showed a reduced abundance at 24 h, significantly increased at 72 h, and almost recovered to normal at 168 h. Phb1 was also present in nuclei, with continuous increase in abundance observed 72 and 168 h after 70% PHx. The altered ultrastructure and reduced mass of mitochondria during LR had almost completely recovered to normal at 168 h. PHBi in BRL-3A cells resulted in increased S-phase entry, a higher number of apoptotic cells, and disruption of mitochondrial membrane potential.

CONCLUSION

Phb1 may contribute to maintaining mitochondrial stability and could play a role in regulating cell proliferation and apoptosis of rat liver cells during LR.

Key Words: Prohibitin 1; Liver regeneration; Subcellular proteomic analysis; Mitochondrial stability; Cell proliferation

Core tip: Using subcellular proteomic analysis, we previously found that prohibitin 1 (Phb1) was downregulated in rat liver mitochondria at 24 h after 70% partial hepatectomy (PHx). Phb1 has various functions, but little is known about its role during liver regeneration (LR). To explore the function of Phb1 in mitochondria during LR, we investigated the changes of Phb1 expression, the alterations of mitochondrial mass and ultrastructure, and the subcellular distribution of Phb1 at 24, 72 and 168 h in rat liver after 70% PHx. Using RNA-interference-mediated knockdown of Phb1, the potential functions of Phb1 were analyzed. Phb1 was differentially expressed during LR.

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INTRODUCTION

It is well known that the liver has the capacity to regenerate and restore its original size and function after 70% partial hepatectomy (PHx), or injury[1,2]. It would be important clinically to develop therapeutic strategies to enhance liver regeneration (LR) or support the liver in its attempt to restore its functional integrity under pathophysiological circumstances[3,4]. However, the complexity of the regulatory mechanisms of LR, together with our limited understanding of the functional priorities of the hepatocytes have rendered difficult the identification of targets for therapeutic interventions.

As the hub of energy metabolism, mitochondria have been investigated due to their direct involvement in the process of LR[5]. In an attempt to identify mitochondrial proteins that are correlated with the early phase of LR, using subcellular proteomic analysis in our recent study, our recent study revealed that Prohibitin 1 (Phb1), a potential tumor suppressor protein, was downregulated in rat liver mitochondria at 24 h after 70% PHx[6].

Phb1 is a ubiquitously expressed highly conserved protein among eukaryotes. Previous research has proposed that Phb1 is involved in many cellular processes, such as cell cycle regulation, senescence, transcription regulation, tumor suppression and apoptosis[7-11]. Phb1 is reported to mainly localize in mitochondria, with its expression upregulated by mitochondrial stress and downregulated during cellular senescence[12]. Therefore, Phb1 is thought to have a crucial role in mitochondria function. One study identified a novel function of Phb1 in the maintenance of mitochondrial DNA (mtDNA). In Phb1-knockdown cells, the status of mtDNA is altered in several ways[13]. Despite such information, our understanding of the overall functions of Phb1 in mitochondria remains incomplete and its potential role during LR is largely unexplored. LR is a complicated biological procedure involving various signal transduction pathways and molecular events[14,15]. Thus, we hypothesized that Phb1 could play a crucial role during LR. This study aimed to investigate the function of Phb1 in mitochondria during changes in Phb1 expression, mitochondrial mass and ultrastructure, and the subcellular distribution of Phb1 at 24, 72 and 168 h post 70% PHx in rat liver. Using RNA-interference-mediated knockdown of Phb1 (PHBi), we also analyzed the potential functions of Phb1. Our results revealed differential expression of Phb1 during LR, with its primary localization in mitochondria, where its altered expression may be associated with the recovery of mitochondrial mass and ultrastructure. Phb1 was also present in the nuclei, with increased abundance during LR. PHBi in BRL-3A cells, a widely used cell line in liver research, led to increased S-phase entry and apoptotic cell count. We also observed disruption of mitochondrial membrane potential following Phb1 knockdown in BRL-3A cells, mirroring our previous findings. Collectively, these results suggest that Phb1 may contribute to maintaining mitochondrial stability and regulating the cell cycle and apoptosis during LR.

MATERIALS AND METHODS

Animals and surgery

Adult male Sprague–Dawley rats (220–250 g) were obtained from the Experimental Animal House at Second Military Medical University (Shanghai, China). The rats were randomly divided into two groups: Five served as the sham-operation control group and the other five comprised the 70% PHx test group. PHx (~70%) was performed according to the method of Higgins *et al*[16]. The experimental rats were anesthetized by intraperitoneal injection of 2% pentobarbital (40 mg/kg). In the test group, the median and left lateral lobes were removed without injuring the remaining liver tissue.

