Life Sciences 216 (2019) 200–206

Contents lists available at ScienceDirect

Life Sciences

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

Histone deacetylase 2 regulates the doxorubicin (Dox) resistance of hepatocarcinoma cells and transcription of *ABCB1*

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ARTICLE INFO

Keywords:
HDAC2
HCC
ABCB1
c-Fos
Doxorubicin

ABSTRACT

Histone deacetylases (HDACs) can regulate cell-cycle, differentiation, and apoptosis of hepatocarcinoma (HCC) cells, while their roles in drug sensitivity remain unclear. Our results showed that the expression of HDAC2 was significantly increased in HCC doxorubicin (Dox) resistant cells as compared with their corresponding control cells. Over expression of HDAC2 can increase the cell viability and decrease the Dox sensitivity. Kaplan-Meier Plotter assay revealed that HCC patients with higher levels of HDAC2 had significantly poor prognosis than that of the lower expression patients. Mechanistically studies revealed that HDAC2 can regulate the transcription of *ABCB1* via directly binding with its promoter and increasing its expression in Dox resistant HCC cells. Knockdown of HDAC2 significantly inhibited the expression of ABCB1. Co-immunoprecipitation revealed that HDAC2 can bind with c-fos, an important transcription factor of ABCB1, in HCC/Dox cells. Knockdown of c-Fos decreased the binding between HDAC2 and promoter of ABCB1 in HCC/Dox cells. Collectively, our data revealed that HDAC2 can regulate Dox sensitivity of HCC cells and the transcription of ABCB1.

1. Introduction

As the fifth most common malignancy worldwide, the incidence of hepatocellular carcinoma (HCC) is increasing in the East Asia and western countries [28]. The incidence of HCC in the United States has increased by more than 90% in the past 3 decades [5]. For early-stage HCC, liver resection, transplantation, and local ablation have high efficiency. For patients with advanced HCC and patients who are not candidates for curative surgery, systematic chemotherapy is an important approach of clinical treatment [6]. However, the chemoresistance has limited the application and therapy efficiency of chemotherapy for the purpose of HCC treatment. Therefore, the understanding of cellular and molecular mechanism responsible for chemoresistance will be great helpful for developing effective chemotherapeutic agents.

Doxorubicin is one of the most widely used anti-HCC drug for chemotherapy. As an anthracycline-based agent, it works in part by interfering with the function of DNA and thereby suppressing various of cancer cells such as HCC, testicular, ovarian, and lung cancers [1,31]. Currently, the transarterial chemoembolization (TACE) by use of Dox has been considered as the standard treatment for patients with intermediate HCC [7]. However, the extensive and long-standing clinical utilization of Dox will lead the clinical chemotherapy failure due to cancer cell resistance to Dox, which also causes recurrence and a poor prognosis of HCC patients.

Since chemoresistance is a multifactorial phenomenon, there are many underlying mechanisms involved in drug resistance such as DNA methylation, gene mutation, drug metabolism alteration, and expression variation of drug resistant proteins [14]. It was reported that increased expression of MDR gene and other transporter genes and/or proteins such as multidrug resistance protein (MRP) are involved in Dox resistance [3,4]. P-glycoprotein (P-gp), a member of ATP-binding cassette (ABC) superfamily of membrane transporter encoded by human *ABCB1* gene [18], has been suggested to be involved in Dox resistance of HCC cells. More specifically, the human P-gp expression has been found in most of human HCC tissues [24]. It has been reported that signalling pathways involved in ABCB1/Pgp-mediated MDR included the mitogen-activated protein kinase (MAPK), c-Jun NH2-terminal kinase (JNK), p38, cyclic adenosine monophosphate dependent protein kinase, phosphatidylino sitol 3-kinase and protein kinase C signalling pathways. Further, the transcription factors such as NF-κB, PTEN, and SP1 can regulate the transcription of ABCB1 [26]. However, the detailed mechanisms for the upregulation of P-gp in HCC have not been well illustrated.

Histone acetylation has been revealed as an important factor for the regulation of cancer progression. Histone deacetylases (HDACs), which

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https://doi.org/10.1016/j.lfs.2018.11.043
Received 24 July 2018; Received in revised form 10 November 2018; Accepted 19 November 2018
Available online 20 November 2018
0024-3205/

can remove the acetyl groups from hyperacetylated histones, can regulate the HCC tumorigenesis and progression [19,20]. Recently, studies suggested that HDACs are involved in the chemoresistance of cancer cells [13,25]. We checked the expression of class I HDACs (HDAC 1, 2, 3, and 8) in Dox resistant HCC cells. Our data showed that HDAC2 was significantly increased in Dox resistant cells, while silencing of HDAC2 can rescue the Dox sensitivity. Mechanistically, HDAC2 can regulate the *ABCB1* promoter activities via regulating the phosphorylation of c-fos.

2. Materials and methods

2.1. Cell lines and cell culture

Human hepatocarcinoma cell lines (HepG2, Huh7) were purchased from the Shanghai Institute for Biological Science (Shanghai, China). To generate Dox resistant HCC cells, HepG2 and Huh7 cells were cultured in starting non-toxic dose of 50 nM Dox. After continuous culture in medium containing stepwise increasing concentration of Dox at a range of 0.5–25 μM over a period of 6 months, the Dox-resistant cells were named as HepG2/Dox and Huh7/Dox respectively. The Dox resistant HCC cells were cultured in medium containing 2 μM Dox to maintain the chemoresistance.

