Original Research

Hepatitis B X-interacting protein promotes cisplatin resistance and regulates CD147 via Sp1 in ovarian cancer

Wei Zou*, Xiangdong Ma*, Hong Yang, Wei Hua, Biliang Chen and Guoqing Cai

Department of Obstetrics and Gynecology, Xijing Hospital, the Fourth Military Medical University, Xi'an 710032, P.R. China
*Wei Zou and Xiangdong Ma contributed equally to the work
Corresponding author: Guoqing Cai. Email: guoqingcaixj@163.com

Impact statement

We found that hepatitis B X-interacting protein (HBXIP) was able to activate the CD147 promoter through Sp1. *In vivo*, depletion of HBXIP decreased the tumor volume and weight induced by CP. Taken together, these results indicate that HBXIP promotes cisplatin resistance and regulated CD147 via Sp1 in ovarian cancer cell lines.

Abstract

Ovarian cancer is the highest mortality rate of all female reproductive malignancies. Drug resistance is a major cause of treatment failure in malignant tumors. Hepatitis B X-interacting protein acts as an oncoprotein, regulates cell proliferation, and migration in breast cancer. We aimed to investigate the effects and mechanisms of hepatitis B X-interacting protein on resistance to cisplatin in human ovarian cancer cell lines. The mRNA and protein levels of hepatitis B X-interacting protein were detected using RT-PCR and Western blotting in cisplatin-resistant and cisplatin-sensitive tissues, cisplatin-resistant cell lines A2780/CP and SKOV3/CP, and cisplatin-sensitive cell lines A2780 and SKOV3. Cell viability and apoptosis were measured to evaluate cellular sensitivity to cisplatin in A2780/CP cells. Luciferase reporter gene assay was used to determine the relationship between hepatitis B X-interacting protein and CD147. The *in vivo* function of hepatitis B X-interacting protein on tumor burden was assessed in cisplatin-resistant xenograft models. The results showed that hepatitis B X-interacting protein was highly expressed in ovarian cancer of cisplatin-resistant tissues and cells. Notably, knockdown of hepatitis B X-interacting protein significantly reduced cell viability in A2780/CP compared with cisplatin treatment alone. Hepatitis B X-interacting protein and cisplatin cooperated to induce apoptosis and increase the expression of c-caspase 3 as well as the Bax/Bcl-2 ratio. We confirmed that hepatitis B X-interacting protein up-regulated CD147 at the protein expression and transcriptional levels. Moreover, we found that hepatitis B X-interacting protein was able to activate the CD147 promoter through Sp1. *In vivo*, depletion of hepatitis B X-interacting protein decreased the tumor volume and weight induced by cisplatin. Taken together, these results indicate that hepatitis B X-interacting protein promotes cisplatin resistance and regulated CD147 via Sp1 in ovarian cancer cell lines.

Keywords: Hepatitis B X-interacting protein, cisplatin, chemoresistance, ovarian cancer, Sp1, CD147

***Experimental Biology and Medicine* 2017; 242: 497–504. DOI: 10.1177/1535370216685007**

Introduction

Ovarian cancer is the most lethal gynecological cancer, with a 60% mortality rate in patients and a five-year survival rate of lower than 30% in advanced stage disease.[1] Efforts at early detection, new therapeutic approaches to reduce mortality, and platinum-based chemotherapy are used as general therapy for ovarian cancer and other malignancies. Although an improvement in median survival has been observed, most patients eventually succumb to recurrent progressive disease because the development of drug resistance has ultimately affected the effectiveness of chemotherapy.[2] Multiple mechanisms are involved in drug resistance, such as drug metabolism, membrane transporters, and evasion of apoptosis.[3] Growing efforts are required to achieve a greater understanding of the molecular mechanisms underlying ovarian cancer drug resistance and to aid the development of novel diagnostic and therapeutic strategies.

Hepatitis B X-interacting protein (HBXIP) is a conserved 19-kD protein identified by its interaction with the C terminus of the hepatitis B virus X protein.[4] Emerging evidence has demonstrated that HBXIP plays a variety of roles in carcinoma. For example, it regulate cell apoptosis and division through bind to survivin,[5] as well as participating in centrosome dynamics and cytokinesis.[6] HBXIP is highly expressed in clinical breast cancer tissues and promotes cell migration.[7] In addition, HBXIP has been implicated in the progression of breast cancer.[8] Furthermore,

HBXIP promoted the migration of ovarian cancer cells through regulation Skp-2.[9] However, the underlying mechanism of HBXIP in the chemoresistance of ovarian cancer is not well documented. In the present study, our findings indicate that HBXIP inhibition is a novel and promising strategy to target cisplatin-sensitivity.

Material and methods

Tissues collected and cell culture

The tissues from patients who received regular cisplatin-based chemotherapy in our department from 2011 to 2013 were collected. Patients had been randomly divided into cisplatin-sensitivity and cisplatin resistance, on the basis of the serum CA125 levels. Briefly, treatment-sensitive patients were defined as those having at least 50% reduction in CA125, otherwise, it belongs to cisplatin-resistant tissues.[10] Cisplatin-sensitive cell lines A2780 and SKOV3 and cisplatin-resistant cell lines A2780/CP and SKOV3/CP were bought from Shanghai Institutes for Biological Sciences. These cells were maintained in PRMI-1640 medium (Gibco BRL, NY, USA) with 10% FBS (Gibco BRL, NY, USA) at 37°C with 5% CO_2.