The control group underwent a sham operation identical to the test group procedure, but without liver removal. After surgery, the rats were kept on a standard diet until they were killed by cervical dislocation under anesthesia.

Electron microscopy

Liver specimens were fixed with 4% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 4 h at 4C. After fixation, they were washed overnight in sodium cacodylate buffer at 4C. The specimens were then postfixed with 1% osmium tetroxide in sodium cacodylate buffer for 1 h at 4C, dehydrated in alcohol, embedded in araldite resin, and semithin sections were removed for optical microscopy. Ultrathin sections were mounted on copper mesh grids and stained with uranyl acetate and lead citrate as described[17] before examination with a Hitachi H-800 electron microscope.

Separation of rat liver subcellular fractions and protein preparation

Rat livers were removed and treated as previously reported[18] for isolation of nuclei, cytosol and mitochondria. Livers were collected and homogenized. Subsequent centrifugation at increasingly higher speeds at 4C yielded the following fractions: Nuclear fraction at 1000 *g* for 10 min; mitochondrial fraction at 15 000 *g* for 15 min; and microsomes at 144 000 *g* for 90 min. The final supernatant was the cytosolic fraction. Purification of mitochondria was performed by Nycodenz density gradient purification[19]. The mitochondrial pellets obtained from differential centrifugation were suspended in 12 mL 25% Nycodenz and placed onto a discontinuous Nycodenz gradient consisting of 5 mL 34% Nycodenz and 8 mL 30% Nycodenz, followed by 8 mL 23% Nycodenz, and finally, 3 mL 20% Nycodenz. The sealed tubes were centrifuged for 90 min at 52 000 *g* at 4C. The mitochondria were in the band at the 25%/30% interface which was collected and diluted with the same volume of homogenization buffer and then centrifuged twice at 15 000 *g* for 20 min. The preparation of each subcellular fraction protein of rat livers was performed as previously described[19]. Protein concentration of each fraction was determined with a Quick Start Bradford Assay Kit (Bio-Rad).

Western blotting

Protein extracts of each sample were separated on 12% SDS-PAGE and transferred to nitrocellulose membranes (Millipore). The blots were probed by anti-Phb1 antibody (Neomarker) and proteins were normalized with anti--actin antibody (Neomarker) or anti-COX IV antibody (Cell Signaling) or anti-histone H3 antibody (Cell Signaling) and were visualized by Amersham ECL system. The digital image was obtained by scanning the membrane, and then subjected to gray value analysis. For a better understanding of western blotting results and derived ratio changes, a detailed methodology introduction can be found in the subsequent figures and legends.

Cell culture

The normal rat liver cell line BRL-3A was obtained from the Shanghai Institute of Biochemistry and Cell Biology. The BRL-3A cells were maintained as a monolayer in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum, 100 U/mL penicillin, and 100 mg /mL streptomycin. The cells were maintained at 37C in an atmosphere with 5% CO_2.

RNA interference

Duplex siRNA was obtained from GeneChem (Shanghai, China). The siRNA sequence targeting rat Phb1 was 5′-GCCAGAUUUGUGGUGGAAAtt-3′ (sense) and 5′-UUUCCACCACAAAUCUGGCtt-3′ (antisense). A nonsense duplex was used as the control (mock). BRL-3A cells were plated on six-well plates with antibiotic-free DMEM overnight and transfected with siRNA by Lipofectamine2000 (Invitrogen). The final concentration of siRNA duplex was 100 nM. Six hours after transfection, the medium was switched to DMEM supplemented with antibiotics.

Reverse transcription polymerase chain reaction (RT-PCR) and quantitative RT-PCR

Total RNA of each sample was isolated by TRIzol reagent (Invitrogen). After treatment with DNase I, each RNA sample was reverse-transcribed with random primers (dN6) (MBI Fermantas, Vilnius, Lithuania). The single-stranded cDNA was used in quantitative real-time PCR to evaluate the relative expression levels of Phb1 (5′-GCGGTGGAAGCCAAACAG-3′ and 5′-TTCTTCTGCTGCTCAGCCTTT-3′), compared to -actin (5′-ATGGTGGGTATGGGTCAGAAG-3′ and 5′-TGGCTGGGGTGTTGAAGGTC-3′) used as an internal control for determining cell number and metabolic status. Quantitative real-time PCR (ABI7300, Applied Biosystems) was done with the SYBR Green I reagents (TOYOBO) and the primers were designed according to the ABI manufacturer's protocol. Forty cycles of PCR were performed with cycling conditions of 15 s at 95C and 60 s at 60C. The real-time PCR signals were analyzed with LightCycler 3.5 software (Roche Diagnostics).

