Original Article

PRDX1 Influences The Occurrence and Progression of Liver Cancer by Inhibiting Mitochondrial Apoptosis Pathway

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Abstract

Objective: The aim of this study is to elucidate the role of *PRDX1* in hepatocellular carcinoma using hepatoma cells.

Materials and Methods: In this experimental study, we elucidated role of *PRDX1*, using hepatoma cell lines.

Results: *PRDX1* was upregulated in different types of cancers, including lung adenocarcinoma, breast cancer and liver cancer reported by several studies. nevertheless, mechanism of inducing liver cell death by *PRDX1* remains largely unknown. Here, we showed that *PRDX1* expression is enhanced in different cell lines. Here, we used western blot, quantitative real time polymerase chain reaction (qRT-PCR) and different biochemical assays to explore the role of PRDX1. We observed that overexpression of *PRDX1* significantly enhanced proliferation of hepatoma cell lines, while knock-down of this gene showed significant inhibitory effects. We found that knock-down of *PRDX1* activated cleaved caspase-3, caspase-9 proteins and Poly [ADP-ribose] polymerase 1 (PARP-1), which further executed apoptotic process, leading to cell death. We found that *PRDX1* knock-down significantly produced mitochondrial fragmentation. We showed that silencing *PRDX1* led to the loss of B-cell lymphoma 2 (Bcl-2) and activated Bcl-2-like protein 11 (Bim) which further induced Bax activation. Bax further released cytochrome c from mitochondria and induced apoptotic proteins, suggesting a significant role of *PRDX1* knock-down in apoptosis. Finally, we showed that knock-down of *PRDX1* significantly activated expression of Dynein-related protein 1 (Drp1), fission 1 (Fis1) and dynamin-2 (Dyn2) suggesting a crucial role of *PRDX1* in mitochondrial fragmentation and apoptosis conditions. This study highlighted an important role of *PRDX1* in regulating proliferation of hepatoma cells and thus future studies are required to validate its effect on hepatcoytes.

Conclusion: We propose that future works on *PRDX1* inhibitors may act as a therapeutic candidate for treatment of liver cancer.

Keywords: Hepatocellular Carcinoma, Liver Cancer, Peroxiredoxins, *PRDX1*

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Introduction

Human hepatcullar carcinoma (HCC) is one of the most common types of liver cancer, which accounts for more than 70% of total liver cancer. It is known for the high mortality rate (>8%) worldwide. Peroxiredoxins are a large family of antioxidant enzymes that play an essential role in antioxidant defense and peroxide detoxification. *PRDX1* is a multifunctional protein involved in cell growth, differentiation and apoptosis (1). *PRDX1* is reported in different types of cancer, including lung adenocarcinoma, breast cancer, soft tissue sarcomas (2), colorectal cancer and prostate cancer. Most studies reported elevated level of *PRDX1* in pathological conditions.

However, Fang et al. (3) showed the lower *PRDX1* expression in HCC cells. *PRDX1* is upregulated in cervical cancers and enhanced proliferation, migration and invasion by inhibiting apoptosis (4). Analysis of a disease model represented higher *PRDX1* expression level in brain, while it is associated with toll like receptor-4 (TLR-4) inflammation and apoptosis. Several studies reported its expression in tumor tissues of the liver (3). However, the cellular role of *PRDX1* in hepatocellular carcinoma and mechanism of this associaition with related protein remains unknown. Caspases are essential proteins. They are activated when cell death is required (5). Poly ADP-ribose polymerase 1 (PARP-1) has multiple functions involved in DNA repair, cell death and transcriptions of some essential genes involved in inflammatory processes (6). It is a well-known substrate of caspase proteins (7). Caspase-3 cleaves PARP-1 upon activation and therefore prevents PARP-1 from repairing the damage (8). It has been demonstrated that overexpression of human Mitochondrial fission 1 protein (hFis1) induced apoptosis, which may suggest a role of mitochondrial fission in apoptosis. Dynein-related protein 1 (DrP1) expression is upregulated in HCC cells and involved in autophagy (9).

Here, in this study, we elucidated role of *PRDX1*

in hepatocellular carcinoma using hepatoma cells. The role of *PRDX1* on hepatocytes cells was poorly known. We We reported for the first time that the mechanism by which *PRDX1* acts on HCC cells via B-cell lymphoma 2 (Bcl-2).

Materials and Methods

RNA sequencing database

In this experimental study, the RNA sequencing data from more than 350 patients of liver cancer were obtained from TCGA database. The expression of *PRDX1* mRNA were analyzed using the database. The Kaplan-Meier and Cox regression survival analysis was performed to see the relationship between *PRDX1* levels and patient survival.

Cell culture

HCC cells were obtained from ATCC (Virginia, USA). Cell culture media and supplements were purchased from Gibco (Sigma, USA). HCC cells were cultured in DMEM high glucose medium supplemented with 2 mM glutamine, 100 units/ml penicillin (both from Gibco, USA), 10 % fetal calf serum (ThermoFisher, USA) and 100 lg/ml, streptomycin (Gibco, USA). Cells were grown at 37°C in the presence of 5% CO_2. Cells were grown for 2-3 days. Following the confluency, the cells were proceeded for analysis of mRNA or protein expressions.