Cell transfection

Small interfering RNA targeting the messenger RNAs of HBXIP (siHBXIP) and negative control for siHBXIP (NC), siSp1 and siCtrl, empty vector pCMV and pCMV-HBXIP were synthesized by Sangon Biotech (Shanghai, China) and transfected into A2780 or A2780/CP using Lipofectamine 2000 for 48 h, according to the manufacturer's instructions.

Quantitative RT-PCR

Total RNA was isolated from cells with TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. Reverse transcription and real-time PCR were performed with the TaqMan Reverse Transcription Regents and TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA). β-actin was used as an internal control for normalization. The primers sequences were as follows: HBXIP (sense): 5′-CGGAAGCGCAGTGATGTTTT-3′, HBXIP (antisense): 5′-CTTAGCTGCTTGCTGGGCTA-3′; β-actin (sense): 5′-CTCCATCCTGGCCTCGCTGT-3′, β-actin (antisense): 5′-GCTGTCACCTTCACCGTTCC-3′. Each experiment was carried out in triplicate. Differences in gene expression, expressed as fold changes, were calculated using the $2^{-\Delta\Delta Ct}$ method.

Cell counting kit-8 (CCK8) assay

Cell viability was measured using the CCK8 assay (Yiyuanbiotech, Guangzhou, China) according to the manufacturer's protocol. A2780/CP cells were plated in 96-well plates (1×10^4 cells/well)and transfected with negative control for siHBXIP (NC) and siHBXIP (Sangon Biotech, Shanghai, China) using Lipofectamine 2000 for 48 h (Invitrogen, CA, USA), followed by 24-h CP treatment at various concentrations. Then, 10 μL of CCK-8 solution was added to each well for another 2.5 h and the absorbance at 450 nm was measured with a microplate reader.

Western blot analysis

Total proteins from tissues or cells were collected by tissue homogenization or cell lysis. The BCA assay was used to assay protein concentration. Proteins (50 μg) from each group were loaded onto gels, resolved by SDS-PAGE, and electrotransferred onto nitrocellulose membranes. Membranes were blocked with 5% milk at room temperature for 1 h, then incubated overnight at 4°C with primary anti-HBXIP (Abcam, Cambridge, MA, USA), anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-cleaved caspase3 (CST, Danvers, MA, USA), anti-Bax (CST) and anti-Bcl-2 (CST) and anti-Sp1 (Santa Cruz Biotechnology), and subsequently, with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibody at room temperature for 1 h. Protein expression was quantified using a Kodak Gel Documentation System.

Apoptosis assay

Apoptosis of A2780/CP cells were assessed with the Annexin V/PI Apoptosis Detection Kit (BD Biosciences, San Jose, CA). Briefly, 1×10^6 A2780/CP cells were plated on a 10 cm^2 plate and incubated for 24 h. The cells were transfected with NC or siHBXIP for 48 h. Cisplatin (50 μM) was added to both NC and siHBXIP-treated cells and the cells were incubated for an additional 48 h. Cells were resuspended, and then 5 μL of FITC annexin V and 5 μL of PI were added. Stained cells were analyzed by flow cytometry during the first 15 min of staining at room temperature. Cells were measured using a flow cytometer FC500/FC500-MPL (Beckman Coulter, Brea, CA, USA) and data were analyzed using FlowJo software (Tree Star Inc.).

Luciferase reporter gene assay

A2780 cells grown in 24-well plates were cotransfected with CD147 luciferase reporter plasmid (0.2 μg) and pRL-TK normalization construct (0.1 μg) using Lipofectamine 2000 (Invitrogen). The pCMV-HBXIP plasmid was co-transfected with reporter plasmids, which lead to a cloud forcing expression of HBXIP. Small interfering RNAs (siRNAs) targeting HBXIP or Sp1 (50 nM) and the reporter plasmids were cotransfected into the cells. Forty-eight hours after transfection, cells were assayed for luciferase activity using the Dual-Luciferase Reporter Assay System (Promega).

Tumor xenografts in mice

Female immune-deficient nude mice (BALB/c-nu; age, four to six weeks) were purchased from Shanghai Slac Laboratory Animal Co. Ltd. All animals were housed in appropriate cages on a 12-h light/dark cycle with access to food and water. A2780/CP cells were transfected with NC or siHBXIP and treated with CP, then harvested and 5×10^6 cells in 200 μL of phosphate-buffered saline were subcutaneously injected at the shoulder of mice

(each group, n = 9). Tumor size was measured with digital calipers every three days, up to 30 days after injection. Tumor volume was calculated according to the following formula: $0.5 \times (\text{length} \times \text{width}^2)$. Four weeks later, the animals were euthanized by anesthesia overdose, and their tumors were removed and weighed.

Statistical analysis

Statistical significance was analyzed by unpaired Student's *t* test and one-way analysis of variance using IBM SPSS Statistics 21.0 (SPSS Inc. IBM, Armonk, NY, USA). Data are shown as mean ± SD from three independent experiments. Results were considered statistically significant at $P < 0.05$.