Flow cytometry

Cells were stained with propidium iodide (PI; BD Clontech) as previously described[20]. A suspension of 10^4 cells was analyzed for each DNA histogram, and from the analysis of DNA histograms, the percentages of cells in different phases of cell cycle were evaluated. Flow cytometry was performed on a FACSCalibur and analyzed using CellQuest software (BD Bioscience). The Annexin V/PI method was used to quantify numbers of apoptotic cells. Cells were washed twice with phosphate-buffered saline and stained with Annexin V and PI for 20 min at room temperature. The level of apoptosis was determined by measuring the fluorescence of the cells by flow cytometry analysis.

Statistical analysis

The data presented are the means ± SD of three independent experiments. Statistical significance was estimated with Student's *t*-test for unpaired observations. $P < 0.05$ was considered significant.

RESULTS

Altered expression of Phb1 mRNA and protein abundance during LR

Phb1 mRNA expression was examined by real-time PCR. Compared with the control group, expression of Phb1 mRNA in the 70% PHx test group was 0.46-fold decreased at 24 h, and 1.54-fold and 1.89-fold increased at 72 and 168 h, respectively (Figure 1A). Western blotting showed that Phb1 protein expression during LR was 0.54-fold decreased at 24 h, and 1.48-fold and 1.73-fold increased at 72 and 168 h, respectively, after 70% PHx (Figure 1B), which was consistent with the expression of Phb1 mRNA.

Subcellular distribution and protein abundance alteration in each subcellular fraction of Phb1 during LR

Previous observations suggest that examining the subcellular distribution of Phb1 might yield important information about physiological or pathological processes that are taking place in cells. To verify the cellular distribution pattern of Phb1 during LR, we fractionated cytosolic, mitochondrial and nuclear fractions of rat liver cells and performed western blotting analysis. The purity of subcellular fractionation was controlled by several marker proteins (Figure 2A). Phb1 was mainly located in mitochondria and its abundance was reduced 0.47-fold at 24 h, and induced 1.47-fold at 72 h and almost recovered to normal at 168 h after 70% PHx (Figure 2B). Phb1 was also located in nuclei and its abundance was increased during LR after 70% PHx (Figure 2C). No Phb1 was found in the cytosol.

Alterations of mitochondria during LR

Mitochondrial mass and ultrastructural alterations during LR were observed to determine whether Phb1 changes were associated with mitochondrial stabilization or biogenesis. The mitochondrial mass was quantified by examining the protein contents of mitochondrial fractions[21] extracted from equivalent weights (1 g) of liver tissues from each experimental group. The results indicated an increase in mitochondrial protein contents during LR with 5.37 ± 0.08, 6.38 ± 0.10 and 7.16 ± 0.16 mg at 24 , 72 and 168 h respectively, after 70% PHx. The mitochondrial protein contents at 168 h in the 70% PHx test group closely mirrored that of the control group (Figure 3A).

Mitochondrial ultrastructural alterations during LR were observed by electron microscopy. The mitochondrial morphologies of control livers (Figure 3B upper panel) (SH 24 h, SH 72 h, SH 168 h), were characterized by a consistent basic architecture featuring a folded internal membrane and a dense matrix. The alterations in mitochondrial ultrastructure following 70% PHx are showed in Figure 3B bottom panel. At 24 h after 70% PHx (PH 24 h), the mitochondria displayed significant swelling, reduction in the number of cristae, dilated and pale matrix, absence of dense granules, and clear matrix compartment vacuolization. At 72 h after 70% PHx (PH 72 h), only slight changes with moderate distension were seen in mitochondrial morphology. By 168 h after 70% PHx (PH 168 h), the mitochondria had mostly returned to their normal morphology, and were rich in cristae, with an electron-dense matrix. These alterations in mitochondrial ultrastructure have been associated with changed in mitochondrial function during LR[17].

PHBi leads to an increase in the number of apoptotic cells

To downregulate Phb1 cellular expression, PHBi was performed in BRL-3A cells. PHBi resulted in a dramatic reduction in both Phb1 mRNA and protein level compared with that of the control group (mock). Detailed results are available in our previous publication[4].

