

Original Research Article

HIPK2 reduces the resistance of gastric cancer cells to cisplatin via p53 pathway

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Sent for review: 9 September 2020

Revised accepted: 24 November 2020

Abstract

Purpose: To uncover the functional effect of homologous domain-associated protein kinase 2 (HIPK2) on the viability of cisplatin (DDP)-resistant gastric cancer (GC) cells and elucidate the possible mechanism of action.

Methods: The effect of DDP on GC viability and apoptotic rate was evaluated using MTT and flow cytometry (FCM) assays. The potential effect of HIPK2 on DDP sensitivity and cell apoptosis was investigated in the presence of cisplatin while the effect of HIPK2 on p53 activation was determined by immunoblot assay.

Results: HIPK2 expression was decreased in DDP-resistant GC cell while upregulation of HIPK2 reduced growth, but promoted apoptosis in DDP-resistant GC cells. Further investigations showed that HIPK2 promoted p53 activation, while suppression of p53 weakened the inhibitory effect of HIPK2 on DDP-resistance in GC cells.

Conclusion: The results suggest that HIPK2 is a promising and important therapeutic factor for the regulation of the resistance of GC cells to DDP. Thus, may have a role to play in the management of gastric cancer

Keywords: Gastric cancer, Cisplatin, HIPK2, Homologous domain-associated protein kinase 2, p53 pathway, Therapeutic target

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INTRODUCTION

Gastric cancer (GC) is one of the deadliest cancers and a major reason of tumor-related deaths [1]. Gastric cancer originates from malignant tumors of the gastric mucosa epithelium [2]. The incidence of GC ranks first among all malignant tumors in China [3]. No significant symptoms are observed in early stage of GC. Chemotherapy is the traditional treatment

for gastric cancer, and cisplatin (DDP) is considered as the most common chemotherapy drug to treat g GC [4]. Chemotherapy resistance often occurs during chemotherapy, resulting in poor therapeutic effect [5]. Therefore, elucidating the mechanism underlying the chemotherapeutic resistance to DDP and identifying new therapeutic targets are improve for the prognosis of GC patients.

Homologous domain-associated protein kinase 2 (HIPK2) is a nuclear localization protein belonging to the DYRK serine/threonine homologous domain-interacting kinase family [6]. A previous study indicated that HIPK2 can regulate gene expression and is activated by signal induction, which affects the progression of renal fibrosis and acute kidney injury [7]. HIPK2 overexpression protects cardiomyocytes from H/R-induced injury by activating Nrf2/ARE antioxidant signaling [8]. HIPK2 depletion protects neurons from mitochondrial toxins through the turnover regulation of Parkin protein [9].

Besides, the mechanism of HIPK2 in the progression and metastasis of multiple tumor types was reported through targeting various proteins, such as p53, p73, and MDM2 [10]. In particular, HIPK2 protein has been reported associated with cancer chemotherapy resistance [11], and its down-regulation could enhance the DDP resistance in bladder cancer cells through regulation of Wip1 [12]. In esophageal squamous cell carcinoma (ESCC), HIPK2 inhibits the cell metastasis and increases chemotherapy sensitivity [13]. In GC, miR-222-3P induces the proliferative, invasive abilities and reduces cell apoptosis through directly targeting HIPK2 [10], indicating the vital involvement of HIPK2 in GC progression. However, its role and mechanism in chemotherapy resistance in GC needs further exploration.

EXPERIMENTAL

Cell culture and transfection

The human GC cell lines (BGC823 and SGC7901) and gastric epithelial cell (GES-1) were purchased from the Cell Bank of the Chinese Academy of Medical Science (Beijing, China). The cells were maintained in DMEM containing 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA) at 37 °C. The transfection of HIPK2 shRNA was conducted using Lipofectamine 2000 reagent (Invitrogen). BGC823 and SGC7901 cells were exposed to DDP (2 µg/ml) (Sigma, St. Louis, MO, USA). HIPK shRNA and control shRNA were purchased from Merck.

Immunoblot assay

The proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then were transferred to a PVDF membrane. Subsequently, the membrane was blocked with 5% BSA in TBST (0.1% Tween) for 2 h and then incubated with HIPK2 (1:1000

dilution; Abcam, Cambridge, UK), p-p53 (1:1000 dilution; Abcam), p53 (1:1000 dilution; Abcam), or GAPDH (1:10000 dilution; Abcam). Next, the membrane was incubated with secondary antibodies and the target bands were analyzed via the ECL kit and quantified.

MTT assay

Cells were grown in 96-well plates for 48 h, DDP was then added to the plate. The adherent cells were fixed and then treated with MTT. Next, for dissolving the formazan crystals, dimethyl sulfoxide (DMSO) was used, and the absorbance was read at 570 nm using a GF-M3000 microplate reader (CAIHONG, Shandong, China).