Quantitative reverse trasncription polymerase chain reaction

Extraction of total RNA was done by Trizol reagent (Ambion, USA). Synthesis of cDNA was done by RevertAid cDNA Synthesis Kit (Thermo Fisher Scientific, USA) for that 1 µg of total RNA of each sample was used. The cDNA samples were kept at -20°C for quantitative reverse transcriptipn PCR (qRT-PCR). The SYBR green dye (Invitrogen, USA) was used to bind to double stranded DNA and emit green light (ƛmax=520 nm), in terms of quantifying cDNA. For qRT-PCR, the master mix was used as the manufacturer's instruction. Specific primers of *PRDX1* and *GAPDH* were used (Table 1). Data was acquired and analyzed using comparative CT method.

Table 1: Primer information

Gene name	Primer sequence (5′-3′)
PRDX1	F: GCACCATTGCTCAGGATTATG
	R: GCCAACAGGGAGGTCATTTAC
GAPDH	F: GGTGTGAACCATGAGAAGTATGA
	R: GAGTCCTTCCACGATACCAAG

Cells transfection

The *PRDX1*-siRNA are 5′GCACCAUUGCUCAGGAUUATT3′ which was synthesized by GenePharma (Shanghai, China). HepG2 Cells were seeded and transfected using Lipofectamine 2000 reagent (Invitrogen, USA) following manufacturer's instructions. 3×10^4 cells were seeded and allowed to 70-90% confluency. Transfection mixture was prepared with 50 ng, 25 µl DNA dilution and 25 µl opti-MEM (Gibco, USA) dilution. Mixing Lipofectamine 2000 diluent and DNA diluent was performed in a centrifuge tube with a capacity of 1.5 ml, followed by 15 minutes incuabation in hood. Optimum medium was removed and DNA/lipofectamine 2000 mixture was gently added dropwise into cells.

Western blotting

Lysis buffer (200 µl/well) was used to lyse HepG2 cells. Lysis buffer was composed of Triton X-100 (1%), Tris (50 mM, pH=7.6) and NaCl (150 mM), with inhibitors of phosphatases and proteases. sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE, 7.5%) was used to separate 40 µg of the total extracted protein. Then Western blotting was done as demonstrated by Moeschel et al. (10). Following the application of antibodies for western blotting anti-*PRDX1* (ab109498), anti-beta actin (ab115777), anti caspase-3 (ab13847 and ab32042), anti-cleave caspase-9 (ab202068 and ab25758), anti-PARP-1 (ab191217), anti-Bim (ab7888), anti-Fis1 (ab189846), anti-APaf-1 (ab254248), anti-cytochrome c (ab133504), anti-Bcl-2 (ab182858), anti-Bax (ab3191), anti-DRP1 (ab184247) and anti-Dyn2 (ab65556; all purchased from AbCam, UK). Nitrocellulose was blocked using skimmed milk (5%) or BSA (2%, both from Merck, Germany) for two hours. Subsequently, membranes were incubated with primary antibodies at 4°C overnight. Before incubation with secondary antibody, washing was performed (four times for 10 minutes), followed by appropriate conjugated secondary incubation for one hour. For visualizing expression level of proteins, enhanced chemiluminescence was performed.

Cell counting assay

For detecting proliferation, the number of living cells was determined with CCK-8 kit (ab228554, AbCam, UK), according to the manufacturer's instructions. In 96-well plate HepG2 cells were cultivated (five thousands cells in each well). In the incubator, the cells were seeded for 24, 48 and 72 hours. After that, four hours incubation was performed on the cells containing CCK-8 reagent (10 µl). Lastly, optical density was determined at wave length of 450 nm.

Annexin V-FITC-PI staining analysis

HepG2 Cells were stained with Annexin V-FITC/PI

(ab14085, AbCam, UK), following the manufacturer's guidelines. Firstly HCC cells were harvested via trypsinization, then washed with PBS buffer. Centrifugation was done and pellet was collected. The cells were re-suspended with binding buffer. Incubation was performed with annexin V-FITC and propidine iodide (PI) for 10 minutes on ice in dark environment. For detection of apoptotic cells flow cytometry was performed. Binding buffer (150 µl) was added to the sample tubes before analysis on flow cytometry. Data was generated on FACS (Becton Dickinson, USA). Then Cell-Quest software (Becton Dickinson) was used for analyzing or processing the given data.

Clone formation assay

Clone forming assay is an *in vitro* assay which enable single cell to form a colony. Suspension of single cell was made by digesting cells in culture media with (0.25%) or EDTA (0.02%) during logarithm stage. HepG2 Cells were counted via counting chamber in 10 µl suspension using inverted microscope and equal amount of cells were plated in 6-well culture plate. Each well was covered by sterile cover slips. Until formation of colonies, medium was changed every four days. After fixation of colonies with methanol, staining was performed by using crystal violet (1%). Differences in colony formation was observed in the all groups.

