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Knockdown of the FoxM1 enhances the sensitivity of gastric cancer cells to cisplatin by targeting Mcl-1

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Resistance to chemotherapy is a main obstacle for effective treatment of gastric cancer, the mechanism of which is still poorly understood. Forkhead box M1 (FoxM1) plays an important role in chemo-resistance of various tumors. This study aimed to explore whether FoxM1 mediated resistance of the gastric cancer cell line SGC7901 to the chemotherapy agent cisplatin (DDP). In the study, we detected FoxM1 and Mcl-1 expression via real time-PCR and western blot and demonstrated that FoxM1 is overexpressed in cisplatin-resistance GC cells and Mcl-1 expression is regulated by FoxM1. We examined SGC7901/DDP cell viability by MTT assay, which revealed that suppression of the FoxM1/Mcl-1 pathway impaired cell viability and thus increased sensitivity to cisplatin in gastric cancer cells. Taken together, the study implied that the FoxM1/Mcl-1 pathway may overcome cispaltin resistance of gastric cancer and provide a new therapeutic target for the treatment of gastric cancer.

1. Introduction

Gastric cancer (GC) is the fourth most frequently occurring cancer around the world and the second leading cause of cancer-related death (Kheir et al. 2011), especially in Eastern Asia. Even though GC incidence has a remarkably decreased in most parts of the world, it is still a great burden to society. The aggressivity of GC is a result of various intracellular events including the inactivation of tumor suppressor genes, activation of various oncogenes, the abnormal expression of growth factors and their receptors (Johnson and Evers 2008; Zheng et al. 2004). However, the molecular mechanisms for GC development and progression need to be further explored. Cisplatin is one of the most widely used and effective chemotherapeutic drugs for treating a variety of tumors including GC (Wood 2012). However, intrinsic and acquired resistance can impair its effectiveness. Thus, discovery of new therapeutic modalities for improving the sensitivity of cancer cells to cisplatin is essential.

Recently, a series of studies suggest that Forkhead box M1 (FoxM1) plays an important role in chemo-resistance of various tumors. FoxM1 is a member of the forkhead transcription factor family, which takes part in cell proliferation, DNA repair and cell cycle progression (Myatt and Lam 2008). Overexpression of FoxM1 has been observed in multiple types of cancer, including liver cancer (Kalinichenko et al. 2004), prostate cancer (Kalin et al. 2006), breast cancer (Bektas et al. 2008), ovarian cancer (Llaurado et al. 2012), colorectal cancer (Uddin et al. 2011), lung cancer (Kim et al. 2006) and gastric cancer (Li et al. 2009). On the other hand, downregulation of FoxM1 can inhibit cell growth, migration and invasion in several cancer types (Ahmad et al. 2010; Wang et al. 2008; Wang et al. 2007). Furthermore, patients with overexpression of FoxM1 were relatively insensitive to chemotherapy, including platinum drugs (Okada et al. 2013; Qu et al. 2013). Other studies showed that overexpression of FoxM1 were associated with poor prognosis in GC patients (Feng et al. 2013; Zeng et al. 2009). These results strongly indicate that FoxM1 may act as a new target for cancer therapy.

Myeloid cell leukemia-1 (Mcl-1) is an anti-apoptotic protein of Bcl-2 family frequently upregulated or overexpressed in malignant cells (Akagi et al. 2013). As an important anti-apoptotic protein, Mcl-1 has been studied in apoptosis and cell cycle in various human cancers such as gastric cancer, hepatocellular carcinoma, colorectal cancer and chronic lymphycytic leukemia (Akagi et al. 2013; Pepper et al. 2008; Schulze-Bergkamen et al. 2008; Sieghart et al. 2006). It has been reported that silencing Mcl-1 could sensitize GC to chemotherapy (Akagi et al. 2013). Even though FoxM1 and Mcl-1 are both related to the chemotherapeutic sensitivity, the FoxM1 mediated Mcl-1-induced insensitivity to cisplatin in GC is still unclear.

In this study, we demonstrate that FoxM1 is overexpressed in cisplatin-resistance GC cells and Mcl-1 expression is regulated by FoxM1. SGC7901/DDP cell viability assay revealed that the knockdown of FxoM1 or Mcl-1 could impair cell viability in the cisplatin-resistant GC cells and thus improve sensitivity to cisplatin in GC cells. Our study strongly suggests that the FoxM1/Mcl-1 signaling pathway may offer an effective method to reverse chemo-resistance in gastric cancer.