Results

Expression of HBXIP is correlated with cisplatin resistance in ovarian tumors

To confirm the relationship between HBXIP and cisplatin resistance in ovarian cancer cells, we examined the mRNA and protein expression of HBXIP in ovarian cancer tissues with cisplatin resistance or cisplatin-sensitivity, A2780, A2780/CP, SKOV3, and SKOV3/CP cells. As shown in Figure 1(a), tissues which showed cisplatin resistance had

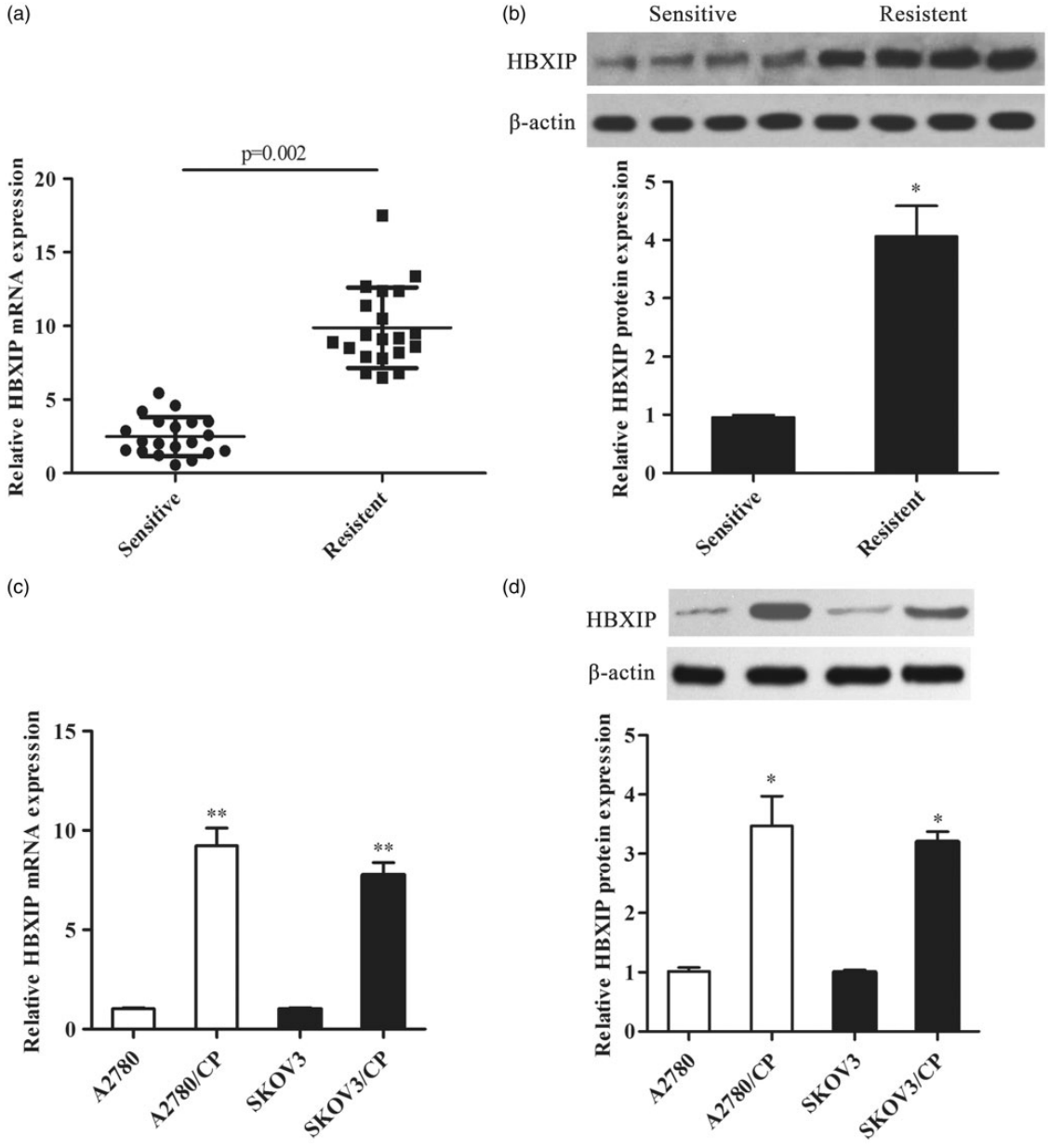

Figure 1 HBXIP expression is elevated in cisplatin-resistant tissues and cells. (a, b) The mRNA (a) and protein (b) levels of HBXIP were assayed using RT-PCR and Western blot in cisplatin-resistant and cisplatin-sensitive tissues (c, d) The mRNA (c) and protein (d) expression of HBXIP were determined using Western blotting in ovarian cancer tissues and cell lines; $^*P < 0.05$, $^{**}P < 0.01$ compared with sensitive or A2780 or SKOV3 groups.

an obviously higher expression of HBXIP in mRNA level. Western blot analysis of HBXIP protein levels determined that HBXIP increased in cisplatin-resistant specimens compared with cisplatin-sensitive specimens (Figure 1(b)). Furthermore, the chemoresistant A2780/CP and SKOV3/CP cells also exhibited markedly higher mRNA levels of HBXIP compared with A2780 and SKOV3 cells (Figure 1(c)), as well as the protein expression of HBXIP (Figure 1(d)). These data indicated that HBXIP could have an important role in the cisplatin resistance of ovarian cancer cells.