Confocal microscopy

MitoTracker red staining was used to analyze mitochondrial filamentous morphology of cells. MitoTracker probes was diluted to 1 mM by adding DMSO. Staining procedure with MitoTracker Red (M22425) and CMXRos (E-max 599 nm; both from ThermoFisher) was performed as described earlier by Harwig et al. (11). Fifty nM MitoTracker™ was used for cells and incubated for 30 minutes at 37°C and. The cells were next washed with FluoroBrite DMEM medium (Gibco, USA), supplemented with 10% fetal cow serum (FCS). Additionally, HepG2 cells were stained with DAPI targeting DNA in nucleus. After staining, the cells were observed undrer confocal microscope.

Statistical analysis

All experiments were performed three times. Represented data were analyzed by using GraphPad Prism (Graphpad software, Inc., San Diego, CA). Multiple groups differences were analyzed using one-way ANOVA. $P<0.05$ was considered to be statistically significant.

Results

PRDX1 is upregulated in liver cancers cells

Given the role of *PRDX1* as an antioxidant system and regulating oxidation reductions, its expression level is critical. Higher-level expression was reported by several studies in different malignancies, including lung (12), cervical (13), prostate (14) and liver cancers (4). However, limited studies reported *PRDX1* expression in human specimens. Significant difference in *PRDX1* expression using TCGA public database was observed, we found that transcripts of *PRDX1* had a significant differences between cancerous and paracancerous tissues of liver cancer patients (Fig.1A). *PRDX1* mRNA expression was found to have a higher expression of 1.3 fold in cancerous tissues compared to control (15). *PRDX1* transcript and protein expression levels were significantly higher in different cancerous cell lines (HepG2, Huh-7, Hep-3B) compared to control cells (Fig.1B-D), which are consistent with the other studies. The highest *PRDX1* mRNA and protein level were found in HepG2 cells with 3-fold higher expression compared to control.

PRDX1 knock-down induced apoptosis in hepatoma cells

Based on the higher expression of *PRDX1* in most of the cancerous tissues and cells, we were interested to see its cellular role. We overexpressed the *PRDX1* construct in HCC cells and tested the efficiency of transfection. We found almost 3-fold higher mRNA and protein expression of *PRDX1* using hepatoma cells, while using siRNA against *PRDX1* (si-PRDX1) significantly inhibited its expression (Fig.2A, 2B). Figure 2C shows relative expression level of PRDX1, in the presence of si-RNA. Cell counting kit-8 (CCK-8) is a widely used colorimetric-based assay used to measure cells viability.We wanted to test the effect of PRDX1 on HCC cell proliferations. We found that knock-down of PRDX1 significantly decreased half maximal inhibitory concentration (IC_{50}) value of HCC cells, suggesting that Knock-down of PRDX1 possesses great anti-tumor activity (Fig.2D). This was further confirmed by clonal formation assay, which was used to assess effect of PRDX1 expression on the proliferation of hepatoma cells. PRDX1 overexpression significantly enhanced proliferation of hepatoma cells (Fig.2E, middle panel) compared to control cells, while knock-down of PRDX1 showed significant inhibitory effects and reduced cell proliferations. Next, we were interested to see its effect on caspase proteins. Interestingly, we found that knock-down of PRDX1 induced cell death via activation of cleaved and active caspase-3 and caspase-9, which further executed an apoptotic process, leading to cell death and inhibited proliferation of hepatoma cells (Fig.2F, G). As overexpression of PRDX1 played a role in cellular proliferation, it regulated PARP-1 to inhibit apoptosis, while knock-down of PRDX1 greatly increased PARP-1 expression, suggesting depletion of NAD^+ levels, thereby induced cellular death (Fig.2G). Figure 2H represents statistical analysis of western blot protein. These results suggested that PRDX1 knock-down inhibited cell proliferation and induced cell death via activation of caspase proteins.