Previous reports have suggested that Phb1 could serve an antiapoptotic role in undifferentiated granulosa cells[22]. In this study, to evaluate whether Phb1 was involved in modulating apoptosis in rat liver cells, flow cytometry was used to evaluate percentage of apoptotic cells by Annexin V/PI staining. Phb1 knockdown cells displayed a 1.56-fold increase in the percentage of apoptotic cells compared with controls (Figure 4A).

PHBi leads to increased S-phase entry

We investigated whether the decrease of Phb1 by PHBi had any effect on cell growth and proliferation. The cell cycle distribution in Phb1 knockdown cells showed a 1.26-fold increase in the S-phase compared to control cells (Figure 4B). Although the increase in the S-phase was not dramatic, the difference was significant. Nuell *et al*[23] also previously reported a cell cycle modulatory role of Phb1, indicating that Phb1 could function as a negative cell cycle regulator.

DISCUSSION

Phb1, a potential tumor suppressor protein, was initially cloned due to its ability to induce G1/S phase arrest. Phb1 is proposed to be involved in numerous cellular processes. However, most studies to date have focused on the role of Phb1 in various types of tumors, with its role during LR remaining largely unexplored. In recent years, some studies have explored the role of Phb1 in liver injury and liver cancer[10,24-27]. However, the role of Phb1 in LR remained unstudied.

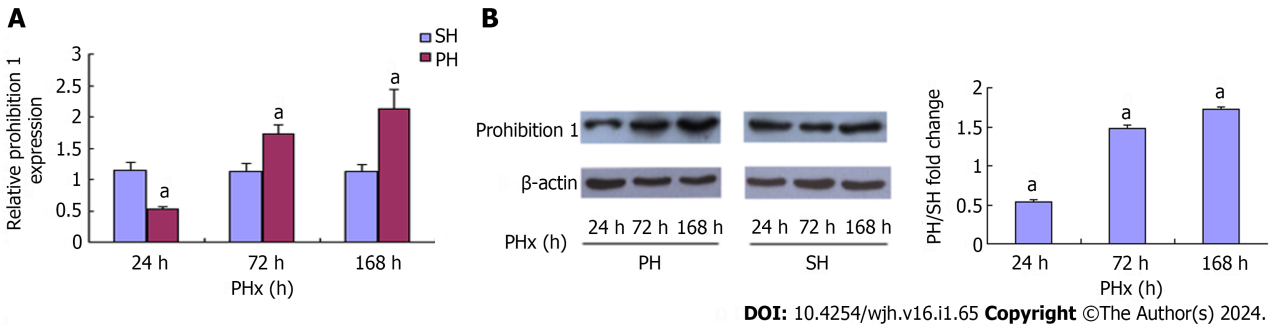

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Figure 1 Altered expression of prohibitin 1 mRNA and protein during liver regeneration. A: Altered expression of prohibitin 1 (Phb1) mRNA during liver regeneration (LR). Rat livers were homogenized and harvested with TRIzol reagent. Real-time polymerase chain reaction (PCR) amplifications were performed, standardized by the amounts of β-actin. The real-time PCR signals were analyzed with LightCycler 3.5 software; B: Altered expression of Phb1 protein during LR. Equal amounts of protein from each sample were separated on 12% SDS-PAGE and immunoblotted with antibodies against Phb1 and control protein, β-actin. On the left are the western blotting results, and on the right is a gray analysis of the results showing the fold changes of Phb1 expression in PH groups in comparison with SH groups, standardized by the amounts of β-actin. All grouping of gels/blots were cropped from different parts of the same gel. PH: 70% PHx test groups; PHx: Partial hepatectomy; SH: Sham-operation control groups;. Results are from three independent experiments and data are represented as means ± SD. $^{a}P < 0.05$, *versus* control group, significant difference.