FCM assay

Cells were collected, washed and fixed (70% ethanol, -20°C, 1 h). Subsequently, the cells were incubated with PI and FITC for 30 min and analyzed using a BD FACSCalibur cytometer instrument.

Statistical analysis

Statistical analysis was conducted using GraphPad 5.0 software in this study. The data were presented as means ± standard deviation (SD). Student's *t*-test was used for statistical analysis. *P* < 0.05 was considered statistically significant.

RESULTS

HIPK2 expression is decreased in DDP-resistant GC cells

BGC823 and SGC7901 cells were administrated with DDP and the resistant cells were named as BGC823/DDP and SGC7901/DDP, respectively. The expression level of HIPK2 was downregulated in GC (BGC823 and SGC7901) cells compared with that in GES-1 cells. Compared with that in GC cells, the HIPK2 level was further decreased in DDP-resistant GC cells (Figure 1 A). DDP at concentrations of $10^{-1.5}$, 10^{-1} , $10^{-0.5}$, 10^0 , $10^{0.5}$, 10^1 , $10^{1.5}$, and 10^2 was added to cells, and the IC₅₀ values were obtained. The mean IC₅₀ value in BGC823/DDP cells was 12.37 µg/mL, which is higher than that in BGC823 cells (4.461 µg/mL). The mean IC₅₀ value in SGC7901/DDP cells was 12.55 µg/mL, which is higher than that in SGC7901 cells (4.651 µg/mL) (Figure 1 B). These resistant cells were used in the following analysis. These data demonstrated that HIPK2 level is decreased in DDP-resistant GC cells.

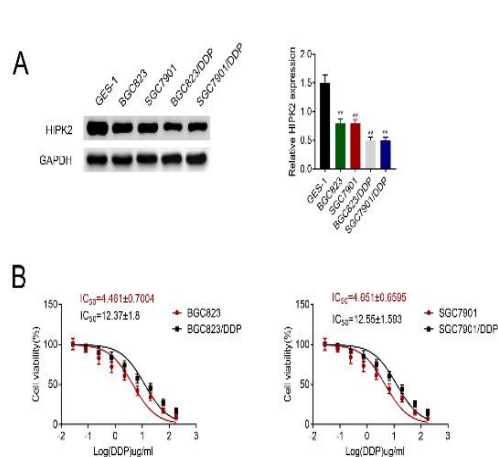


Figure 1: HIPK2 expression level in DDP-resistant GC cells. (A) Immunoblotting was used to detect the level of HIPK2. (B) Viability of cells treated with different doses of DDP, and the IC_{50} was detected using MTT assays; $**p < 0.01$.

HIPK2 upregulation relieves DDP resistance in GC cells

To further reveal the function of HIPK2 in the regulation of GC (BGC823 and SGC7901) cell proliferation, HIPK2 shRNA and HIPK plasmid were transfected into GC cells to knockdown and overexpress its expression, respectively. The transfection efficiency was determined by immunoblot assays (Figure 2 A and B). HIPK2 overexpression sharply sensitized to DDP, with an IC_{50} of 14.44 $\mu\text{g/ml}$ in control cells and 7.225 $\mu\text{g/ml}$ in HIPK2-overexpressed BGC823 cells, and 16.24 $\mu\text{g/ml}$ in control cells and 6.678 $\mu\text{g/ml}$ in HIPK2-overexpressed SGC7901 cells (Figure 2 C). In addition, HIPK2 down-regulation led to increased DDP resistance, with IC_{50} values of 14.75 $\mu\text{g/ml}$ in control cells and 26.12 $\mu\text{g/ml}$ in HIPK2-downregulated BGC823/DDP cells, and 12.45 $\mu\text{g/ml}$ in control cells and 22.44 $\mu\text{g/ml}$ in HIPK2-downregulated SGC7901/DDP cells. Taken together, HIPK2 upregulation relieved DDP resistance in GC cells.

HIPK2 contributes to DDP-induced cell apoptosis

To further explore HIPK2 function in DDP resistance, flow cytometry was performed in HIPK2-depleted or -overexpressed cells. HIPK2 overexpression induced cell apoptosis in DDP-treated BGC823/DDP and SGC7901/DDP cells (Figure 3). By contrast, HIPK2 knockdown reduced the apoptosis of BGC823/DDP and SGC7901/DDP cells induced by DDP. Therefore, HIPK2 contributed to DDP-induced cell apoptosis.