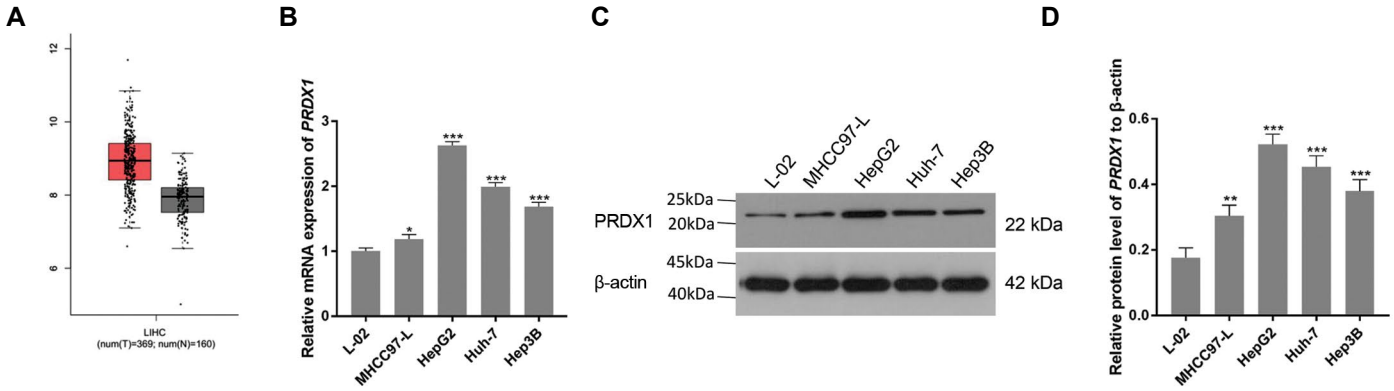

Fig.1: Expression of peroxiredoxin 1 (*PRDX1*) in different cell lines of liver cancers. **A.** Box diagram of *PRDX1* expression, with significant difference between cancerous and paracancerous tissues of liver cancer patients. **B.** *PRDX1* mRNA expression was significantly higher in cancerous cells. **C.** Western blot results showed an increased expression of PRDX1 protein in cancerous cells, with the highest expression level observed in hepatoma (HepG2) cells. **D.** Quantifications of protein levels. The experiment was repeated three times. Data represent mean ± SEM. *; P<0.05, **; P<0.01, and ***; P<0.001.