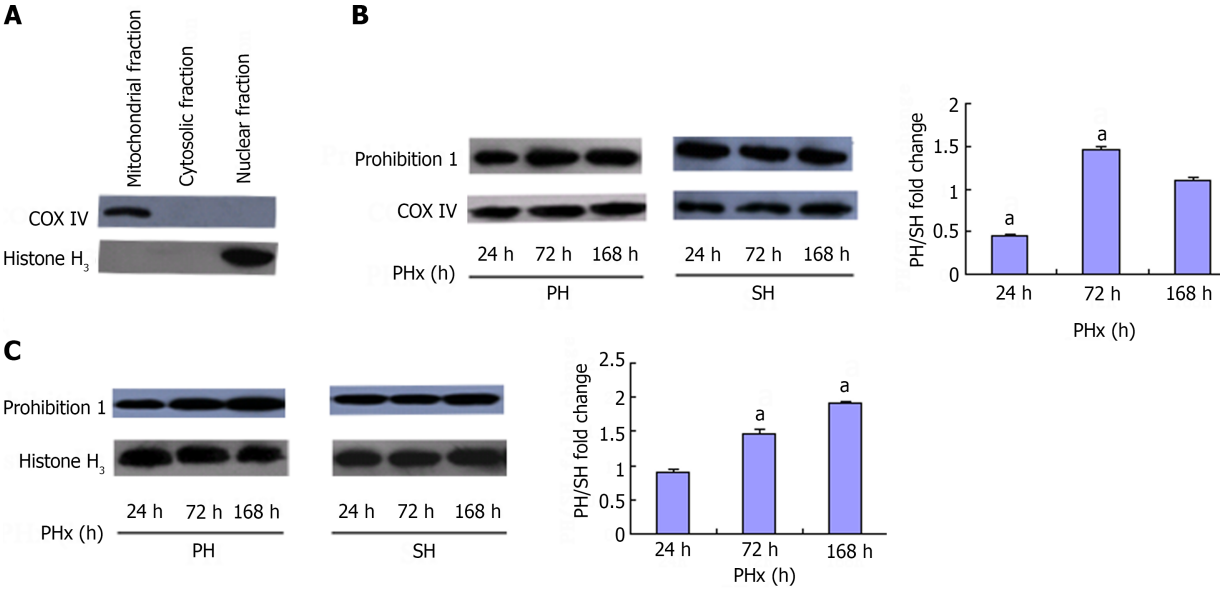

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Figure 2 Subcellular distribution and protein abundance alterations of prohibitin 1 during liver regeneration. Equal amounts of protein extracts obtained from each subcellular fraction were separated on 12% SDS-PAGE and immunoblotted with antibodies against prohibitin 1 (Phb1), cyclooxygenase IV (COX IV) and histone H3. A: Control western blotting for subcellular fractionation (COX IV for mitochondria and Histone H3 for nuclei). The grouping of gels/blots were cropped from different parts of different gels; B: Changes in Phb1 protein abundance in mitochondria. On the left are the western blotting results, and on the right is a gray analysis of the results showing the fold changes of Phb1 expression in mitochondria in PH groups in comparison with SH groups, standardized by the amounts of COX IV. The grouping of gels/blots in PH groups were cropped from different parts of the same gel. The grouping of gels/blots in SH groups were cropped from different parts of the same gel; C: Changes of Phb1 protein abundance in nucleus. On the left are the Western blotting results, and on the right is a gray analysis of the results showing the fold changes of Phb1 expression in nucleus in PH groups in comparison with SH groups, standardized by the amounts of Histone H3. The grouping of gels/blots for Phb1 were cropped from different parts of the same gel. The grouping of gels/blots for histone H3 were cropped from different parts of the same gel. PH: 70% PHx test groups; PHx: Partial hepatectomy; SH: Sham-operation control groups. Results are from three independent experiments and data are represented as means ± SD. $^{a}P < 0.05$, *versus* control group, significant difference.

In this study, Phb1 mRNA and protein expression underwent concordant changes during LR after 70% PHx. Compared to sham-operation control groups, 70% PHx test groups showed downregulation of Phb1 mRNA and protein expression at 24 h, and upregulation at 72 and 168 h (Figure 1). A previous study found that the gene encoding Phb1 might have additional antiproliferative effects that do not require translation[11]. Manjeshwar *et al*[28] reported that the 3′ untranslated region of the *Phb1* gene encoded a functional RNA that arrested cell-cycle proliferation between the G1 and S phases. In light of previous reports, we propose that Phb1 might regulate cell proliferation during LR in a complex manner, potentially involving mechanisms mediated by both Phb1 mRNA and protein.