2.2. Cell viability assay

The Cell Counting Kit-8 (CCK-8; Dojindo; Kumamoto, Japan) was used to test the cell viability according to the manufacturer's instructions. Briefly, cells were plated into 96-well plates (3000 cells/well; 100 μl medium) and treated as indicated. The medium was removed and replaced with 100 μl media containing 10 μl CCK-8 solution for 2 h. Absorbance was then measured at 450 nm using an MRX II microplate reader (Dynex, Chantilly, VA, USA).

2.3. Western blot assay

After treatment, cells were washed with ice-cold PBS and lysed by use of cell lysis buffer (Cell Signalling; Danvers, MA, USA). BCA Protein assay kit (Thermo Fisher Scientific Inc.; Rockford, IL, USA) was used to measure the protein concentration in cell lysis. After denatured by boiling for 10 min, 20 μg of proteins were separated by use of 10% SDS-PAGE gels, and then transferred to polyvinylidene fluoride (PVDF) membranes (Millipore; Billerica, MA, USA). The membrane was blocked by us of 5% milk in PBST and incubated with primary antibody at 4 °C overnight. Then appropriate HRP-conjugated secondary antibody was further incubated for 2 h before visualized using an autoradiography kit (Kodak; Rochester, NY, USA). GAPDH was used as the loading control for the whole cell lysis.

2.4. Real-time reverse transcription-polymerase chain reaction (RT-PCR)

Total RNAs were extracted by use of Trizol Reagent (Invitrogen; Carlsbad, CA, USA). The concentration of RNA was measured by use of Nanodrop before reverse transcribed using Prime Script Reagent RT Kit (Takara Biotechnology Co.; Dalian, China) following the manufacturer's protocol. The PCR primers were designed and purchased from Takara. The primer sequences are as follows: *HDAC1*, forward: 5-TCC AAC ATG ACC AAC CAG AA-3; reverse: 5-TTG TCA GGG TCC TCC TCA TC-3′; *HDAC2*, forward: 5-TGG AGG AGG CTA CAC AAT CC-3; reverse: 5-TTT GAA CAC CAG GTG CAT GT-3′; *HDAC3*, forward: 5-CTC CCC TTT CCC TCA AAC TC-3; reverse: 5-TTG CAT GGA AGG AGG AAC TG-3′; *HDAC8*, forward: 5-AGG TGA TGA GGA CCA TCC AG-3; reverse: 5-ACC CTC CAG ACC AGT TGA TG-3′; *ABCB1*, forward: 5-CCC ATC ATT GCA ATA GCA GG-3; reverse: 5-TGT TCA AAC TTC TGC TCC TGA-3′; *ABCC1*, forward: 5-ATG TCA CGT GGA ATA CCA GC-3; reverse: 5-GAA GAC TGA ACT CCC TTC CT-3′; and *ABCG2*, forward: 5-AGA TGG GTT TCC AAG CGT TCA T-3; reverse: 5-CCA GTC CCA GTA CGA CTG TGA CA-3′. RT-PCR was performed using ABI 7900 Prism HT (Applied Biosystems Inc.; Shanghai, China) followed by melting curve analysis. The mRNA expression was normalized to β-actin (forward: 5-TGGCACCCAGCACA ATGAA-3; reverse: 5-CTAAGTCATAGTCCGCCTAGAAGCA-3).

To investigate the half-life of mRNA, cells were pre-transfected with si-NC or si-HDAC2 for 24 h and further treated with 5 μg/ml actinomycin D (Act-D) as previously described [2]. Samples were collected at the indicated time periods and the mRNA of *ABCB1* was checked by use of qRT-PCR.

2.5. HDAC2 expression analysis

Kaplan-Meier survival curves of RFS based on HDAC mRNA expression in 364 HCC patients according to the previous study [27]. Patients were assigned to two subgroups according to the median expression of HDAC2. The log-rank test was used to compare differences between two groups.

2.6. Cell transfection

For transfection, cells were seeded into 6-well plates in 2 ml of growth medium. The pcDNA (vector) and pcDNA/HDAC2 were transfected by use of Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. To knockdown of HDAC2 or c-fos, cells were transfected with the negative control siRNA (si-NC, Guangzhou RiboBio Co., Ltd., Guangzhou, China) and siRNAs by use of lipofectamine RNAimax (Life Technologies) according to the manufacturer's protocol.

2.7. Drug efflux assay

The intracellular amount of Dox was quantitatively determined by flow cytometry (Beckman Coulter, U.S.A.) according to the previous study [30]. Briefly, HCC/Dox cells were transfected with si-NC or si-HDAC2 for 24 h and then treated with 5 μM Dox for an additional 1 h in darkness at 37 °C, respectively. Then cells were washed and collected for analysis by use of flow cytometry (Beckman Coulter, U.S.A.).