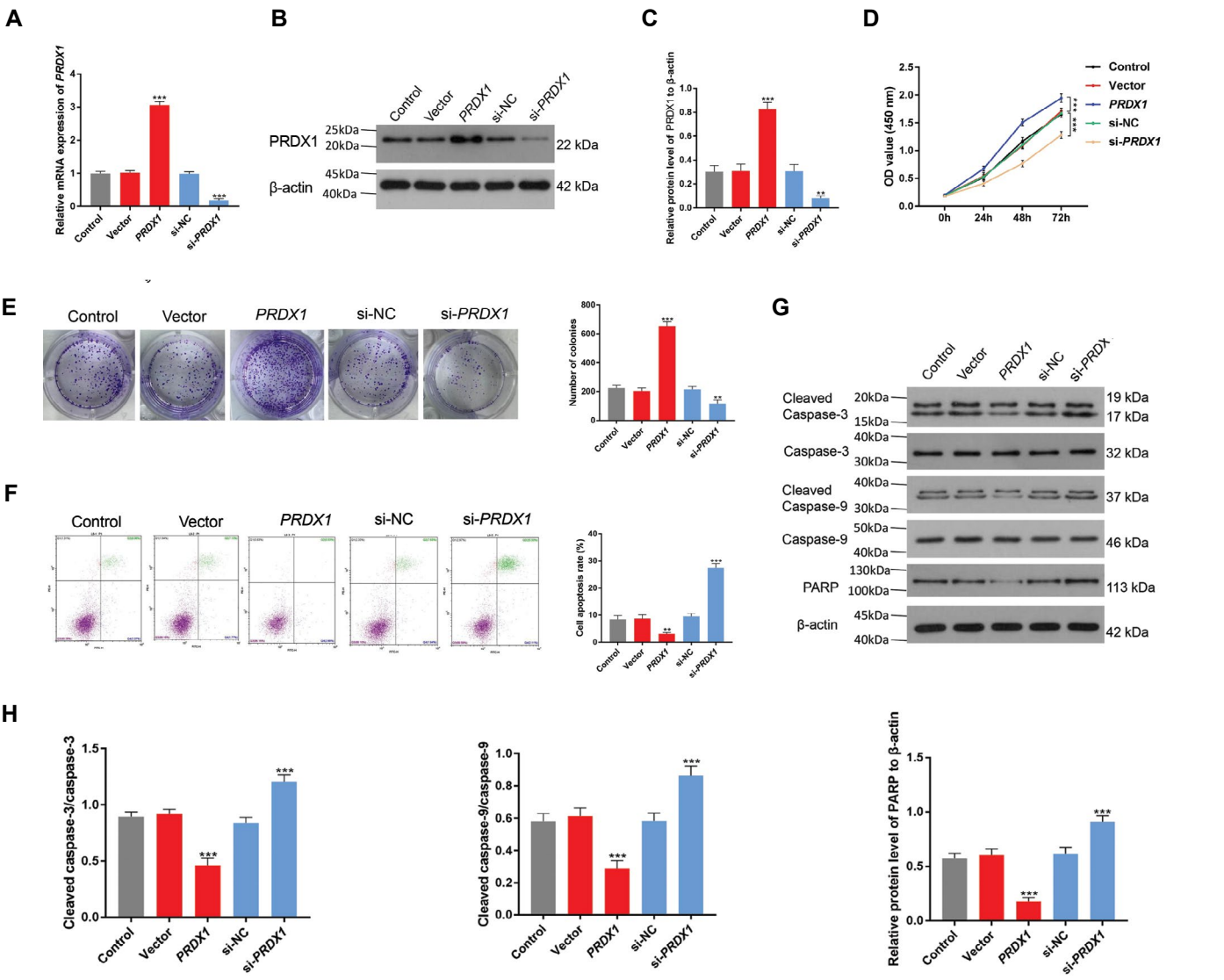

Fig.2: *PRDX1* knock-down induced cell death via apoptosis in HCC cells. **A.** Significant increase of *PRDX1* mRNA expression level when overexpressed in hepatoma cells. **B.** Western blot showed that PRDX1 overexpression enhanced expression level of PRDX1, while knock-down of this gene substantially reduced the expression. **C.** Quantification of the protein levels. **D.** CCK-8 assay showed a significant reduction in the IC_{50} value of hepatoma cells. **E.** Clonal formation assay demonstrated effect of *PRDX1* expression on the proliferation of hepatoma cells. Knock-down *PRDX1* showed a significant inhibitory effect on cells proliferation. **F.** Quantification of the number of colonies. **G.** Western results showed the effect of knock-down of PRDX1 protein on caspase proteins in hepatoma cells. **H.** Quantification of the protein levels. The experiment was repeated three times. Data represent mean ± SEM. **; P<0.01, and ***; P<0.001.

PRDX1 knock-down induced apoptosis via Bax activation

PRDX1 mediated apoptosis in hepatoma cells was unknown. We wanted to explore effect of PRDX1 in hepatoma cells. We found that PRDX1 overexpression significantly increased Bcl-2 expression, while knock-down of this gene showed a significant reduction of Bcl-2 and enhanced Bax expression (Fig.3A), suggesting an apoptotic condition. Mitochondrial function is essential which is required for normal cellular metabolism. Mitochondrial fragmentation is associated with increased fission (16). Next, we assessed mitochondrial morphology using mitotracker, whether or not PRDX1 regulate mitochondrial morphology. We found that PRDX1 kncok-down significantly produced mitochondrial fragmentation (Fig.3B). All of these cellular events are closely associated with each other. These results indicated that higher expression of Bax may induce apoptosis via dysregulating mitochondrial membrane potenital and induced fragmentation. While increased mitochondrial fragmentation and expression of Bax suggested a favorable condition of cellular death. Taken together, these results implied that PRDX1 played a crucial role in inducing cell death of hepatoma cells via regulating Bcl-2 and Bax (Fig.3A).