HBXIP inhibition contributes to the sensitivity to cisplatin

A2780/CP cells were transfected with NC or siHBXIP for 48 h, and the mRNA (Figure 2(a)) and protein (Figure 2(b)) levels of HBXIP were significantly reduced. A CCK-8 assay

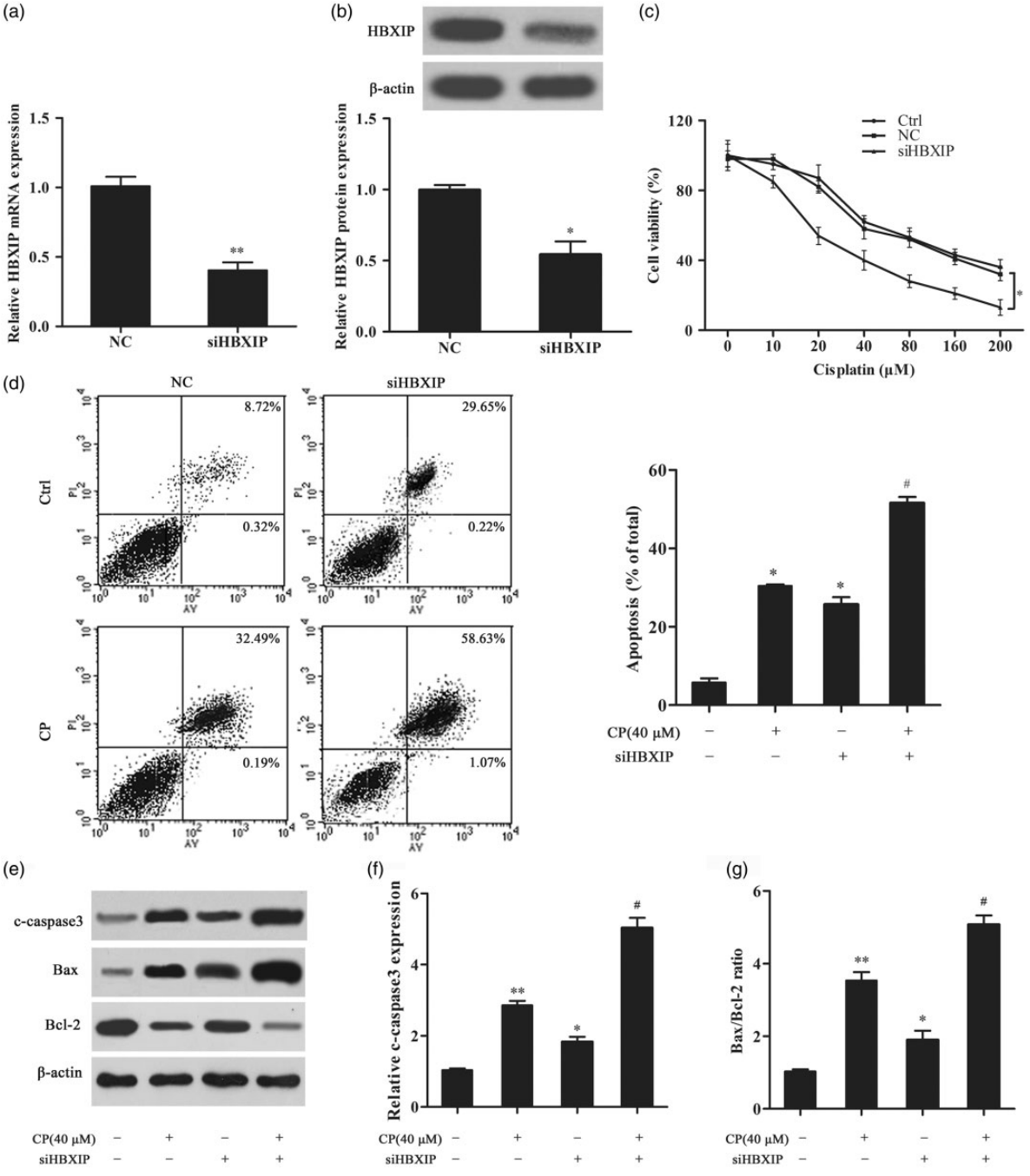

Figure 2 Depletion of HBXIP enhanced cisplatin-sensitivity of A2780/CP cells. A2780/CP cells were transfected with NC or siHBXIP for 48 h using lipofectamine 2000. (a, b) The mRNA (a) and protein (b) levels of HBXIP were assayed using RT-PCR and western blotting respectively. (c) Cell viability was measured using CCK-8 assay. (d) The apoptosis rate was detected using flow cytometry. (e–g) The expression of c-caspase3 (e, f) and Bax/Bcl ratio (e, g) were evaluated using Western blotting. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with NC or vehicle (neither CP nor siHBXIP) group. $^{\#}P < 0.05$ compared with CP group

demonstrated that cell viability was decreased by CP in a dose-dependent manner, and depletion of HBXIP leads to a significant reduction in cell viability compared with the NC group, and contributed to decreased viability at the remaining time points (Figure 2(c)). Following 40-μM cisplatin treatment, the cell apoptosis rate was clearly increased in A2780/CP cells, as well as in the siHBXIP group, and with both cisplatin and siHBXIP treatment, A2780/CP cells exhibited a large increase in the apoptosis rate in comparison to the group treatment with CP alone (Figure 2(d)). Western blot revealed that c-caspase3 was increased in the CP or siHBXIP group, and sharply increased in the CP + siHBXIP group compared with the CP group (Figure 2(e) and (f)). Moreover, the Bax/Bcl-2 ratio was markedly elevated when A2780/CP cells were treated with CP and transfected with siHBXIP (Figure 2(e) and (g)).