2.8. Promoter activity assay

The promoter activity of ABCB1 in HCC cells were tested according to the previous study [32]. Briefly, cells were transfected with *ABCB1* promoter luciferase reporter construct pTL-MDR1 (Affymetrix) and pBABE-puro plasmid by use of Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's instructions. After treatment as indicated, the luciferase activity (relative luciferase units, RLU) was normalized to protein amount as determined by the BCA assay (ThermoFisher, Waltham, MA).

2.9. Chromatin immunoprecipitation (ChIP)

The binding between HDAC2 and promoter of ABCB1 was measured by use of Chromatin Immunoprecipitation (ChIP) Assay Kit (Millipore Corporation). Cells were crosslinked by 1% formaldehyde and sonicated for 30 min. HDAC2 was immunoprecipitated overnight at 4 °C. The bound ABCB1 was amplified by use of the following primer: forward: 5′-CAA CTC GTC AAA GGA ATT AT-3′ and reverse: 5′-TTG TAC CTT TGA TCA ACA CC-3′.

2.10. Co-immunoprecipitation (Co-IP)

Cells were lysed by use of NP40 lysis buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1% NP40, 1 mM ethylenediaminetetraacetic acid (EDTA), 10 mM sodium butyrate). The cell lysis was incubated with anti-HDAC2 at 4 °C overnight. After washed with lysis buffer for three times, the immunoprecipitated complexes were resolved by sodium

dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with the indicated antibodies.

2.11. Statistical analyses

The data were presented as the mean and standard deviation (SD). Statistical analysis was performed using Prism5 (Version 5.0; GraphPad; SanDiego, CA, USA). Data were analyzed by two-tailed unpaired Student's *t*-test between two groups and by One-Way ANOVA followed by Bonferroni test for multiple comparison. Statistical significance was defined as a p-value < 0.05.

3. Results

3.1. HDAC2 is upregulated in HCC/Dox cells and essential for Dox resistance

In order to test whether HDACs are involved in the drug resistance of HCC cells, we measured the expression of class I HDAC including HDAC1, 2, 3, 8 in HepG2/Dox, Huh7/Dox and their corresponding parental cells by qRT-PCR. Our data showed that mRNA of both HDAC 1 and 2 were upregulated in HepG2/Dox as compared with HepG2 cells (Fig. 1A). Only upregulation of HDAC2 was observed in Huh7/Dox cells as compared with Huh7 cells (Fig. 1B). Western blot analysis showed that HDAC2, while not HDAC1, was over expressed in both HepG2/Dox and Huh7/Dox when compared to their corresponding parental cells (Fig. 1C). To investigate the roles of HDAC2 in Dox resistance, we knocked down the expression of HDAC2 in both HepG2/Dox and Huh7/Dox cells (Fig. 1D). Cell viability assay showed that si-HDAC2 can significantly rescue the Dox resistance of both HepG2/Dox (Fig. 1E) and Huh7/Dox cells (Fig. 1F). Collectively, these data confirmed that HDAC2 is upregulated in HCC/Dox cells and essential for Dox resistance.

3.2. HDAC2 triggers the progression of HCC

Kaplan-Meier Plotter assay revealed that HCC patients with higher levels of HDAC2 had significantly poor prognosis than that of the lower expression patients (Fig. 2A). We over expressed HDAC2 cells in HepG2 and Huh7 cells (Fig. 2B). Our data showed that over expression of HDAC2 can trigger the cell viability of both HepG2 and Huh7 cells (Fig. 2C). In addition, over expression of HDAC2 can abolish the suppression effects of Dox on the cell viability of both HepG2 and Huh7 cells (Fig. 2C). These results showed that HDAC2 triggers the progression of HCC.