2. Investigations and results

2.1. FoxM1 is upregulated in cisplatin-resistant GC cells

To evaluate the role of FoxM1 in cisplatin-resistant GC cells, we measured the expression of FoxM1 in human GC cell line (SGC7901 and SGC7901/DDP) and found that FoxM1 mRNA and protein was highly expressed in SGC7901/DDP cells compared with SGC7901 cells (Fig. 1). The results indicated that the expression of FoxM1 maybe associated with cisplatin-resistance of GC cells.

2.2. Depletion of FoxM1 impaired cell viability in cisplatin-resistant GC cell line

To further determine the effects of FoxM1 on cisplatin-resistant GC cells, we constructed FoxM1 siRNA tranfected SGC7901/DDP cells. Firstly, the transfection efficiency of FoxM1 siRNA was confirmed by detecting the expression of FoxM1 in transfected cells. Compared with the control-treated (no siRNA) GC

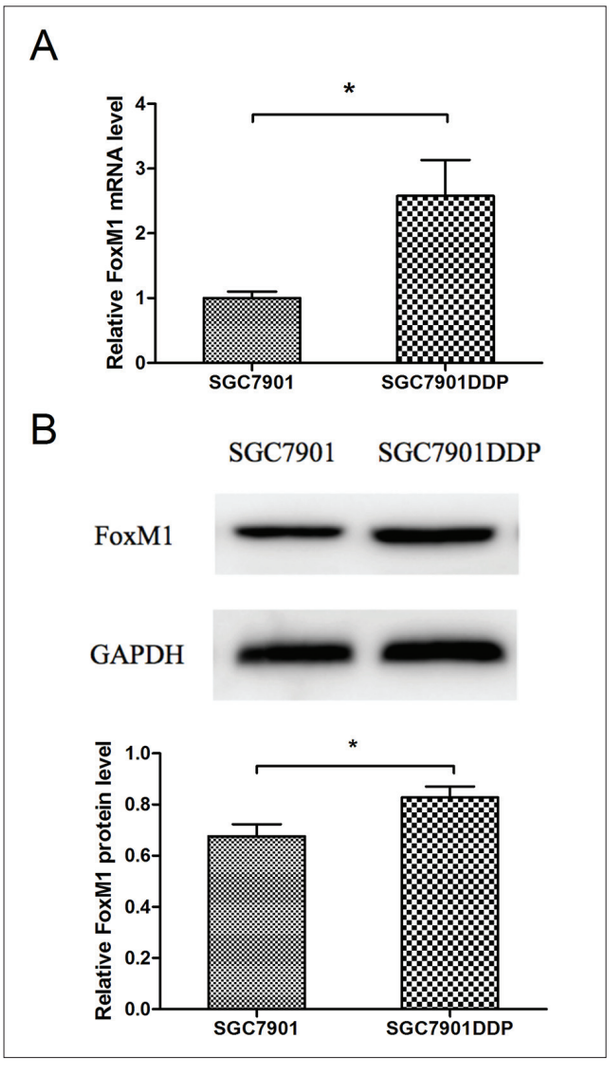

Fig. 1: FoxM1 level was elevated in cisplatin-resistant gastric cancer cells. The FoxM1 expression in SGC7901 and SGC7901/DDP cells was analyzed by real-time PCR (A) and Western blot (B) respectively. The relative mRNA and protein levels were normalized to GAPDH. Data are presented as the mean ± SD of three independent experiments. *, $P < 0.05$

cells, the expression of FoxM1 was obviously downregulated in SGC7901/DDP cells transfected with FoxM1 siRNA at both the mRNA and protein levels (Fig. 2A, 2B). Afterwards, we examined SGC7901/DDP cell viability by MTT assay. As shown in Fig. 2C, the viable cells rate in FoxM1 siRNA group was from 100 % to 33.8 %, which was lower than NC group (the rate was from 100 % to 60.8%), indicating that the knockdown of FxoM1 could impair cell viability in the cisplatin-resistant GC cells.

2.3. Mcl-1 was downregulated in FoxM1 deficient cisplatin-resistant GC cells

To investigate whether FoxM1 could affect Mcl-1 expression in cisplatin-resistant GC cells, RT-PCR and western blot assays were employed to examine Mcl-1 expression in FoxM1 deficient SGC7901/DDP cells. As shown in Fig. 3, depletion of FoxM1 obviously decreased Mcl-1 expression in SGC7901/DDP cells at both the mRNA and protein levels.