HBXIP activates the CD147 promoter through transcription factor Sp1

CD147 has been implicated in promoting chemoresistance in cancer stem cells and prostate cancer.[11,12] Importantly, the regulation of CD147 in cancer development occurs at the transcriptional and post-transcriptional levels.[13,14] In breast cancer cells, a transcriptional factor Sp1 stimulates its binding activity to the CD147 promoter, and CD147 also promotes Sp1 phosphorylation at T453 and T739.[15] To determine whether CD147 is involved in HBXIP-mediated chemoresistance in ovarian cancer cells, we tested whether HBXIP is able to upregulate CD147. As shown in Figure 3(a), we observed that the overexpression of HBXIP enhanced the expression of CD147 at the protein level in A2780 cells, whereas the knockdown of HBXIP by siHBXIP reduced the expression of CD147 in A2780/CP cells. Moreover, we found that forcing the expression of HBXIP activated the promoter of CD147 in A2780 cells (Figure 3(b)); however, CD147 promoter activity was decreased with HBXIP inhibition (Figure 3(c)). It has been reported that Sp1 can bind to sites in the region (−108/+37) of the CD147 promoter. We supposed that HBXIP might activate the CD147 promoter via activation Sp1. We constructed the fragment of the pGL3-CD147 mut promoter in which the Sp1 sites were mutated (Figure 3(d)). Our data demonstrated that overexpression of HBIXP significantly increased the luciferase activities of pGL3-CD147 but failed to influence that of pGL3-CD147 mut in A2780 cells (Figure 3(d)). Moreover, depletion of Sp1 significantly attenuated the promoting effect of HBXIP on the activity of the CD147 promoter (Figure 3(e)), suggesting that HBXIP binds to the CD147 promoter via activating Sp1. Western blot analysis confirmed that silencing Sp1 reduced the expression of CD147 by pCMV-HBXIP at the protein level in A2780 cells (Figure 3(f)). In addition, knockdown of HBXIP decreased the expression of CD147 and the protein level of CD147 was further diminished when Sp1 was silenced by siRNA in A2780/CP (Figure 3(g)). We conclude that HBXIP activates the CD147 promoter through Sp1.

Depletion of HBXIP reverses cisplatin resistance in ovarian cancer in vivo

To confirm that HBXIP knockdown could inhibit cisplatin resistance in ovarian cancer *in vivo*. We established the xenograft model of ovarian cancer in nude mice, and then injected A2780/CP cells that had been treated with cisplatin alone or combined with siHBXIP. As shown in Figure 4(a), CP treatment resulted in a significant increase in tumor volume compared with the vehicle group and the reduction of tumor volume induced by CP could be further diminished by siHBXIP. In addition, CP also decreased tumor weight and a further in the reduction of tumor weight when cells were treated with both CP and siHBXIP (Figure 4(b)). Overall, we conclude that HBXIP contributes to cisplatin resistance in ovarian cancer *in vivo*.

Discussion

Ovarian cancer shows a high rate of platinum sensitivity, but resistance frequently develops in recurrent disease.[16] Resistance to chemotherapy is a primary cause of treatment failure and relapse in various cancer types, including ovarian cancer.[17] As such, understanding the signaling networks that regulate chemoresistance is critical for successful treatment. Comprehending how cisplatin resistance occurs is the basis for developing strategies to improve prognosis prediction. In this study, we confirmed the role of HBXIP in the development of cisplatin-resistant ovarian cancer cells and its possible relationship with CD147.

Many studies show that HBXIP acts as an oncoprotein in cell proliferation and migration.[18,19] The expression of HBXIP was detected in human ovarian cancer specimens with cisplatin-sensitivity and cisplatin resistance and cell lines. RT-PCR and Western blotting showed that HBXIP was highly expressed in cisplatin-resistant specimens or cell lines, which may relate to the positive regulative function of HBXIP in the proliferation and migration of ovarian cancer cells.[9]

An abnormal cell proliferation contributes to the pathogenesis of cancer and according to this point of view, cisplatin resistance might relate to the imbalance between cell proliferation and apoptosis. Apoptosis seems to be the main mechanism whereby chemotherapy and radiation induce the killing of tumor cells. There is presently a line of evidence indicating that deregulation of programmed cell death, including apoptosis and autophagy, is an important mechanism for tumor resistance to anticancer drugs.[20] It has been reported that HBXIP functions as a cofactor for survivin in apoptosis suppression,[5] which is consistent with our data that depletion of HBXIP resulted in the reduction of cell viability and augmentation of apoptosis in A2780/CP cells. In addition, depletion HBXIP further aggravated apoptosis when A2780/CP cells were treated with CP, suggesting that HBXIP contributes to cisplatin-sensitivity in ovarian cancer cells. Caspases, a family of cysteinyl aspartate-specific proteases, are best known as executioners of apoptotic cell death: their activation is considered to be a commitment to cell death and c-caspase3 is a biomarker of cell apoptosis.[21] Survivin associates with procaspase 9 via HBXIP to suppress caspase activation and