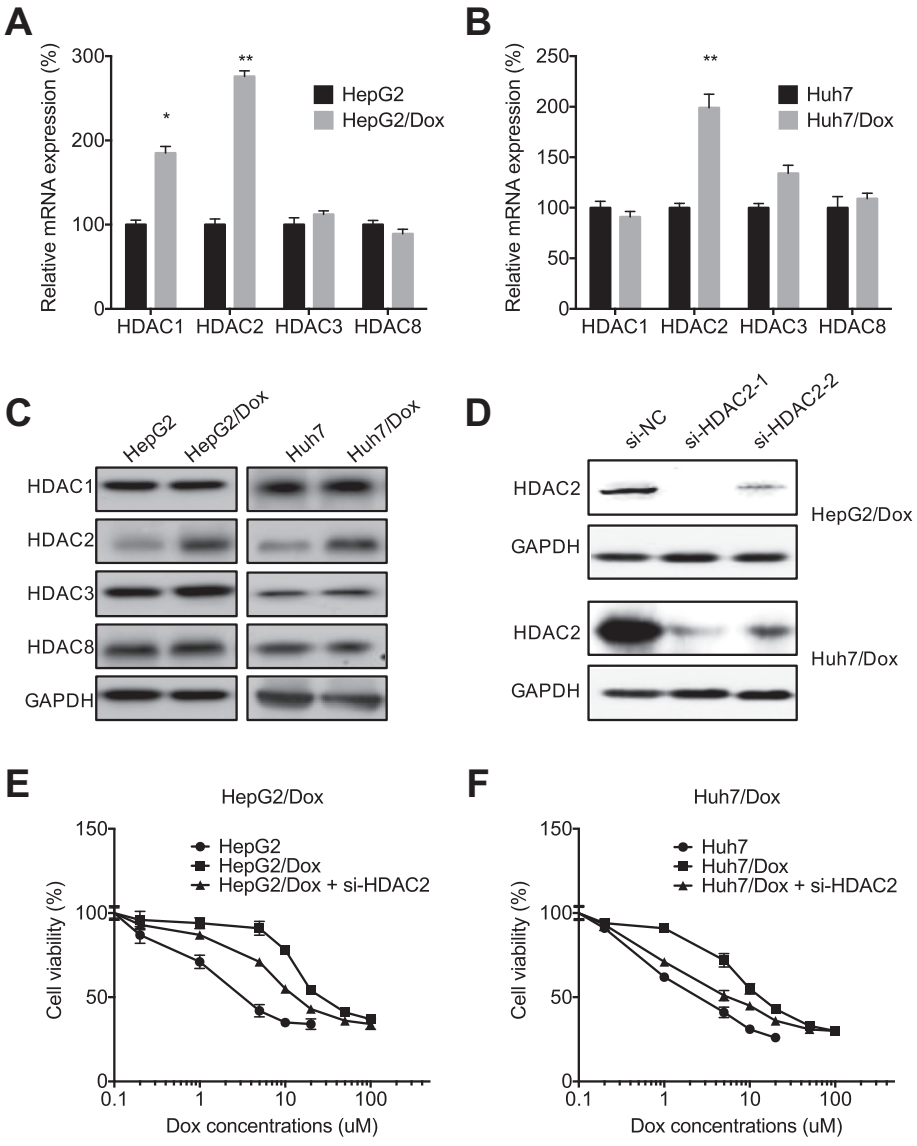

Fig. 1. HDAC2 is upregulated in HCC/Dox cells and essential for Dox resistance. The mRNA of class I HDACs (HDAC3, 2, 3, 8) were detected in HepG2/Dox (A), Huh7/Dox (B) and their corresponding parental cells by qRT-PCR. (C) The protein expression of class I HDACs (HDAC1, 2, 3, 8) was detected in HepG2/Dox, Huh7/Dox and their corresponding parental cells by western blot analysis; (D) Both HepG2/Dox and Huh7/Dox cells were transfected with si-NC or si-HDAC2 for 24 h. si-HDAC2-1 was used for further studies; After transfected with or without si-HDAC2 for 12 h, HepG2/Dox (E) or Huh7/Dox (F) cells were treated with increasing concentrations of Dox for another 48 h, the cell viability was measured by CCK-8 kit. Data were presented as means ± SD of three independent experiments. *$p < 0.05$; **$p < 0.01$.

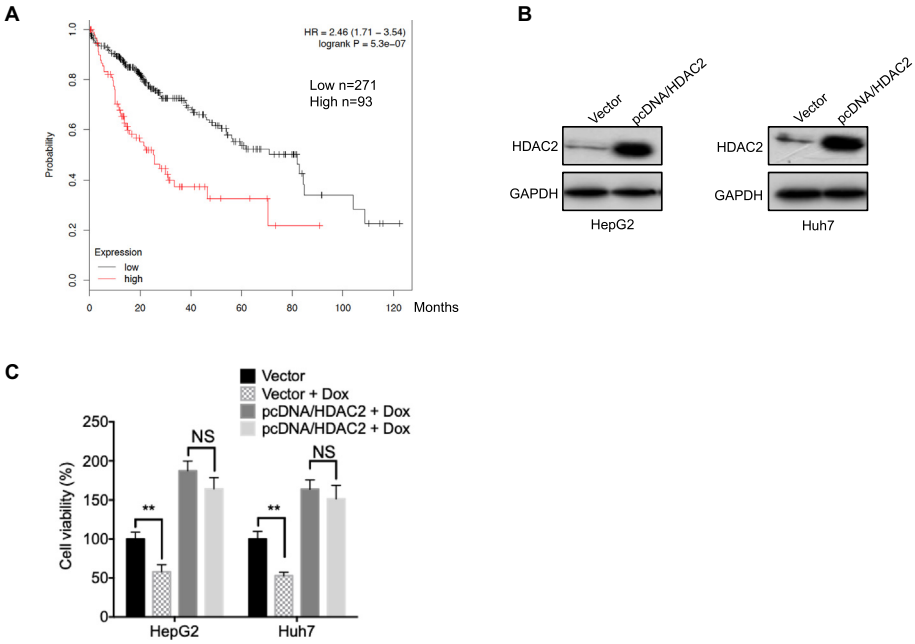

Fig. 2. HDAC2 triggers the progression of HCC. (A) Kaplan-Meier survival curves of overall survival rate based on HDAC2 mRNA expression in 364 HCC patients. Patients were assigned to two subgroups according to the median expression of HDAC2. The log-rank test was used to compare differences between two groups; (B) HepG2 and Huh7 cells were transfected with pcDNA (Vector) or pcDNA/HDAC2 for 24 h; (C) Cell were transfected with pcDNA (Vector) or pcDNA/HDAC2 for 24 h and then further treated with or without 2 μM Dox for 48 h, the cell viability was tested by use of CCK-8 kit. Data were presented as means ± SD of three independent experiments. $^{**}p < 0.01$, NS, no significant.