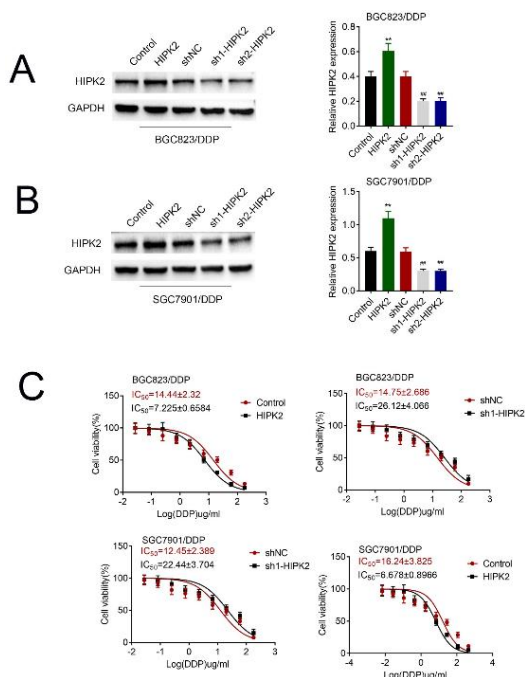


Figure 2: HIPK2 upregulation relieves DDP resistance in GC cells. (A, B) Transfection efficiency of HIPK2 shRNA or HIPK plasmid in cells was detected by immunoblot assays. (C) The viability assay of cells transfected with control or HIPK2 shRNA or plasmid following DDP treatment; $*p < 0.05$; $**p < 0.01$

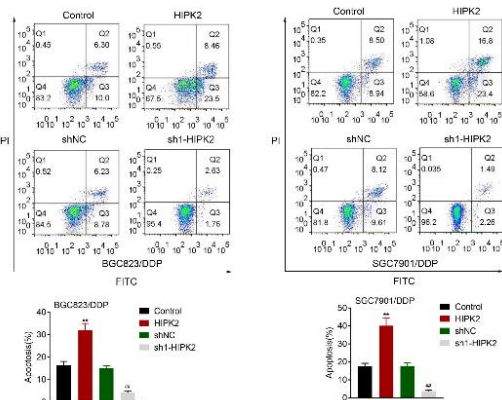


Figure 3: HIPK2 contributes to DDP-induced cell apoptosis. (A) Flow cytometry analysis in GC cells transfected with control, HIPK2, shRNA or sh-HIPK2; $**p < 0.01$

p53 inhibition weakens the suppressive effects of HIPK2 the DDP resistance in GC cells

Tumor suppressor *p53*, together with HIPK2, is important for solid tumor chemosensitivity. Therefore, we assessed whether *p53* is involved in HIPK2-mediated DDP resistance. Consistently, HIPK2 overexpression sensitized GC cells exposed to DDP. *p53* knockdown

decreased cell sensitivity to DDP in BGC823/DDP and SGC7901/DDP cells (Figure 4 A). In addition, the increased cell apoptosis induced by HIPK2 overexpression was reversed by p53 knockdown in BGC823/DDP and SGC7901/DDP cells (Figure 4B). HIPK2 overexpression also activated phosphorylated p53 without affecting the total p53 level in BGC823/DDP and SGC7901/DDP cells. This activated effect was then abrogated by p53 depletion (Figure 5). The results suggest that p53 inhibition weakens the suppressive role of HIPK2 in the DDP resistance in GC cells.

DISCUSSION

As one of the most common malignant tumor in China, GC ranks first among malignant tumors due to its mortality rate [14]. The onset of gastric cancer is insidious and rapid, and most patients are diagnosed in the advanced stage [15]. The 5-year cumulative survival rate is approximately 20% [16]. Cisplatin, with few side effects, is a standard chemotherapy drug for gastrointestinal cancer. Its therapeutic effect has been widely recognized [5], playing a therapeutic role by blocking the G2 phase of the cell cycle. Although DDP is the most commonly used chemotherapy drug to treat advanced GC, its long-term use leads to chemotherapy resistance, markedly limiting the therapeutic effect [17]. Therefore, elucidating the mechanism underlying the chemotherapy resistance to DDP in GC is crucial. In this study, a nuclear localization protein, HIPK2, was observed to reduce the resistance of GC cells to DDP. The data showed that HIPK2 expression was reduced in DDP-resistant GC cells. Importantly, the upregulation of HIPK2 suppressed growth and promoted apoptosis of DDP-induced GC cells. Therefore, HIPK2 serves as a potential and important molecular factor to regulate the resistance of GC cells to DDP.