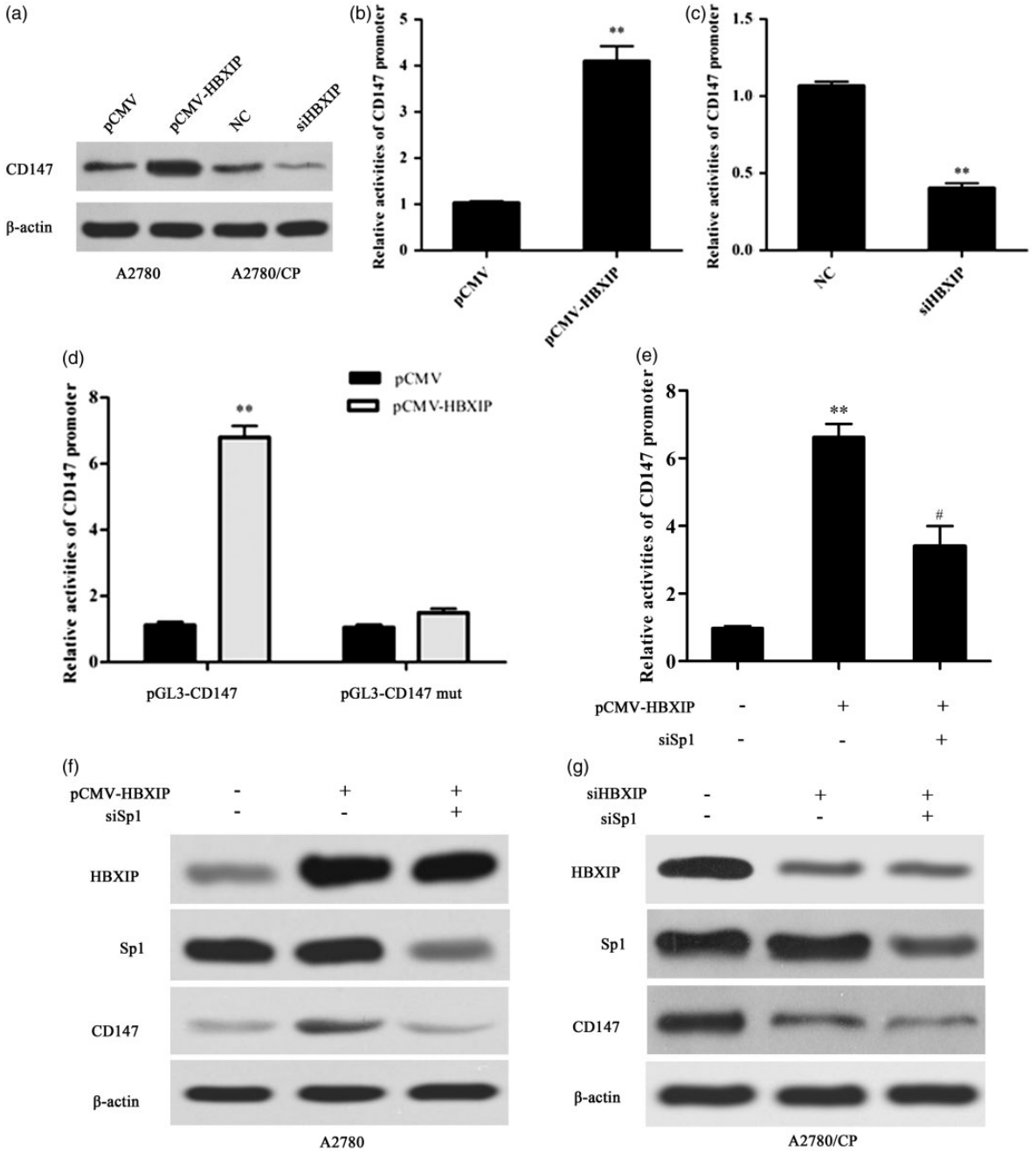

Figure 3 HBXIP activates the promoter of CD147 via the transcription factor Sp1. (a) The expression of CD147 was examined using Western blotting. (b, c) The activities of the CD147 promoter were measured by luciferase reporter gene assay in pCMV-HBXIP-transfected A2780 cells (b) or siHBXIP-transfected A2780/CP cells (c). (d, e) The activities of CD147 and CD147 mut were assayed using luciferase reporter gene assays in A2780 cells transfected with pCMV-HBXIP. (f, g) The expression of HBXIP, Sp1 and CD147 was detected using Western blotting in A2780 (f) or A2780/CP (g). $^{*}P < 0.05$, $^{**}P < 0.01$ compared with NC or pCMV or vehicle (neither pCMV-HBXIP or siSp1) group. $^{\#}P < 0.05$ compared with the pCMV-HBXIP group

cellular apoptosis.[22] Our results indicated that HBXIP inhibition resulted in upregulation induced even further by CP as well as the Bax /Bcl-2 ratio in A2780/CP cells.

CD147, also as known as basigin or Emmprin, is a type I glycoprotein that belongs to the immunoglobulin superfamily. It is highly expressed in tumor cells and stimulates adjacent fibroblasts or tumor cells to produce matrix metalloproteinases (MMPs).[23] CD147 is overexpressed in a number of carcinomas and is associated with tumor progression.[23,24] Previous findings have shown that CD147 promotes migration, invasion, and metastasis in cancer cells, enhancing the activity of MMPs by digesting the components of the extracellular matrix in breast cancer, oral squamous cell carcinoma, and ovarian cancer.[25–27] Sp1 was a eukaryotic transcriptional activator identified and involved in many physiological and pathological processes by regulating gene expression, which is affected by the rate of Sp1 protein synthesis, nuclear translocation, and DNA-binding affinity.[28] Kong *et al.*[29] reported that the expression of Sp1 directly regulated the expression level of CD147 in