3.3. HDAC2 regulates the expression and function of P-gp in HCC/Dox cells

It was reported that candidate transporters responsible for doxorubicin efflux include *ABCB1*, *ABCG2*, and *ABCC1* [23,29]. Over expression of *ABCB1* and *ABCG2*, while not *ABCC1*, was observed in HepG2/Dox cells as compared with the HepG2 cells (Fig. 3A). Knockdown the expression of HDAC2 decreased the protein levels of P-gp (ABCB1 protein), while not BCRP (ABCG2 protein), in both HepG2/Dox and Huh7/Dox cells (Fig. 3B). The doxorubicin efflux function in HepG2/Dox cells transfected with si-HDAC2 was investigated. Our data showed that knockdown of HDAC2 can impair the Dox efflux functions in both HepG2/Dox and Huh7/Dox cells (Fig. 3C). Furthermore, knockdown of HDAC2 can also decrease the mRNA expression of ABCB1 in both HepG2/Dox and Huh7/Dox cells (Fig. 3D). Taken together, these results suggested that HDAC2 regulates the expression and function of P-gp in HCC/Dox cells.

3.4. HDAC2 triggers the transcription of ABCB1

Considering that knockdown of HDAC2 can impair the mRNA expression of ABCB1, we firstly tested whether HDAC2 can affect the mRNA stability of ABCB1. Our data showed that si-HDAC2 had no

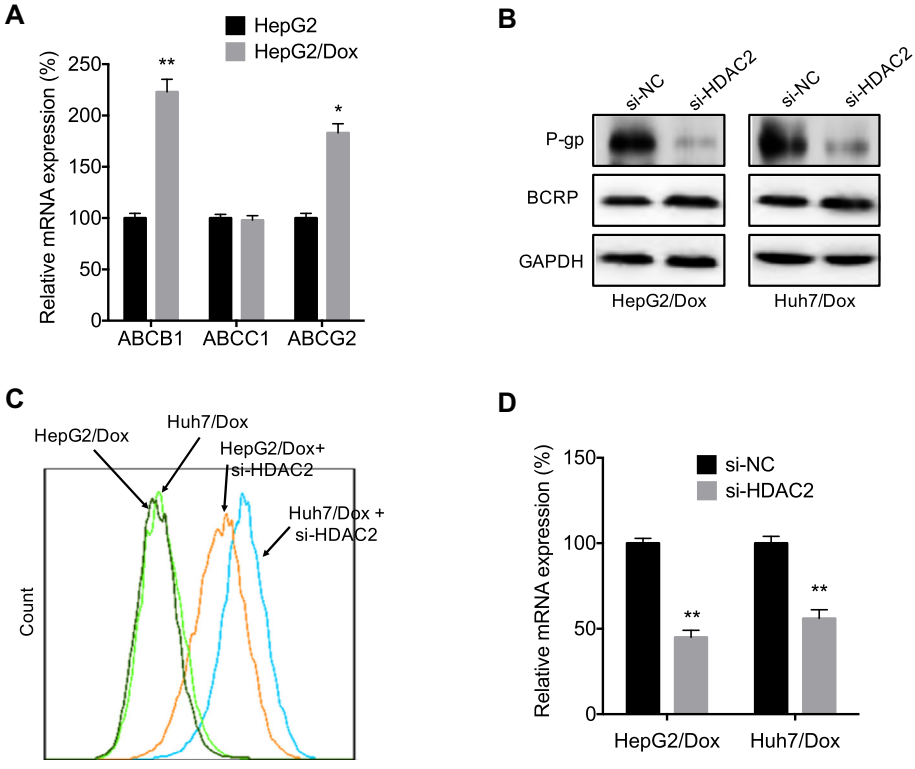

Fig. 3. HDAC2 regulates the expression and function of P-gp in HCC/Dox cells. (A) The relative mRNA expression of ABCB1, ABCC1, ABCG2 in HepG2/Dox and HepG2 cells; (B) Cells were transfected with si-NC or si-HDAC2 for 48 h, the expression of P-gp and BCRP was measured by western blot analysis; (C) HepG2/Dox and Huh7/Dox cells were transfected with siNC or si-HDAC2 for 24 h and then further incubated with 5 μM Dox for 1 h, the intracellular fluorescence was analyzed by flow cytometry; (D) The mRNA expression of ABCB1 in HepG2/Dox and Huh7/Dox cells transfected with siNC or si-HDAC2 for 24 h. Data were presented as means ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$.