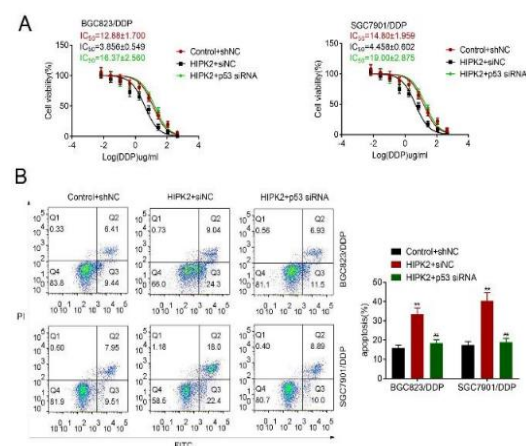


Figure 4: Inhibition of p53 weakens the inhibitory effect of HIPK2 on the DDP resistance in GC cells. (A) The viability assay of GC cells transfected with control, HIPK2 plasmid or HIPK2 plasmid plus p53 depletion following DDP treatment. (B) Flow cytometry analysis in cells transfected with control plasmid, HIPK2 plasmid or HIPK2 plasmid with p53 depletion following DDP treatment.

HIPK2 is a multi-functional tumor suppression protein that affects cancer cell growth and apoptosis [18]. Importantly, HIPK2 suppresses the pathways of cancer chemoresistance [19]. For instance, decreased HIPK2 expression is induced by hypoxia and hyperglycemia treatment [19]. Moreover, its depletion promotes cancer progression and resistance to chemotherapy drugs [20].

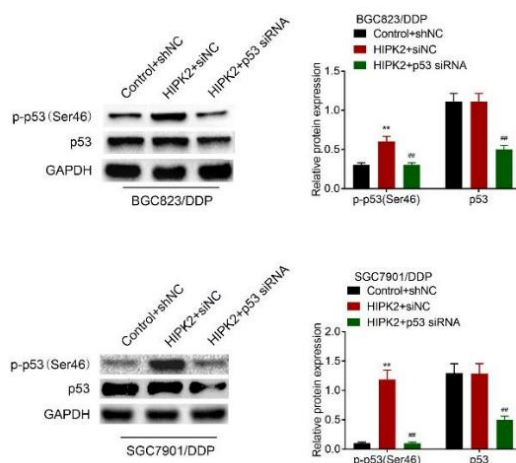


Figure 5: The expression of p53 and phospho-p53 in cells with HIPK2 overexpression or HIPK2 overexpression with p53 depletion was detected by immunoblot assays. ***p* < 0.01

Similarly, this study provided evidence that HIPK2 expression was decreased in DDP-resistant GC cells, suggesting its important participation in DDP resistance in GC. Further analysis showed that HIPK2 could regulate the resistance to DDP in GC cells by p53 pathway, indicating the participation of HIPK2/p53 axis in chemoresistance to DDP in GC. Other studies also attached importance to the importance of HIPK2/p53 axis in chemoresistance. 4-hydroxybenzoic acid (4-HBA) increases the sensitivity of breast cancer cells to adriamycin via promoting the HIPK2/p53 axis [21]. HIPK2 also inhibits cell metastasis and improves the chemosensitivity levels of ESCC [13].

HIPK2 affects the progression of multiple cancer types. HIPK2 overexpression was reported in papillomavirus-positive tonsillar squamous cell carcinoma and correlated with the prognosis of

patients [22]. HIPK2 regulates malignant growth through the phosphorylation of Notch1 [23]. Many studies also revealed the anti-tumorigenic mechanisms of HIPK2, such as apoptosis stimulation, angiogenesis inhibition, and metastasis prevention [17]. These studies, together with the findings in this study, confirmed the key cancer suppressive role of HIPK2.

One of the limitations in the present study is the lack of mechanism underlying the interactive relationship between HIPK2 and p53. A previous study indicated that HIPK2 protein neutralizes MDM2 inhibition, rescuing the transcriptional activity of p53 and apoptotic function [17]. p53 is a cancer suppressor and the HIPK2/p53 activity is important for solid tumor chemosensitivity [24]. Another study showed that Daxx cooperates with the HIPK2/p53 complex to stimulate cell death [25]. Whether the HIPK2/p53 axis also participates in the regulation of chemotherapy drug resistance in GC through these mechanisms need further investigations.

CONCLUSION

HIPK2 expression is decreased in DDP-resistant GC cells, but its upregulation inhibits growth and promotes apoptosis of GC cells. Additionally, HIPK2 promotes p53 activation and reduces the resistance of GC cells to DDP. Thus, HIPK2 is a potential and important molecular factor for the regulation of the resistance of GC cells to DDP.

DECLARATIONS

Acknowledgement

This work was supported by the Scientific Research Task of Sichuan Provincial Health Department. (Grant no. 130493).

Competing interests

There are no conflicts of interest to disclose.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Jiali Mi designed the study and supervised the data collection. Xing Zhang analyzed and interpreted the data. Yingdong Jia prepared the manuscript for publication and reviewed the draft of the manuscript for publication. All the authors have read and approved the manuscript.

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