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ORIGINAL ARTICLE – CANCER RESEARCH

METase/lncRNA HULC/FoxM1 reduced cisplatin resistance in gastric cancer by suppressing autophagy

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Abstract

Background Autophagy plays an important role in regulating cisplatin (CDDP) resistance in gastric cancer cells. However, the underlying mechanism of methioninase (METase) in the regulation of autophagy and CDDP resistance of gastric cancer cells is still not clear.

Materials and methods Western blot was used to detect the levels of autophagy-related proteins, multidrug-resistant 1 (MDR-1), and FoxM1 protein. LncRNA HULC was detected by qRT-PCR. Cell viability was detected using CCK-8 assay. The interaction between lncRNA HULC and FoxM1 was confirmed by RNA pull-down and RIP assay.

Results Lentiviral vector carrying METase (LV-METase) suppressed autophagy and CDDP resistance of drug-resistant gastric cancer cells. LncRNA HULC was significantly downregulated in drug-resistant gastric cancer cells transfected with LV-METase. Besides, we found that lncRNA HULC interacted with FoxM1. In addition, METase suppressed autophagy to reduce CDDP resistance of drug-resistant gastric cancer cells through regulating HULC/FoxM1, and interfering HULC suppressed autophagy to reduce CDDP resistance of drug-resistant gastric cancer cells through regulating FoxM1. Finally, interfering HULC inhibited tumor growth in vivo.

Conclusion METase suppressed autophagy to reduce CDDP resistance of drug-resistant gastric cancer cells through regulating HULC/FoxM1 pathway.

Keywords Methioninase · Cisplatin resistance · lncRNA HULC · FoxM1 · Gastric cancer

Introduction

Gastric cancer is one of the most common malignant cancers and the third leading cause of cancer-related death in the world (Siegel et al. 2018). Although preoperative and postoperative combined chemotherapy improves the overall survival rate of gastric cancer patients, drug resistance remains one of the major obstacles for effective cancer chemotherapy (Orditura et al. 2014). Currently, cisplatin (CDDP) is the main chemotherapy drugs for the treatment of gastric cancer patients, especially for patients with advanced gastric cancer (Pasini et al. 2011). However, recurrence of gastric cancer is common for patients who received CDDP chemotherapy, and the underlying mechanism of CDDP resistance is unclear. Fortunately, studies have shown that the inhibition of autophagy can increase the sensitivity of gastric cancer cells to CDDP and reduce multidrug resistance of gastric cancer cells (An et al. 2015; Kumar et al. 2015). Therefore, inhibition of autophagy may overcome CDDP resistance and provide new strategies for the treatment of gastric cancer.

Methioninase (METase), also called L-methionine-α-amino-γ-mercaptoethane lyase, has been reported to play important roles in inhibiting the growth of cancers (Kawaguchi et al. 2019). METase has anti-tumor effects and can overcome drug resistance in vivo and in vitro (Hu and Cheung 2009; Kawaguchi et al. 2018b). In patient-derived orthotopic xenograft mice model, intraperitoneal injection of recombinant METase (rMETase) reduced the tumor size and inhibited the growth of pancreatic cancer, melanoma, and Ewing's sarcoma (Kawaguchi et al. 2017, 2018a). In addition, combination of METase and chemotherapy drugs showed synergistic effects when overcome the doxorubicin

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resistance of undifferentiated spindle cell sarcoma (Igarashi et al. 2018). Importantly, our previous report has found that overexpressed METase increased the sensitivity of drug-resistant gastric cancer cells to CDDP (Xin et al. 2018a). And overexpressed METase promoted the apoptosis of gastric cancer cells through regulating the autophagy (Xin et al. 2019). However, the regulation role of METase in the autophagy of drug-resistant gastric cancer cells is not clear.