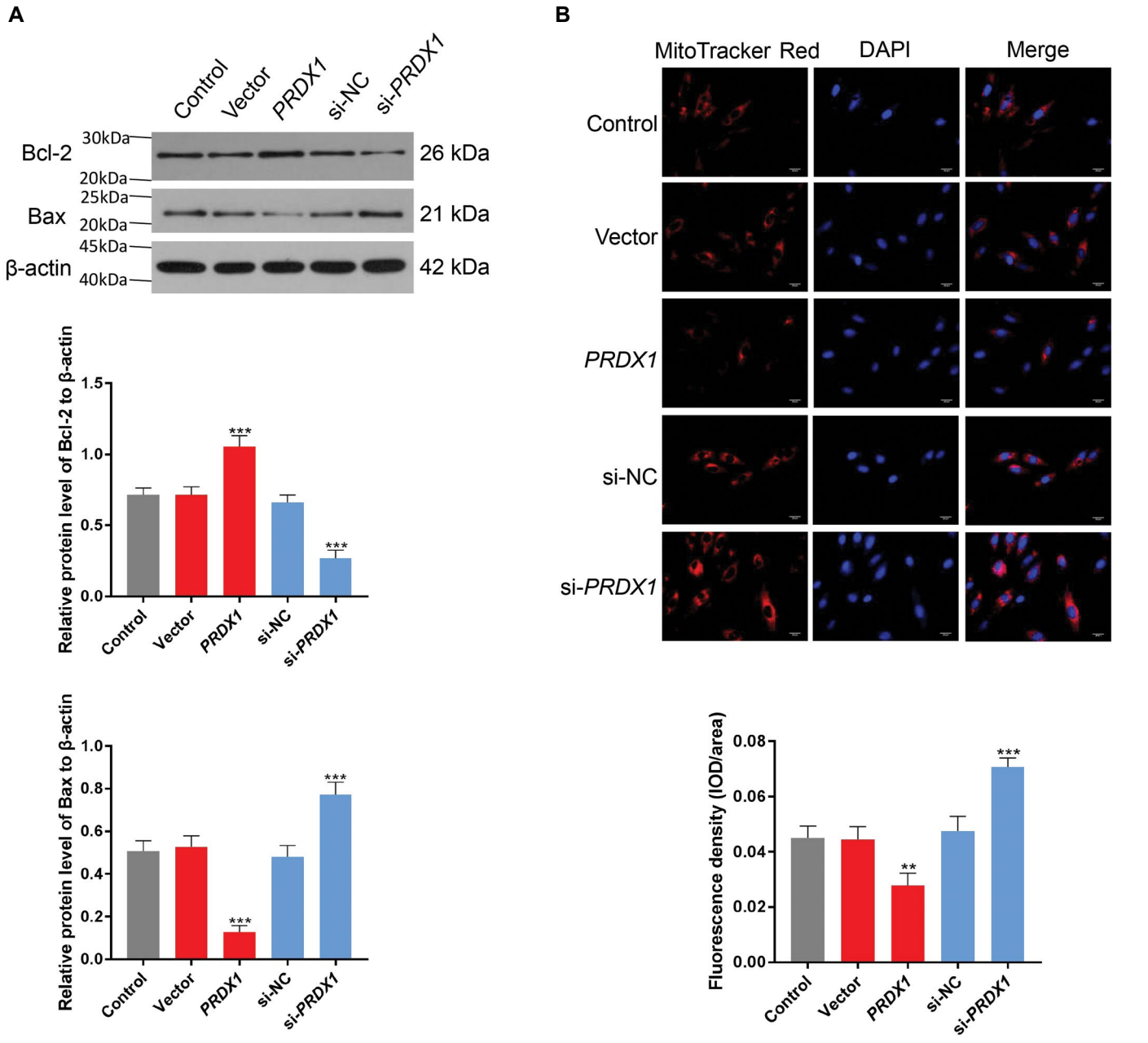

Fig.3: *PRDX1* knock-down reduced mitochondrial transmembrane potential in HCC cells. **A.** Western blot results depicted that PRDX1 knock-down substantially reduced expression of Bcl-2 and increased Bax expression. The lower bottom showed quantifications of protein levels. **B.** Mitotracker red assay was used to see the effect of si-PRDX1 expression on mitochondrial morphology of hepatoma cells. PRDX1 knock-down induced significantly increased filamentous mitochondrial morphology, assessed by fluorescent intensity. Representative graphs show quantifications. The experiment was repeated three times. Data represent mean ± SEM. **; P<0.01, and ***; P<0.001.

PRDX1 knock-down induces apoptosis via activation of mitochondrial fission

Next, we assessed expression level of proteins which are crucial for mitochondrial fission, whether or not accumulation of these proteins regulate mitochondrial fragmentation (Fig.3B). We found that silencing *PRDX1* greatly enhanced expression of Drp1, Fis1 and Dyn2 proteins, reflecting the abnormal function of mitochondria and initiation of apoptosis process. Inhibition of GTPase activity of Drp1 by dominant-negative protein (Drp1K38A) has been shown to delay cell death (17). They demonstrated that overexpression of hFis1 induced apoptosis, suggesting a role of mitochondrial fission in apoptosis. Next, to investigate effect of PRDX1 knock-down on mitochondrial fission machinery, we found that expression of Drp1, Fis1 and Dyn2 were significantly activated (Fig.4A), proposing mitochondrial fragmentation and apoptosis conditions. These results suggested that PRDX1 knock-down had a significant role in regulating critical molecules of mitochondrial fission and apoptosis.

Reduction of Bcl-2 in PRDX1 knocked-down cells (Fig.3B) reflected release of cytochrome c, followed by activation of downstream caspase signaling. Therefore, we checked expression of cytochrome c, Apaf-1 and BH3-only proteins (Bim) to confirm its correlation with PRDX1 knock-down (Fig.4B). Silencing *PRDX1* led to the loss of Bcl-2 and activated Bim protein, which further induced Bax protein activation. Bax further released cytochrome c from mitochondria for the induction of apoptotic proteins. Taken together, these data suggested that knock-down of PRDX1 facilitated mitochondrial fission and activated caspase proteins, i.e. release of cytochrome c, Bim and Apaf-1, to induce death of hepatoma cells.