2.4. Knockdown of Mcl-1 expression decreases cisplatin-resistant GC cell viability

Compared with the control-treated (scrambled siRNA group) GC cells, the expression of Mcl-1 decreased obviously in SGC7901/

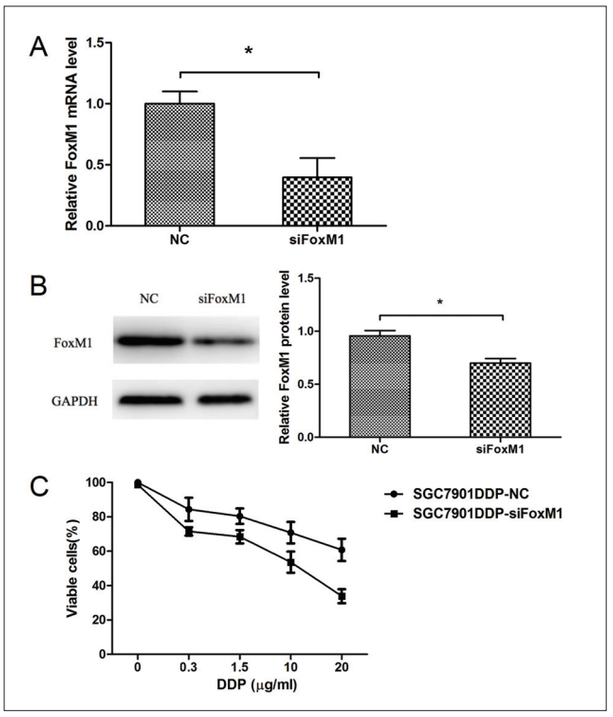

Fig. 2: Depletion of FoxM1 impaired cell viability in cisplatin treatment SGC7901/DDP cells. FoxM1 mRNA (A) and protein (B) expression in SGC7901/DDP cells transfected with FoxM1 siRNA were assayed. (C) MTT tests were performed to detect cell viability of SGC7901/DDP cells. SGC7901/DDP cells were transfected with siFoxM1 or siNC. After transfection, the cells were cultured with cisplatin at different concentrations. The relative mRNA and protein levels were normalized to GAPDH. Data are presented as the mean ± SD of three independent experiments. *, $P < 0.05$

DDP cells transfected with Mcl-1 siRNA at both the mRNA and protein levels (Fig. 4A, 4B), which suggested that Mcl-1 expression was downregulated with Mcl-1 siRNA treatment.

In Fig. 4C, the MTT assay showed that the SGC7901/DDP cell number in Mcl-1 knockdown group (the viable cells rate was from 100 % to 45 %) was significantly decreased compared with NC group (the viable cells rate was from 100 % to 64 %), which revealed that the knockdown of Mcl-1 could attenuate cell viability in cisplatin-resistant GC cells.

3. Discussion

In this study, we showed that FoxM1 was overexpressed in human SGC7901/DDP cells. Moreover, FoxM1 could positively regulate Mcl-1 expression. MTT assays demonstrated that knockdown of FoxM1 or Mcl-1 expression attenuated cell viability of a cisplatin-resistant GC cell line. In other words, inhibition of the FoxM1/Mcl-1 pathway can improve sensitivity to cisplatin in GC cells. Taken together, our study provides a novel signaling pathway and new therapeutic target for gastric cancer patients.

Cisplatin, which is one of the most widely used and effective chemotherapeutic drugs for treating a variety of tumors including GC, triggers DNA crosslinking induced cell death (Xu et al. 2014). It has been reported that overexpression of FoxM1 can increase cisplatin-resistance in cisplatin-sensitive and low FoxM1-expressing ovarian cancer cells (Chiu et al. 2015). In our study, we found that knockdown of FoxM1 downregulated the expression of Mcl-1 and significantly impaired cisplatin-resistant GC cell viability.