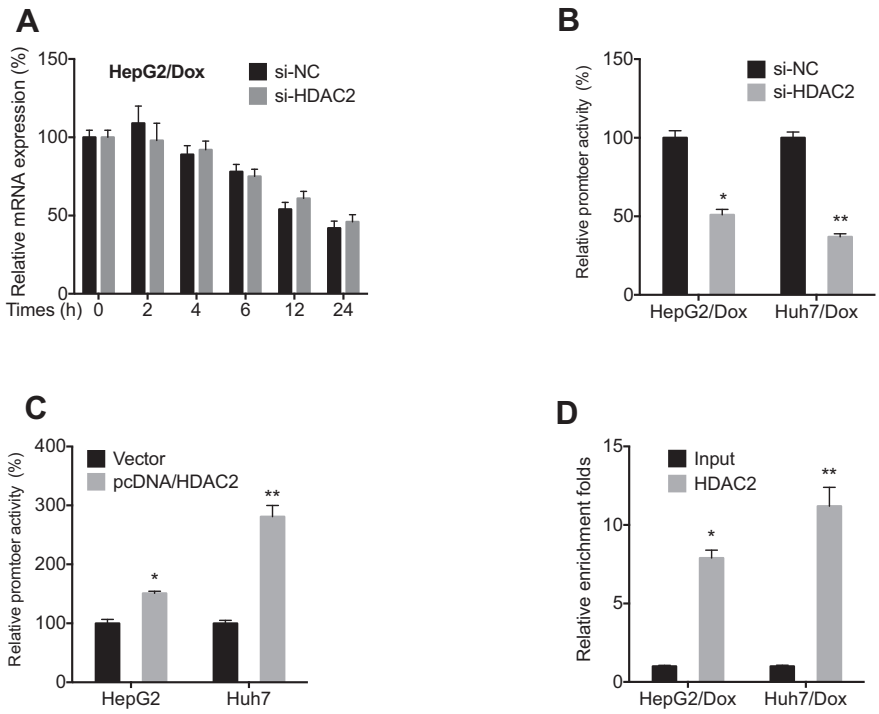

Fig. 4. HDAC2 triggers the transcription of ABCB1. (A) HepG2/Dox cells were pre-transfected with si-NC or si-HDAC2 for 24 h and then further treated with actinomycin D (Act-D, 5 μg/ml). After incubated for the indicated times, the mRNA expression of ABCB1 was measured by qRT-PCR; (B) HepG2/Dox and Huh7/Dox cells were transfected with siNC or si-HDAC2 for 24 h, the promoter activities of ABCB1 were measured by dual luciferase assay; (C) HepG2 and Huh7 cells were transfected with vector or pcDNA/HDAC2 for 24 h, the promoter activities of ABCB1 were measured by dual luciferase assay; (D) The binding between HDAC2 and ABCB1 promoter was measured by ChIP assay. Data were presented as means ± SD of three independent experiments. $^{**}p < 0.01$, $^{*}p < 0.05$.

effect on the mRNA stability of ABCB1 in HepG2/Dox cells (Fig. 4A). We then tested the promoter activity of ABCB1 in HCC/Dox cells transfected with si-HDAC2. The data showed that si-HDAC2 can decrease the promoter activity of ABCB1 in both HepG2/Dox and Huh7/Dox cells (Fig. 4B). Inversely, over expression of HDAC2 in HepG2 and Huh7 cells can increase the promoter activity of ABCB1 (Fig. 4C). To verify whether HDAC2 can directly regulate the transcription of ABCB1 in HCC cells, we used ChIP assay to test the binding between HDAC2 and promoter of ABCB1 in HCC/Dox cells. The data showed that HDAC2 can directly bind with the promoter of ABCB1 in both HepG2/Dox and Huh7/Dox cells (Fig. 4D). The results indicated that HDAC2 triggers the transcription of ABCB1.

3.5. HDAC2 regulates the transcription of ABCB1 via binding with c-fos

Considering that HDAC2 may intact the transcription factors and then regulate the transcription of ABCB1, the binding between HDAC2 with c-fos, p65, and PTEN was investigated in HCC/Dox cells [26]. Our data showed that the binding between HDAC2 with c-fos, while not p65 or PTEN, was significantly increased in HepG2/Dox cells as compared with that in HepG2 cells (Fig. 5A). Similar results were also observed in Huh7/Dox cells (Fig. 5B). Knockdown of HDAC2 can decrease the expression of c-fos, while had no effect on the relative level of p-c-fos/c-fos, in both HepG2/Dox and Huh7/Dox cells (Fig. 5C). Knockdown of HDAC2 can impair the nuclear translocation of c-fos in both HepG2/Dox (Fig. 5D) and Huh7/Dox (Fig. 5E) cells. We then knocked down the expression of c-Fos in HCC/Dox cells by use of siRNA (Fig. 5F). Our data showed that knockdown of c-Fos can suppress the mRNA expression of ABCB1 in both HepG2/Dox and Huh7/Dox cells (Fig. 5G). Furthermore, the knockdown of c-Fos can decrease the binding between HDAC2 and promoter of ABCB1 in both HepG2/Dox and Huh7/Dox cells (Fig. 5H). These data suggested that HDAC2 regulates the transcription of ABCB1 via binding with c-fos.

3.6. HDAC2/c-fos complex regulates the Dox resistance of HCC cells

We further evaluated the nuclear translocation of HDAC2 and c-fos in HCC/Dox cells. Our data showed that the nuclear accumulation of HDAC2 and c-fos were upregulated in both HepG2/Dox (Fig. 6A) and Huh7/Dox (Fig. 6B) cells compared with their corresponding control cells. In addition, silencing of HDAC2 and c-fos together can synergistically increase the Dox sensitivity of HepG2/Dox cells (Fig. 6C). These results indicated that HDAC2/c-fos complex regulates the Dox resistance of HCC cells.