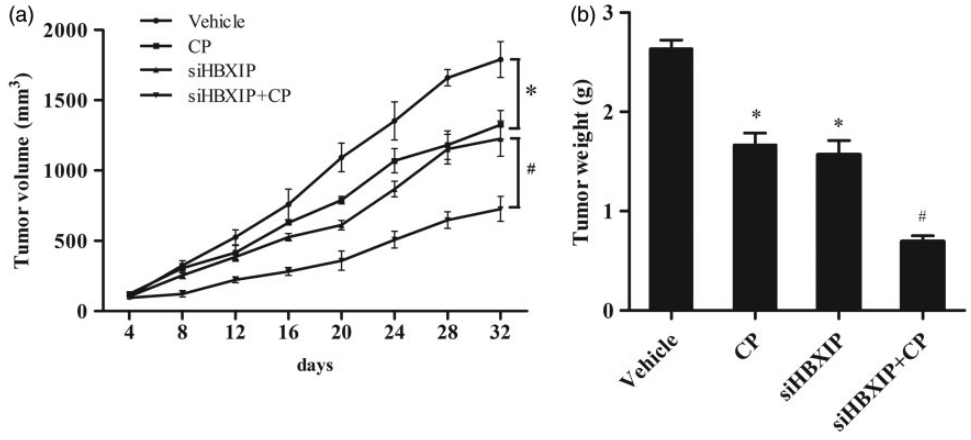

Figure 4 Knockdown of HBXIP reverses cisplatin resistance in ovarian tumors *in vivo*. (a) Tumor volumes were measured with digital calipers. (b) Tumor mass was measured. $^{*}P < 0.05$ compared with the vehicle group; $^{\#}P < 0.05$ compared with the CP group

lung cancer cells. In addition, CD147 promoted Sp1 phosphorylation through the PI3K/AKT and MAPK/ERK pathways.[15] Moreover, T453 and T739 mutations decreased the activity of Sp1 binding to the promoter of CD147 in ovarian cancer cells.[15] Importantly, forcing the expression of CD147 contributes to the chemoresistance of head and neck squamous cell carcinoma cells and cancer stem cell-like cells.[12,30] Interestingly, HBXIP activates the promoter of LMO4 and PDGFB via Sp1.[31,32] Our data revealed that forcing the expression of HBXIP upregulated the expression and promoter activity of CD147; moreover, overexpression of HBIXP significantly increased the luciferase activity of pGL3-CD147, but failed to influence that of pGL3-CD147 mut.

As expected, depletion of HBXIP reduced the expression of CD147, which was abolished by Sp1 inhibition. Functionally, we determined that CD147 was implicated in HBXIP-modulated cisplatin resistance in ovarian cancer. *In vivo*, our data indicated that tumor volume and weight were furtherly reduced after knockdown of HBXIP.

In conclusion, our present study demonstrated that HBXIP inhibition promotes the sensitivity to cisplatin in A2780/CP cells. HBXIP up-regulates CD147 via activation of Sp1 to reduce cisplatin resistance in ovarian cancer cells. Accordingly, CD147 as a determinant of cisplatin resistance should be further explored as a potential theranostics biomarker.

Authors' contributions: WZ, XM, HY designed research; WH, BCh, GC provided technical assistance and reviewed manuscript; WZ, XM performed research and wrote the paper.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to all those who have helped in the course of my writing this paper.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

We are grateful for financial support from the NSFC (81101959 and 81101958).

REFERENCES

1. Siegel R, Naishadham D, Jemal A. Cancer statistics. *CA: Cancer J Clin* 2013;**63**:11–30
2. Holohan C, Van Schaeybroeck S, Longley DB, Johnston PG. Cancer drug resistance: An evolving paradigm. *Nat Rev Cancer* 2013;**13**:714–26
3. Kachalaki S, Ebrahimi M, Khosroshahi LM, Mohammadinejad S, Baradaran B. Cancer chemoresistance; biochemical and molecular aspects: A brief overview. *Eur J Pharm Sci* 2016;**89**:20–30
4. Melegari M, Scaglioni PP, Wands JR. Cloning and characterization of a novel hepatitis B virus x binding protein that inhibits viral replication. *J Virol* 1998;**72**:1737–43
5. Marusawa H, Matsuzawa Si, Welsh K, Zou H, Armstrong R, Tamm I, Reed JC. HBXIP functions as a cofactor of survivin in apoptosis suppression. *EMBO J* 2003;**22**:2729–40
6. Fujii R, Zhu C, Wen Y, Marusawa H, Bailly-Maitre B, Matsuzawa S-i, Zhang H, Kim Y, Bennett CF, Jiang W. HBXIP, cellular target of hepatitis B virus oncoprotein, is a regulator of centrosome dynamics and cytokinesis. *Cancer Res* 2006;**66**:9099–107
7. Shi H, Li Y, Feng G, Li L, Fang R, Wang Z, Qu J, Ding P, Zhang X, Ye L. The oncoprotein HBXIP up-regulates FGF4 through activating transcriptional factor Sp1 to promote the migration of breast cancer cells. *Biochem Biophys Res Commun* 2016;**471**:89–94
8. Cheng D, Liang B, Li Y. HBXIP expression predicts patient prognosis in breast cancer. *Med Oncol* 2014;**31**:1–7
9. Xu F, Zhu X, Han T, You X, Liu F, Ye L, Zhang X, Wang X, Yao Y. The oncoprotein hepatitis B X-interacting protein promotes the migration of ovarian cancer cells through the upregulation of S-phase kinase-associated protein 2 by Sp1. *Int J Oncol* 2014;**45**:255–63
10. Rustin GJ, Quinn M, Thigpen T, Du BA, Pujade-Lauraine E, Jakobsen A, Eisenhauer E, Sagae S, Greven K, Vergote I. Re: New guidelines to evaluate the response to treatment in solid tumors (ovarian cancer). *J Natl Cancer Inst* 2004;**96**:487–8
11. Hao J, Madigan MC, Khatri A, Power CA, Hung T-T, Beretov J, Chang L, Xiao W, Cozzi PJ, Graham PH. In vitro and in vivo prostate cancer metastasis and chemoresistance can be modulated by expression of either CD44 or CD147. *PloS One* 2012;**7**:e40716
12. Kang MJ, Kim HP, Lee Ks, Yoo YD, Kwon YT, Kim KM, Kim TY, Yi EC. Proteomic analysis reveals that CD147/EMMPRIN confers chemoresistance in cancer stem cell-like cells. *Proteomics* 2013;**13**:1714–25