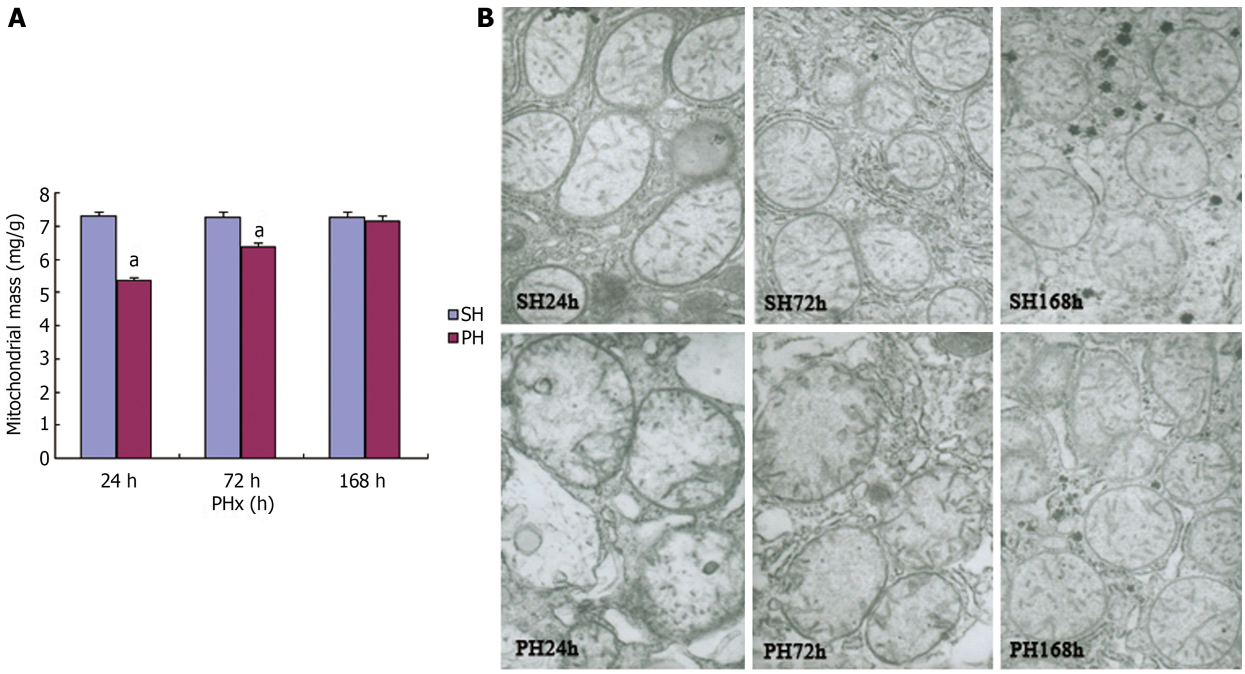

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Figure 3 Mitochondrial mass and ultrastructural alterations during liver regeneration. A: Changes of mitochondrial mass during liver regeneration (LR). Mitochondrial mass was measured by examining the protein contents of mitochondrial fractions extracted from the same weight (1 g) liver tissues of each experimental group; B: Electron micrographs of mitochondria in liver tissues during LR. Three maps in the upper panel are detail of hepatocytes in control groups, showing normal mitochondria. The other three maps in the bottom panel are detail of hepatocytes after 70% PHx, showing altered mitochondria during LR. PH: 70% PHx test groups; PHx: Partial hepatectomy; SH: Sham-operation control groups; PH: 70% PHx test groups. Ten randomly selected electron micrographs of the same magnification (15 000×) were examined from one hepatic lobule of five rats for each experimental group.