4. Discussion

The roles of HDACs on the Dox resistance of HCC cells were not well illustrated. Our present study revealed that among class I HDACs, HDAC2 was over expressed in HCC/Dox cells. The increased expression of HDAC2 was correlated with worse prognosis of HCC patients. Over expression of HDAC2 can trigger the cell viability of HCC cells, while silencing of HDAC2 increased the Dox sensitivity of HCC/Dox cells. HDAC2 can regulate the transcription of ABCB1 via directly binding to its promoter. It can bind with c-fos and then increase the promoter activity of ABCB1. Silencing of HDAC2/c-fos can rescue the Dox sensitivity of HCC/Dox cells. Collectively, our present study suggested that HDAC2 can positively regulate the Dox resistance of HCC cells and modulate ABCB1 transcription.

Our present study revealed that HDAC2 can positively regulate the Dox resistance of HCC cells and trigger the progression of HCC. Recent study revealed that the expression of HDAC2 in HCC cancer cells was significantly greater than that in their corresponding normal tissues, furthermore, high HDAC2 expression was associated with poor survival in low-grade and early-stage tumors ($p < 0.05$) [12]. Increased cancer-specific mortality of HCC patient was significantly associated with HDAC2 expression [12]. Cellular studies indicated that HDAC2 regulated cell cycle and that disruption of HDAC2 caused G1/S arrest in cell cycle of HCC cells [17], while targeted inhibition of HDAC2 was sufficient to blunt the growth of human HCC in a murine xenograft model by upregulation of p27 [11]. The HDAC inhibitor panobinostat has synergistic effects with Dox in acute myeloid leukemia to control DNA repair [15]. For breast cancer, HDAC2 overexpression is a poor prognostic factor of patients with increased multidrug resistance-associated protein expression who received anthracyclines therapy [33]. Our results, together with published data, suggested that HDAC2 might be a