Long non-coding RNAs (lncRNAs) are a group of RNAs that are greater than 200 nucleotides in length, which have been reported to be oncogene or tumor suppressors in gastric cancer (Wu et al. 2019; Yu et al. 2019). In addition, lncRNAs have been identified to be involved in autophagy-associated drug resistance (CDDP or vincristine) in gastric cancer (Xi et al. 2019; YiRen et al. 2017). LncRNA highly up-regulated in liver cancer (HULC) was first discovered in liver cancer and is related with the chemosensitivity of hepatocellular carcinoma cells (Xiong et al. 2017). Researchers also found that lncRNA HULC was highly expressed in drug-resistant gastric cancer cells and silencing HULC increased CDDP-induced apoptosis of gastric cancer cells (Zhang et al. 2016). However, the underlying mechanism of lncRNA HULC in drug resistance of gastric cancer is still not clear. Based on the regulation of METase on lncRNA (Xin et al. 2019), we further investigated the abnormally expressed lncRNAs in drug-resistant gastric cancer cells under the treatment of overexpressed METase, and found that lncRNA HULC was remarkably downregulated after the treatment of overexpressed METase, indicating that METase may affect the autophagy of drug-resistant gastric cancer cells through regulating lncRNA HULC.

In this study, the results showed that lncRNA HULC and its downstream protein FoxM1 were regulated by METase. In addition, METase suppressed autophagy through regulating HULC/FoxM1 to reduce CDDP resistance of drug-resistant gastric cancer cells in vitro. Knockdown of HULC also reduced tumor volume and autophagy of gastric cancer in vivo.

Materials and methods

Cell lines and cell transfection

Human gastric cancer cell lines SGC7901 and MGC-803 were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). Cells were incubated in RPMI 1640 medium (Gibco, NY, USA) supplemented with 1.5 g/l NaHCO3, 2.5 g/l glucose, 0.11 g/l sodium pyruvate, and 10% fetal bovine serum (FBS; Gibco) in a humidified incubator with 5% CO_2 at 37 °C. Cisplatin (CDDP) was purchased from Sigma-Aldrich (Missouri, USA). The CDDP-resistant SGC7901/CDDP and MGC-803/CDDP cells were developed from parental SGC7901 and MGC-803 cells that were persistently treated with increasing concentration of CDDP from 0.06 μg/ml to 1 μg/ml (Xin et al. 2018a).

METase lentiviral vector (LV-METase) and negative control lentiviral vector (LV-NC) were purchased from Shanghai Cancer Institute. pcDNA-HULC was constructed by inserting HULC cDNA into pcDNA3.1 (Invitrogen, CA, USA), and pcDNA-FoxM1 was constructed by inserting FoxM1 cDNA into pcDNA3.1. Small interference RNA-targeting HULC (si-HULC), si-FoxM1, and negative control (si-control) were purchased from RiboBio Co., Ltd. (Guangzhou, China). Cells (2×10^4) were seeded into 96-well plates and transfected with appropriate concentrations of vectors using Lipofectamine 2000 transfection reagent (Invitrogen) in accordance with the manufacturer's instructions.

Western blot

Total proteins were collected from gastric cancer cells or tumor tissues using RIPA lysis and extraction buffer (Thermo Scientific, CA, USA), and the concentration of proteins was measured using Bradford protein concentration assay kit (Beyotime Biotechnology, Nantong, China). Then, proteins were separated by 12% SDS-PAGE and transferred to PVDF membranes. The membranes were blocked with 5% non-fat milk and incubated with primary antibodies against LC3B (LC3-I and LC3-II are the proteins that are labeled by LC3B antibody; 1:3000; Abcam, Cambridge, UK), p62 (1:10,000; Abcam), Beclin 1 (1:2000; Abcam), MDR-1 (Cell Signaling Technology, MA, USA), FoxM1 (1:500; Abcam), β-actin (1:5000; Abcam) overnight at 4 °C. Subsequently, the membranes were incubated with HRP-conjugated secondary antibody (1:3000; Abcam) for 2 h. The bands were visualized using enhanced chemiluminescence (ECL) kit (Santa Cruz Biotechnology, CA, USA) and recorded with ChemImager 5500 V2.03 software. β-actin was used as an internal reference.