FoxM1, a member of the Forkhead superfamily of transcription factors, regulate the process of cancer progression, tumorigenesis and chemotherapy resistance. Recently, it has been reported that FoxM1 may play a key role in determining sensitivity to anticancer drugs (Koo et al. 2012; Myatt and Lam 2008). In this study, we found that FoxM1 was overexpressed in SGC7901/DDP cells

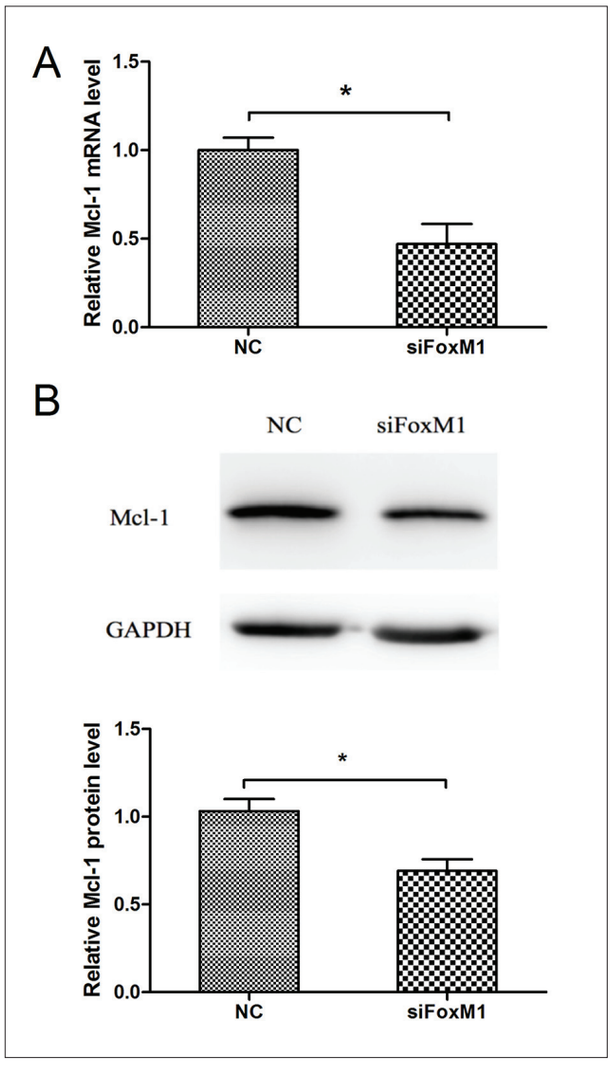

Fig. 3: Mcl-1 was down-regulated with knockdown of FoxM1. The expression of Mcl-1 was assayed by real-time PCR (A) and Western blot (B) respectively. The relative mRNA and protein levels were normalized to GAPDH. Data are presented as the mean ± SD of three independent experiments. *, $P < 0.05$

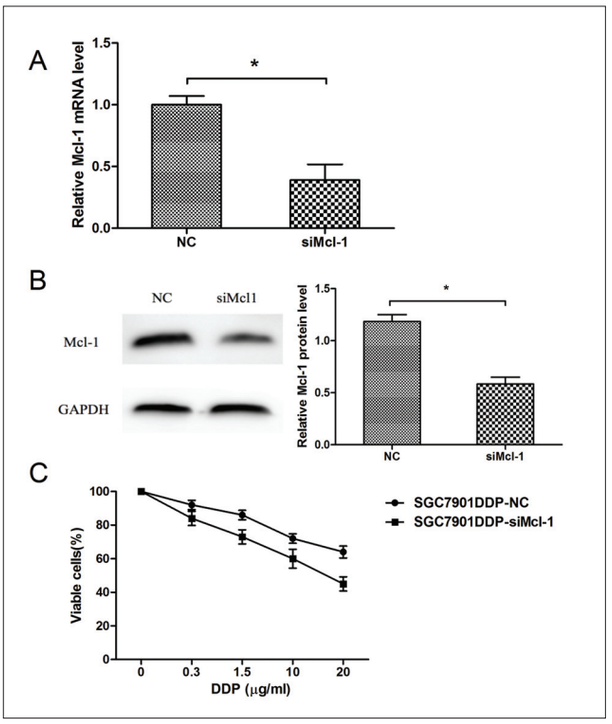

Fig. 4: Knockdown of Mcl-1 impaired cell viability in the cisplatin treatment SGC7901/DDP cells. Mcl-1 mRNA (A) and protein (B) expression in SGC7901/DDP cells transfected with Mcl-1 siRNA were assayed. (C) MTT tests were performed to detect cell viability of SGC7901/DDP cells. SGC7901/DDP cells were transfected with siMcl-1 or siNC. After transfection, the cells were cultured with cisplatin at different concentrations. The relative mRNA and protein levels were normalized to GAPDH. Data are presented as the mean ± SD of three independent experiments. *, $P < 0.05$

compared with SGC7901 cells. Moreover, we have shown that knockdown of FoxM1 in SGC7901/DDP cells increased sensitivity and attenuated cisplatin-resistant GC cell viability.