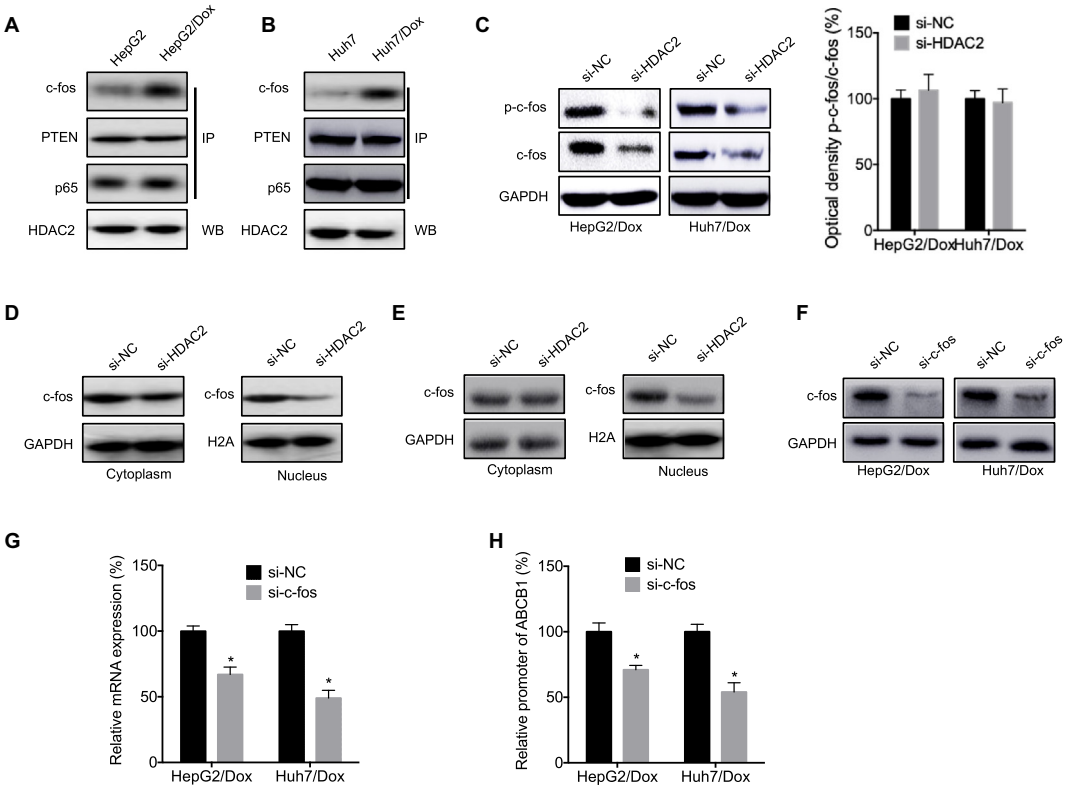

Fig. 5. HDAC2 regulates the transcription of ABCB1 via binding with c-fos. (A) HDAC2 in HepG2 and HepG2/Dox cells were pulled down by its antibody, the binding with c-fos, PTEN, and p65 was measured by western blot analysis; (B) HDAC2 in Huh7 and Huh7/Dox cells were pulled down, its binding with c-fos, PTEN, or p65 was measured by western blot analysis; (C) HepG2/Dox or Huh7/Dox cells were transfected with si-NC or si-HDAC2 for 24 h, the expression of p-c-fos and c-fos was measured by western blot analysis (left), the relative levels of p-c-fos/c-fos were quantitatively analyzed (right); HepG2/Dox (D) or Huh7/Dox (E) cells were transfected with si-NC or si-HDAC2 for 24 h, the expression of c-fos in cytoplasm and nuclear fractions of cells were measured by western blot analysis; HepG2/Dox and Huh7/Dox cells were transfected with si-NC or si-c-fos for 24 h, the c-fos was measured by western blot analysis (F), the mRNA of ABCB1 was measured by qRT-PCR (G); (H) Promoter activity of ABCB1 in HepG2/Dox or Huh7/Dox cells transfected with si-NC or si-c-fos for 24 h. Data were presented as means ± SD of three independent experiments. $^{**}p < 0.01$, $^{*}p < 0.05$.

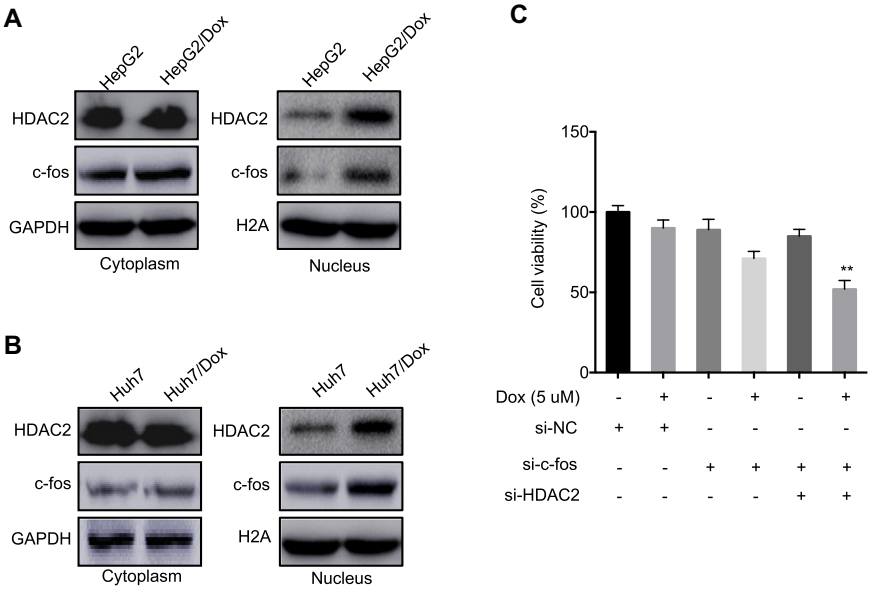

Fig. 6. HDAC2/c-fos complex regulates the Dox resistance of HCC cells. The subcellular distribution of HDAC2 and c-fos in HepG2/Dox (A), Huh7/Dox (B) and their corresponding control cells were measured by western blot analysis; (C) HepG2/Dox cells were transfected with si-NC, si-c-fos, si-HDAC2 alone or together for 6 h, then cells were further treated with 5 μM Dox for 48 h. The cell viability was tested by CCK-8 kit. Data were presented as means ± SD of three independent experiments. $^{**}p < 0.01$, $^{*}p < 0.05$.

potential important target for drug development and overcome of chemoresistance for HCC patients.

We also revealed that HDAC2 can bind with c-fos and then directly regulate the transcription of ABCB1 in HCC/Dox cells. Previously, it was reported that CAGE confers drug resistance by regulating expression of p53 through HDAC2 in human hepatic cancer cell line SNU387 [10]. Similarly, class I HDAC inhibitor mocetinostat can reverse the ZEB1 associated drug resistance in cancer cells [16]. Further, HDAC2 can regulate the Dox resistance of colorectal cancer (CRC) cells via targeting ABCB1 transcription [32]. It was reported that the

expression of ABCB1 can be regulated by mRNA turnover and translational regulation [9]. We found that HDAC2 had no effect on mRNA stability of ABCB1, while it can positively regulate its promoter activity in HCC/Dox cells. Similarly, knockdown of HDAC2 can decrease the phosphorylation of c-fos and c-Jun, which can directly interact with the *ABCB1* promoter and then promote its transcription [8,22]. It was reported that p65 is involved in HDAC2 regulated Dox resistance of CRC cells [32]. However, our data showed that there is no variation for the binding between HDAC2 and p65 in HCC/Dox and their parental cells, which suggested that the mechanism of HDAC2 regulated Dox resistance might be cell line dependent.

Collectively, our present study revealed that HDAC2 can bind with c-fos and then positively regulate the transcription of ABCB1 in HCC/Dox cells. It suggested that HDAC2 might be an important target for HCC therapy. In addition, the combination of HDAC2 specific inhibitor and anticancer drugs including Dox might be an efficiency approach to elevate the treatment outcome of HCC.

Conflict of interest

The authors declare no conflict of interest.

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