CCK-8 assay

Cell viability of SGC7901/CDDP and MGC-803/CDDP cells was detected using Cell Counting Kit-8 (CCK-8) assay. Cells (4×10^3) with different treatments were seeded into 96-well plates with 100 μl RPMI 1640 medium per well. Then, 10 μl CCK-8 solution was added into each well and incubated at 37 °C for 2 h. The absorbance was measured at 450 nm using a microplate reader (SpectraMax M5; Molecular Devices, CA, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from gastric cancer cells or tumor tissues using TRIzol reagent (Beyotime Biotechnology).

cDNA was synthesized using High-Capacity RNA-to-cDNA Kit (Applied Biosystems), and qRT-PCR was performed using SYBR Green Real-Time PCR Master Mixes (Applied Biosystems, CA, USA) and conducted on Mx3000P real-time PCR system (Stratagene, CA, USA) under the following conditions: pre-denaturation at 95 °C for 10 min, denaturation at 95 °C for 10 s, annealing at 60 °C for 20 s, and extension at 72 °C for 34 s, for 40 cycles. The relative HULC, MRUL, AK022798, ANRIL, and GHET1 were calculated using the $2^{-\Delta\Delta CT}$ method and normalized to GAPDH expression.

Gene	Primer sequence
HULC	F: 5′-CTGGCAATAAACTAAGCA-3′ R: 5′- CAACATAATTCAGGGAGAA-3′
MRUL	F: 5′-ACCCACAGACAACTGTGGACCC-3′ R: 5′-GCCGCCCCTATTGTTGCCCA-3′
AK022798	F 5′-TGTGTCAGGGTGAGATGGTT-3′ R 5′-TTGGCAAAATTCACAGCATT-3′
ANRIL	F: 5′-TGCCGGAGCTGTCGACCC-3′ R: 5′-CTTTGATCTCTGCTGTTGAATCAGAATG-3′
GHET1	F: 5′-CAACAAAGCAGGTAAACATTGG-3′ R: 5′-GCAAAGGCAGAGTGAAAGGT-3′
GAPDH	F: 5′-GAACAAAGCAGGTAAACATTGG-3′ R: 5′-GACAAGCTTCCCGTTCTCAG-3′

RNA pull-down assay

Pierce Magnetic RNA–Protein Pull-Down Kit (Thermo Scientific) was used to conduct RNA pull-down assay. Cells were rinsed in cold phosphate-buffer solution and lysed using Pierce IP Lysis Buffer containing protease inhibitor. Pierce RNA 3′ End Desthiobiotinylation kit was used to label HULC RNA. Streptavidin magnetic beads were washed with 20 mM Tris and re-suspended with 1X RNA capture buffer. Then, 50 pmol labeled HULC RNA was added into proteins isolated from cells and incubated with the buffer containing streptavidin magnetic beads. Eluent was used to wash the beads, and the HULC-FoxM1 complex binding to beads was detected using western blot.

RNA immunoprecipitation (RIP) assay

Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore, MA, USA) was used to conduct RIP assay. Cells were collected and lysed using RIP lysis buffer containing 0.25 µl protease inhibitor and 0.125 µl RNase inhibitor. The supernatant was incubated with magnetic beads washed with RIP buffer and conjugated with FoxM1 antibody or normal rabbit IgG (negative control). The precipitated RNA was collected and stored for qRT-PCR analysis.

Ubiquitination assay

HA-Ub, FLAG-FoxM1, si-HULC, and si-control were transfected into SGC7901/CDDP and MGC-803/CDDP cells. After 36 h transfection, MG132 (10 nM; Sigma-Aldrich) was added to RPMI 1640 medium for 8 h. Then, cells were collected and lysed, and cell extraction was immunoprecipitated with labeled antibodies for 12 h at 4 °C. FoxM1 protein level was detected using western blot.