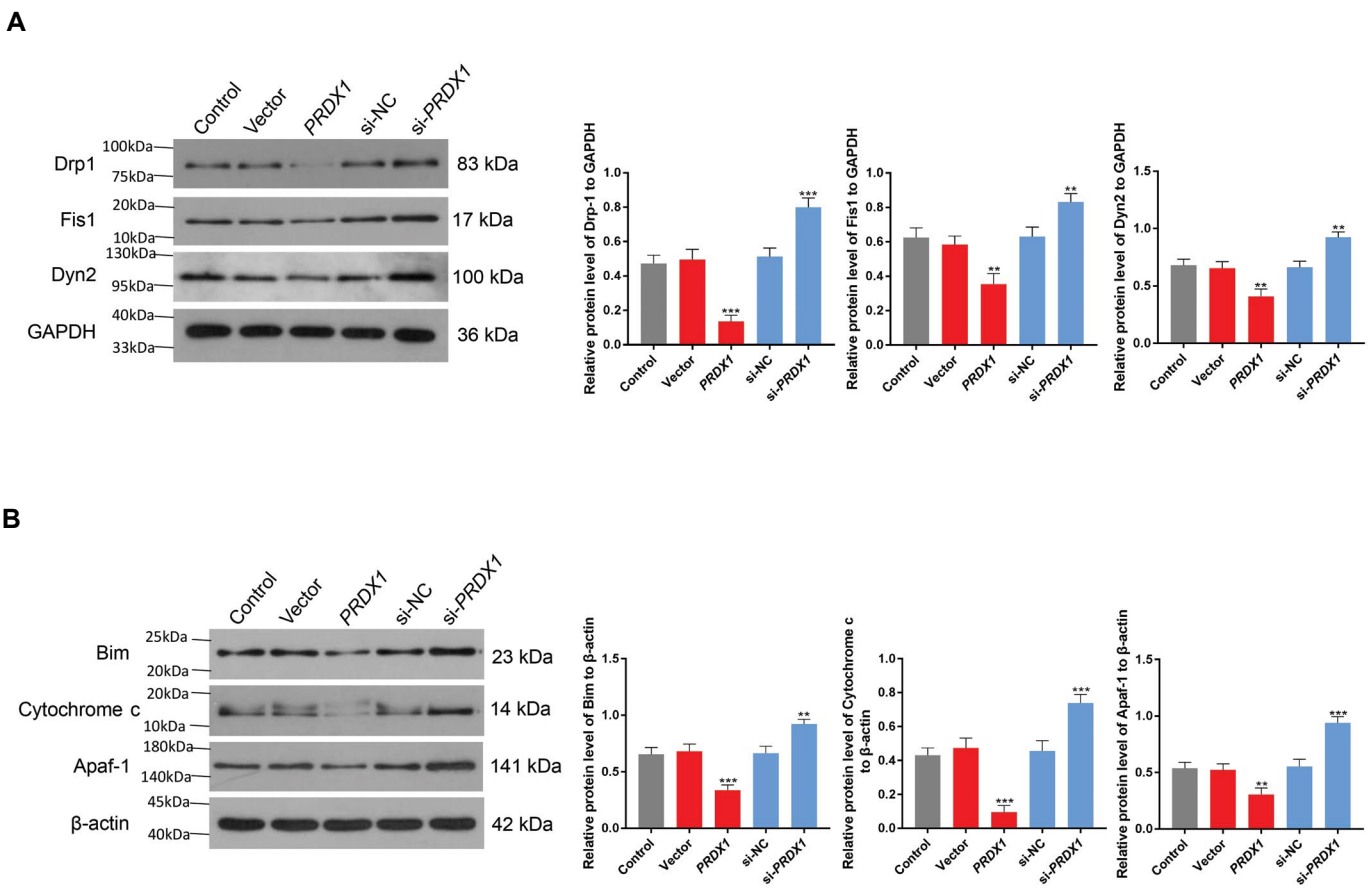

Fig.4: Effect of PRDX1 knock-down on apoptosis and mitochondrial fission machinery of HCC cells. **A.** Western results showed that knock-down of PRDX1 significantly enhanced expression of Drp1, Fis1 and Dyn2 in hepatoma cells. **B.** Images and graphs showed that overexpression of PRDX1 decreased apoptotic proteins (Bim, cytochrome C and Apaf-1) expression level, while knock-down of this gene significantly enhanced expression of caspase proteins. The right panel shows quantifications of protein levels. The experiment was repeated three times. Data represent mean ± SEM. **; P<0.01, and ***; P<0.001.

Discussion

In this study, we explored effect of silencing *PRDX1* in hepatoma cells. Several studies showed that *PRDX1* is upregulated in different types of cancer, including lung adenocarcinoma (19, 20), soft tissue sarcoma (2) and prostate cancer (14, 21). However, cellular role of *PRDX1* in liver cells remains to be understood. Sun et al. analyzed RNA sequences from the TCGA database and reported that *PRDX1* mRNA expression level was increased 1.3 fold in the malignant compared to the control tissues (15). We tested *PRDX1* expression and found an increased expression of *PRDX1* in different HCC cell lines, which is in line with the other studies and suggested a vital role in cellular proliferation. We found relatively higher *PRDX1* mRNA and protein expression levels in HepG2 cells.

There are studies which reported role of *PRDX1* in cellular proliferation. Gong et al. (22) demonstrated that *PRDX1* regulated proliferation of esophageal squamous cell carcinoma. Lu et al. (4) reported upregulation level of *PRDX1* in cervical cancer and found that *PRDX1* enhanced proliferation, migration and invasion by inhibiting apoptosis. Next, we were interested to explore association between *PRDX1* and hepatoma cells. We found that knock-down of *PRDX1* significantly decreased the IC50 value of hepatoma cells, suggesting that knock-down of *PRDX1* possessed great anti-tumor activity. We assessed proliferation effect by clonal formation assay and found that *PRDX1* overexpression significantly enhanced proliferation of the hepatoma compared to control cells, while knock-down of this gene showed significant inhibitory effects and reduced proliferation of cells, suggesting an important role of *PRDX1* silencing in hepatoma cells. Next, we were interested to explore molecular mechanism of cell death. We asked question whether silencing *PRDX1* is associated with caspase activation? We found that knock-down of *PRDX1* induced cell death via activation of active and cleaved caspase-3 and caspase-9 proteins, which further executed apoptotic process, leading to cell death.