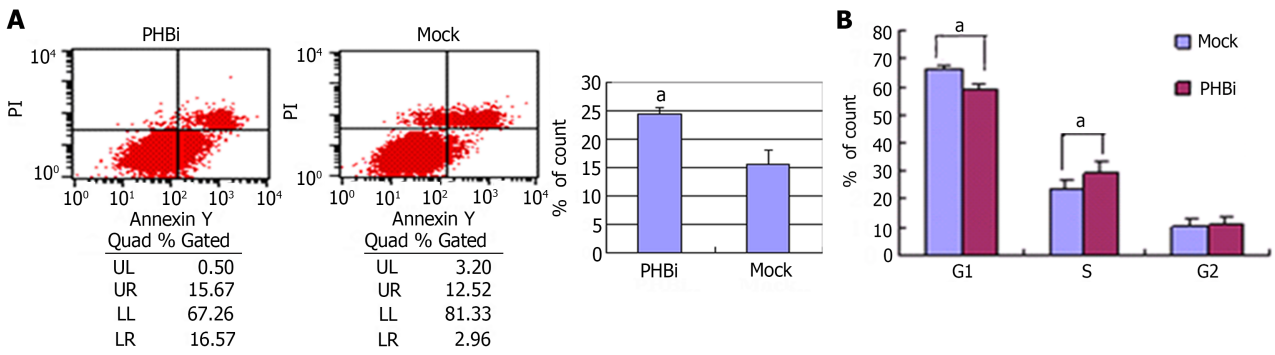

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Figure 4 RNA-interference-mediated knockdown of Phb1 induced an increase in the number of apoptotic cells and increased S-phase entry. A: An increase in the number of apoptotic cells in RNA-interference-mediated knockdown of Phb1 (PHBi) cells. At 72 h post-transfection, stained with Annexin V and propidium iodide (PI), PHBi cells showed a significant increase in the percentage of Annexin V/PI positive cells compared with controls (cells transfected with nonsensing duplex); B: Increased S-phase entry in PHBi cells. At 48 h post-transfection, FACS analysis was performed with PHBi and mock (cells transfected with nonsensing duplex, used as control). Results are from three independent experiments and data are represented as means ± SD. $^{a}P < 0.05$, *veruss* mock, significant difference. UL: Upper left; UR: Upper right; LL: Lower left; LR: Lower right; G1: G1 phase; S: S phase; G2: G2 phase.

The well-characterized function of Phb1 is as a chaperone involved in the stabilization of mitochondrial proteins. Mitochondrial-localized Phb1 is confirmed as a high-molecular-weight hetero-complex (ring-shaped structure) by single-particle structures[7]. The interaction of no assembled respiratory chain subunits with the Phb1 complex has led to the proposal of a chaperone activity of Phb1 during the biogenesis of the respiratory chain[29]. Recently, Phb1 was reported to be essential for normal mitochondrial development, and Phb1 deficiency was showed to be associated with deficient mitochondrial biogenesis[30]. PHBi showed enhanced sensitivity to anthralin-induced cell death due to enhanced loss of mitochondrial membrane potential in psoriatic lesions[31]. Mitochondria are the center of energy metabolism and play a crucial role in regulating cell life. Various stimuli can induce dysfunction and structural injury in mitochondria, which triggers a series of cellular events ultimately leading to apoptosis or necrosis. We found that Phb1 was mainly located in the mitochondria in rat liver, and its abundance underwent a 0.47-fold reduction at 24 h, a 1.47-fold induction at 72 h, and nearly recovered to normal level at 168 h after 70% PHx (Figure 2B). Mitochondria showed significant changes in the

ultrastructure at 24 h, and nearly recovered to normal at 168 h after 70% PHx (Figure 3B). The reduced mitochondrial mass also nearly recovered to normal at 168 h after 70% PHx. Mitochondrial membrane potential is an important parameter of mitochondrial function. In our previous study, we found that knockdown of Phb1 in BRL-3A cells resulted in disruption of mitochondrial membrane potential, implying a potential role of Phb1 in maintaining mitochondrial integrity[6]. Ross *et al*[32] also reported that siRNA-mediated knockdown of Phb1 in Kit225 cells resulted in disruption of mitochondrial membrane potential and Phb1 proteins were novel phosphoproteins upregulated during T-cell activation that function to maintain mitochondrial integrity. In this study, using PHBi, we also observed that Phb1 knockdown cells exhibited a 1.56-fold increase in the number of apoptotic cells (Figure 4A). Although these results provide evidence for a functional role of Phb1 in suppressing apoptosis in rat liver cells, the involved molecular mechanisms remain unknown. It is likely that the mechanism by which knockdown of Phb1 results in apoptosis targets the mitochondria in agreement with previous findings[22]. All these results suggest that Phb1 has a role in regulating stabilization of mitochondria during LR, which might affect mitochondrial function.

Although it has been reported that Phb1 is primarily located in mitochondria[12,30,33,34], other studies have reported that Phb1 is also located in the nuclei[35,36]. We found that Phb1 was located in nuclei as well as mitochondria in rat liver and its abundance increased during LR (Figure 2B). Previous studies reported that Phb1 was present in the nuclei and interacted with transcription factors important in cell-cycle progression[35,36]. In this study, using PHBi, we observed that Phb1 knockdown cells showed an increase in S-phase entry (Figure 4B). The involvement of Phb1 in the cell cycle was also observed in a prostate cancer cell line, in which downregulation of Phb1 led to an increase in cell-cycle entry from G1 to S phase[30]. Although most data suggest that Phb1 has an antiproliferative effect by interacting with the p53 and pRb pathways in the nuclei[9,37], it appears that Phb1 can also have antiapoptotic effects. In osteosarcoma cells, *Phb1* was identified as a gene with downregulated expression in response to cytotoxic drugs, and the transient overexpression of the Phb1 coding sequence significantly reduced cytotoxic drug-induced apoptosis in these cells[38]. In this study, we also observed that Phb1 knockdown cells showed an increase in the number of apoptotic cells (Figure 4A). It has been reported that the subcellular localization of Phb1 may depend on the cell type examined and its physiological status, and Phb1 might have distinct but overlapping functions in each of these cellular compartments[39]. Although there is controversy concerning the function of nuclear-localized Phb1, in combination with previous reports, we suggest that the upregulated Phb1 in the nuclei in rat liver cells might have a function, at least in part, in regulating cell-cycle progression of rat liver cells. It might regulate the balance between proliferation and apoptosis during LR after 70% PHx, but this needs further investigation.