Nude mouse xenograft

Female BALB/c nude mice (4–6 weeks) were obtained from the laboratory animal center of Nanchang University, and kept in a 12 h light/12 h dark cycle environment at 24 °C with free access to water and food. SGC7901/CDDP cells (2×10^6) stably transfected with sh-HULC or sh-NC were subcutaneously injected into the left posterior ventral side of the mice. The nude mice were divided into sh-NC and sh-HULC groups, with six mice in each group. Seven days after injection of SGC7901/CDDP cells, 3.0 mg/kg CDDP was intraperitoneally injected every 3 days. The mice were sacrificed 35 days after SGC7901/CDDP cells injection. Tumor size was measured using a caliper and the volume was calculated using the formula: volume = length × width^2/2. The tumor tissues were collected for the detection of HULC, FoxM1, and autophagy-related proteins expressions. All animal experiments were approved by the Ethics Committee of The Second Affiliated Hospital of Nanchang University, and were conducted in accordance with the Declaration of Helsinki.

Statistical analysis

All data were presented as mean ± standard deviation (SD), and the results were analyzed using SPSS 18.0 software. Results were compared using Student's t test or one-way analysis of variance (ANOVA). *P* value less than 0.05 was considered statistically significant.

Results

Overexpressed METase suppressed autophagy and CDDP resistance of drug-resistant gastric cancer cells

When compared with LV-NC group, autophagy-related protein beclin 1 and the ratio of LC3-II/LC3-I were obviously reduced in CDDP-resistant gastric cancer cell lines (SGC7901/CDDP and MGC-803/CDDP) transfected with LV-METase, whereas autophagy-related protein p62 was obviously increased (Fig. 1a). Cell viability of SGC7901/

CDDP and MGC-823/CDDP cells was significantly reduced in LV-METase group after the stimulation of 1 μg/ml CDDP for 0, 24, 48, and 72 h (Fig. 1b). Besides, protein level of multidrug-resistant 1 (MDR-1) was obviously reduced in LV-METase group (Fig. 1c). These findings indicated that METase suppressed autophagy and CDDP resistance of SGC7901/CDDP and MGC-803/CDDP cells.

Selection of abnormally expressed lncRNAs in drug-resistant gastric cancer cells by transfecting with LV-METase

To determine whether METase affect the autophagy of drug-resistant gastric cancer cells through regulating lncRNAs, we detected the expressions of several lncRNAs in SGC7901/CDDP and MGC-803/CDDP cells transfected with LV-METase. As shown in Fig. 2, lncRNA HULC, lncRNA MRUL, lncRNA AK022798, lncRNA ANRIL, and lncRNA GHET1 expressions were detected, and only lncRNA HULC was significantly downregulated in SGC7901/CDDP and MGC-803/CDDP cells transfected with LV-METase, indicating that METase may affect the autophagy of drug-resistant gastric cancer cells through inhibiting lncRNA HULC expression.

LncRNA HULC interacted with FoxM1

According to the prediction of bioinformatics software (RPISeq), lncRNA HULC can bind to FoxM1 protein. To verify the interaction between lncRNA HULC and FoxM1, we conducted RNA pull-down assay and RIP assay. As shown Fig. 3a, b, FoxM1 was detected in HULC pull-down