PARP-1 has multiple functions, involved in DNA repair, cell death and transcriptions of some essential genes involved in inflammatory processes (6). It is a well-known substrate of caspase proteins (7, 23). Caspase-3 cleaves PARP-1 upon activation and prevents PARP-1 from repairing the damage (8, 24). Our results showed that knock-down of *PRDX1* increased PARP-1 expression, suggesting depletion of NAD^+ levels, thereby induced cellular death.

Bcl-2, as an essential protein, is a member of the Bcl-2 family, which act as a negative regulator of apoptosis. Moreover, Bcl-2 has shown to be protective and Bax up-regulation has pro-apoptotic role (25, 26). Several evidences reported that Bcl-2 regulate cytochrome c and therefore prevented activation of apoptotic genes (27, 28). Overactivation of Bcl-2 inhibited release of cytochrome c and initiation of apoptosis (29). Lu et al. (4) found that *PRDX1* overexpression increased Bcl-2 expression, while down-regulated Bax expression. This is in line with our findings, representing that *PRDX1* overexpression significantly increased Bcl-2 expression, while knock-down of this gene showed a significant reduction of Bcl-2 and enhanced Bax expression in hepatoma cells, suggesting a vital role of *PRDX1* knock-down in cancerous cells. Once cytochrome is released in the cytosol, cytosolic cytochrome c further mediates apoptosis-protease activating factor 1 (Apaf-1) to induce activation of the other caspase proteins (30). Researchers reported the role of Bax protein in facilitating release of cytochrome c from mitochondria to induce apoptosis process (31, 32). While, the other studies demonstrated that Bim protein directly activated Bax protein. This may suggest that BIM protein plays an indirect role by antagonizing Bcl-2 proteins, thereby allowing Bax activation to proceed (33, 34). In our study, we found that silencing *PRDX1* led to the loss of Bcl-2 and activated Bim protein which further induced activation of Bax protein. We showed that Bax further released cytochrome c from mitochondria to induce apoptotic proteins, suggesting a significant role of *PRDX1* knock-down in apoptosis. Researchers reported the role of *PRDX1* and found that overexpression of *PRDX1* enhanced Bcl-2 expression, while at the same time down-regulated Bax expression (4). Our findings showed that *PRDX1* overexpression significantly increased Bcl-2 expression, while knock-down of this gene enhanced Bax expression in hepatoma cells. This is consistent with studies previously reported in different cell lines.

Next, we asked question whether *PRDX1* has any role in regulating mitochondrial fission proteins of HCC cells. Drp1, Fis1 and Dyn2 played a role as fission mediators (35). The mitochondrial fission machinery played a vital role in mitochondrial function. Disruption in fission machinery led to the abnormal division of mitochondrial membrane. It was required to produced new mitochondria and maintained quality control of mitochondria (36). Lee et al. (37) found that down-regulation of Drp1 and Fis1 inhibited apoptosis. While in another study, James et al. showed that overexpression of hFis1 may induce apoptosis, suggesting a possible role of mitochondrial fission in apoptosis in yeast cells (18). There are other studies demonstrating the role of Drp1 and Fis1. Thus, they have been shown to inhibit mitochondrial fission and prevent apoptosis (38). In our findings, we analyzed that *PRDX1* knock-down significantly activated mitochondrial fission proteins (Drp1, Fis1 and Dyn2), which may suggest its role in inducing apoptosis. We also showed that mitochondrial fission, induced by silencing *PRDX1*, resulted in mitochondrial fragmentation. These results suggested a significant role of silencing *PRDX1* on mitochondrial fragmentation and fission associated proteins, thereby leading to apoptosis conditions.

Conclusion

A little attention has been given to study effect of *PRDX1* on live cancer via inhibiting mitochondrial apoptosis

pathway. We reported that *PRDX1* acted via Bcl-2 to inhibit cell death and apoptosis in hepatoma cells. Furthermore, silencing *PRDX1* simulated apoptosis pathways by activating Bax protein, which facilitated release of cytochrome c from the mitochondria followed by activation of the other related apoptotic proteins to induce cell death. Future studies targeting *PRDX1* inhibitors are required, which may act as a therapeutic candidate for the treatment of liver cancer.

Acknowledgments

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Authors' Contributions

H.h.S., X.l.J.; Contributed to conception and design. H.h.S.; Were responsible for overall supervision, and drafted the manuscript. H.h.S., X.l.J., Y.l.L., H.J., X.h.Y.; Contributed to all experimental works, data and statistical analyses and interpretation of data. All authors read and approved the final manuscript.

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