CONCLUSION

In summary, our results demonstrate that Phb1 plays two roles in the LR process: one is to regulate cell cycle and apoptosis, and the other is to regulate and maintain mitochondrial stability. Whether the two effects are directly linked or show two different effects remains unclear. Further in-depth studies will aid in us better understanding the complexities and roles of Phb1 in the LR process.

ARTICLE HIGHLIGHTS

Research background

It is clinically important to develop therapeutic strategies to enhance liver regeneration (LR) or support the liver in its attempt to restore its functional integrity under pathophysiological circumstances. However, the complexity of the regulatory mechanisms of LR, together with our limited understanding of the functional priorities of the hepatocytes have rendered difficult the identification of targets for therapeutic interventions.

Research motivation

Prohibitin 1 (Phb1) is a ubiquitously expressed highly conserved protein among eukaryotes. Previous research has proposed that Phb1 was involved in many cellular processes. Phb1 was reported to mainly localize in mitochondria, with its expression upregulated by mitochondrial stress and downregulated during cellular senescence. Therefore, Phb1 is thought to have a crucial role in mitochondrial function. One study identified a novel function of Phb1 in the maintenance of mitochondrial DNA (mtDNA). In Phb1-knockdown cells, the status of mtDNA is altered in several ways. Despite such information, our understanding of the overall functions of Phb1 in mitochondria remains incomplete and its potential role during LR is largely unexplored. LR is a very complicated biological procedure involving various signal transduction pathways and molecular events. Thus, we hypothesized that Phb1 could play a crucial role during LR.

Research objectives

This study aimed to further investigate the function of Phb1 in mitochondria during changes in Phb1 expression, mitochondrial mass and ultrastructure, and the subcellular distribution of Phb1 at 24, 72 and 168 h post 70% partial hepatectomy (PHx) in rat liver. Using RNA-interference-mediated knockdown of Phb1 (PHBi), we also analyzed the potential functions of Phb1.

Research methods
We examined changes in Phb1 mRNA and protein levels, subcellular distribution, and abundance in rat liver during LR following 70% PHx. We also evaluated mitochondrial changes and apoptosis levels using electron microscopy and flow cytometry. PHBi was performed in BRL-3A cells.

Research results
Compared with sham-operation control groups, Phb1 mRNA and protein levels in 70% PHx test groups were downregulated at 24 h, then upregulated at 72 and 168 h. Phb1 was mainly located in mitochondria, showed a reduced abundance at 24 h, significantly increased at 72 h, and almost recovered to normal at 168 h. Phb1 was also present in nuclei, with continuous increase in abundance observed at 72 and 168 h after 70% PHx. The altered ultrastructure and reduced mass of mitochondria during LR had almost completely recovered to normal at 168 h. PHBi in BRL-3A cells resulted in increased S-phase entry, a higher number of apoptotic cells, and disruption of mitochondrial membrane potential.

Research conclusions
In summary, our results demonstrate that Phb1 plays two roles in the LR process: one is to regulate cell cycle and apoptosis, and the other is to regulate and maintain mitochondrial stability.

Research perspectives
Whether the two effects are directly linked or show two different effects remains unclear. Further in-depth studies will aid in us better understanding the complexities and roles of Phb1 in the LR process.

FOOTNOTES

Author contributions: Sun QJ designed the research study; Sun QJ and Liu T performed the research; Sun QJ and Liu T analyzed the data and wrote the manuscript; All authors have read and approve the final manuscript.

Institutional review board statement: This study is reported in accordance with the ARRIVE guidelines (https://arriveguidelines.org). All methods were performed in accordance with the relevant guidelines and regulations.

Institutional animal care and use committee statement: In this study, the ethics approval was obtained from the Medical Research and Ethics Committees at Navy 971 hospital in Qingdao, Shandong, China.

Conflict-of-interest statement: All the authors declare that they have no conflict of interest.

Data sharing statement: All data generated or analyzed during this study are included in this published article.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

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