As an important anti-apoptotic protein and therapeutic target, Mcl-1 draws more attention in cancer therapy, especially in drug resistance. We have found that FoxM1 can positively regulate Mcl-1 expression in SGC7901/DDP cells and influence the cisplatin sensitivity of gastric cancer cells. Here, we demonstrated that Mcl-1 could be as a target of FoxM1 in SGC7901/DDP cells. Thus, we conclude that FoxM1 might take part in cisplatin drug-resistance by positively regulating Mcl-1 expression in SGC7901/DDP cells.

In conclusion, this findings may provide a new therapeutic target for improving the sensitivity of gastric cancer cells and can be used for treating chemotherapy resistance in gastric cancer patients. However, more research is needed to further study the mechanism of FoxM1 in cisplatin resistance of gastric cancer cells. Of course, a clear investigation of the FoxM1/Mcl-1 pathway will provide new ways to overcome FoxM1-mediated cisplatin resistance.

4. Experimental

4.1. Cell culture

The human gastric carcinoma cell line SGC7901 was obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The cell line was cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere containing 5% CO_2 at 37 °C. Cisplatin was obtained from Sigma-Aldrich. The cisplatin-resistant SGC7901/DDP cells were developed from the parental SGC7901 cells that were subjected to persistent gradient exposure to cisplatin for about 12 months, through increasing cisplatin concentration from 0.05 μg/ml until the cells acquired resistance to 1 μg/ml (Xu et al. 2014). Prior to each experiment, SGC7901/DDP cells were cultured in drug-free RPMI 1640 medium for 2 weeks.

4.2. Small interfering RNA and transfection

Human gastric cancer cells were tranfected with 100 nmol/L human FoxM1 or Mcl-1 siRNA using Lipofectamine 2000 (Invitrogen). The medium was removed after 24 h transfection. The cells were incubated in RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere containing 5% CO_2 at 37 °C.

4.3. RT-PCR

The primer sequences for PCR amplification were as follows, FoxM1: 5'-CACCCCAGTGCCAACCGCTACTTG-3' (forward) and 5'-AAAGAGGAGC-TATCCCCTCCTCAG-3' (reverse), Mcl-1: 5'-TCAGCGACGGCGTAACAAACT-3' (forward) and 5'-ACAAACCCATCCCAGCCTCTT-3' (reverse), the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control: 5' -GGTCTCCTCTGACTTCAACA-3' (forward) and 5'-GTGAGG-GTCTCTCTCTTCCT-3' (reverse). Complementary DNA (cDNA) was generated with the high-capacity cDNA reverse transcription kit (Roche). The expression of mRNA of human FoxM1 and Mcl-1 was measured by real-time with a LightCycler 3.5 instrument (Roche Diagnostics, Mannheim, Germany). The PCR cycling conditions were 95 °C for 5 min and 26 cycles of (95 °C for 15 s, 58 °C for 30 s and 72 °C for 45 s).

4.4. Western blotting

Western blots were performed based on the standard procedures. Briefly, the cells were washed with PBS (pH 7.4) and lysed by an ice-cold lysis buffer. After the gastric cancer cells were lysed, the protein concentrations were measured by a bicinchoninic acid (BCA) protein assay, and western blotting analyses were performed as previously described (Ni et al. 2014). An anti GAPDH antibody (Invitrogen) was used to normalize sample loading and transfer. The intensities of the bands were quantified using NIH ImageJ software package (http://rsb.info.nih.gov/ij/).

4.5. Cell viability assay

Cell viability was assessed by MTT assay. Cells were seeded onto 96-well plates and incubated overnight under the usual culture conditions. Then cells were exposed to ciaplatin at various concentrations. After 20 µl of MTT solution was added into each well, the plates were incubated for 4 h at 37 °C. 150 µl DMSO was added into each well and incubated for 10 min. The optical density at 490 nm was read by an enzyme-linked immunosorbent assay (ELISA) reader.

4.6. Statistical analysis

Data were expressed as mean±SD. SPSS statistical software package, version17.0 (SPSS, Chicago, IL, USA) was used for statistical tests. The data were compared between two groups using the two-tailed Student's t-test. P value < 0.05 is considered as statistically significant.

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