13. Liang L, Major T, Bocan T. Characterization of the promoter of human extracellular matrix metalloproteinase inducer (EMMPRIN). *Gene* 2002;**282**:75–86
14. Kong LM, Liao CG, Chen L, Yang HS, Zhang SH, Zhang Z, Bian HJ, Xing JL, Chen ZN. Promoter hypomethylation up-regulates CD147 expression through increasing Sp1 binding and associates with poor prognosis in human hepatocellular carcinoma. *J Cell Mol Med* 2011;**15**:1415–28
15. Zhao J, Ye W, Wu J, Liu L, Yang L, Gao L, Chen B, Zhang F, Yang H, Li Y. Sp1-CD147 positive feedback loop promotes the invasion ability of ovarian cancer. *Oncol Rep* 2015;**34**:67–76
16. Solár P, Sytkowski AJ. Differentially expressed genes associated with cisplatin resistance in human ovarian adenocarcinoma cell line A2780. *Cancer Lett* 2011;**309**:11–8
17. Galluzzi L, Senovilla L, Vitale I, Michels J, Martins I, Kepp O, Castedo M, Kroemer G. Molecular mechanisms of cisplatin resistance. *Oncogene* 2012;**31**:1869–83
18. Liu S, Li L, Zhang Y, Zhang Y, Zhao Y, You X, Lin Z, Zhang X, Ye L. The oncoprotein HBXIP uses two pathways to up-regulate S100A4 in promotion of growth and migration of breast cancer cells. *J Biol Chem* 2012;**287**:30228–39
19. Xu F, You X, Liu F, Shen X, Yao Y, Ye L, Zhang X. The oncoprotein HBXIP up-regulates Skp2 via activating transcription factor E2F1 to promote proliferation of breast cancer cells. *Cancer Lett* 2013;**333**:124–32
20. Pan ST, Li ZL, He ZX, Qiu JX, Zhou SF. Molecular mechanisms for tumor resistance to chemotherapy. *Clin Exp Pharmacol Physiol* 2016;**43**:723–37
21. Abu-Qare AW, Abou-Donia MB. Biomarkers of apoptosis: Release of cytochrome c, activation of caspase-3, induction of 8-hydroxy-2′-deoxyguanosine, increased 3-nitrotyrosine, and alteration of p53 gene. Journal of toxicology and environmental health Part B. *Crit Rev* 2000;**4**:313–32
22. Cheng C-W, Chow AK, Pang R, Fok EW, Kwong Y-L, Tse E. PIN1 inhibits apoptosis in hepatocellular carcinoma through modulation of the antiapoptotic function of survivin. *Am J Pathol* 2013;**182**:765–75
23. Nabeshima K, Iwasaki H, Koga K, Hojo H, Suzumiya J, Kikuchi M. Emmprin (basigin/CD147): Matrix metalloproteinase modulator and multifunctional cell recognition molecule that plays a critical role in cancer progression. *Pathol Int* 2006;**56**:359–67
24. Weidle Uh, Scheuer W, Eggle D, Klostermann S, Stockinger H. Cancer-related issues of CD147. *Cancer Genom Proteom* 2010;**7**:157–69
25. Marchiq I, Albrengues J, Granja S, Gaggioli C, Pouysségur J, Simon M-P. Knock out of the BASIGIN/CD147 chaperone of lactate/H+ symporters disproves its pro-tumour action via extracellular matrix metalloproteases (MMPs) induction. *Oncotarget* 2015;**6**:24636–48
26. Cao Z, Xiang J, Li C. Expression of extracellular matrix metalloproteinase inducer and enhancement of the production of matrix metalloproteinase-1 in tongue squamous cell carcinoma. *Int J Oral Maxillofacial Surg* 2009;**38**:880–5
27. Davidson B, Goldberg I, Berner A, Kristensen GB, Reich R. EMMPRIN (extracellular matrix metalloproteinase inducer) is a novel marker of poor outcome in serous ovarian carcinoma. *Clin Exp Metastasis* 2003;**20**:161–9
28. Chu S. Transcriptional regulation by post-transcriptional modification – Role of phosphorylation in Sp1 transcriptional activity. *Gene* 2012;**508**:1–8
29. Kong LM, Liao CG, Fei F, Guo X, Xing JL, Chen ZN. Transcription factor Sp1 regulates expression of cancer-associated molecule CD147 in human lung cancer. *Cancer Sci* 2010;**101**:1463–70
30. Huang Z, Wang L, Wang Y, Zhuo Y, Li H, Chen J, Chen W. Overexpression of CD147 contributes to the chemoresistance of head and neck squamous cell carcinoma cells. *J Oral Pathol Med* 2013;**42**:541–6
31. Yue L, Li L, Liu F, Hu N, Zhang W, Bai X, Li Y, Zhang Y, Fu L, Zhang X. The oncoprotein HBXIP activates transcriptional coregulatory protein LMO4 via Sp1 to promote proliferation of breast cancer cells. *Carcinogenesis* 2013;**34**:927–35
32. Zhang Y, Zhao Y, Li L, Shen Y, Cai X, Zhang X, Ye L. The oncoprotein HBXIP upregulates PDGFB via activating transcription factor Sp1 to promote the proliferation of breast cancer cells. *Biochem Biophys Res Commun* 2013;**434**:305–10

(Received August 13, 2016, Accepted November 21, 2016)