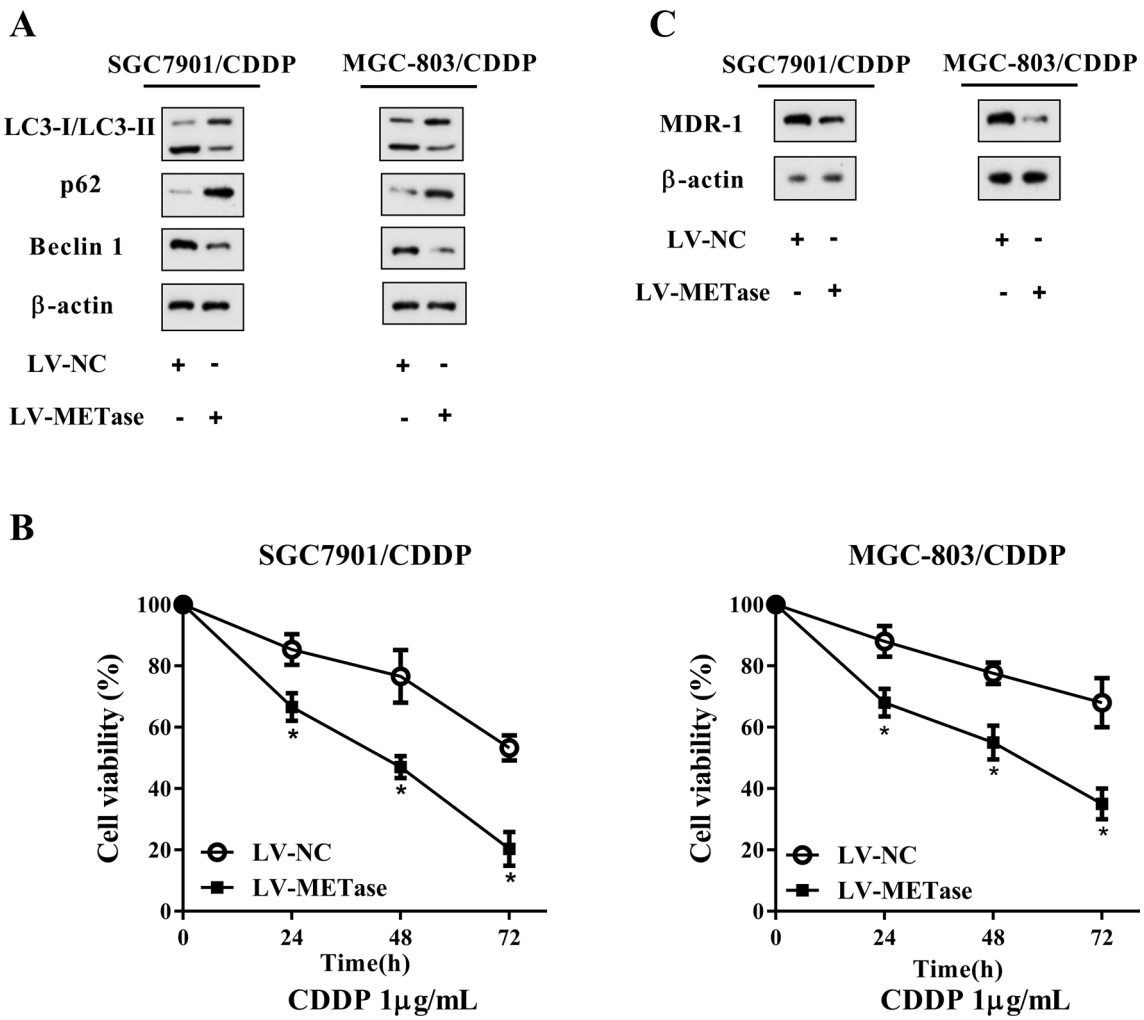

Fig. 1 Overexpressed METase suppressed autophagy and drug resistance of drug-resistant gastric cancer cells. Cisplatin-resistant gastric cancer cell lines (SGC7901/CDDP and MGC-803/CDDP) were transfected with LV-METase or LV-NC. **a** After 24 h, autophagy-related proteins (LC3-I, LC3-II, p62, and beclin 1) were detected using western blot. **b** Cell viability in SGC7901/DDP and MGC-823/DDP cells was detected under the stimulation of 1 μg/ml CDDP for 0, 24, 48, and 72 h using CCK-8 assay. **c** Protein level of multidrug-resistant 1 (MDR-1) was detected using western blot. *$P < 0.05$ vs LV-NC

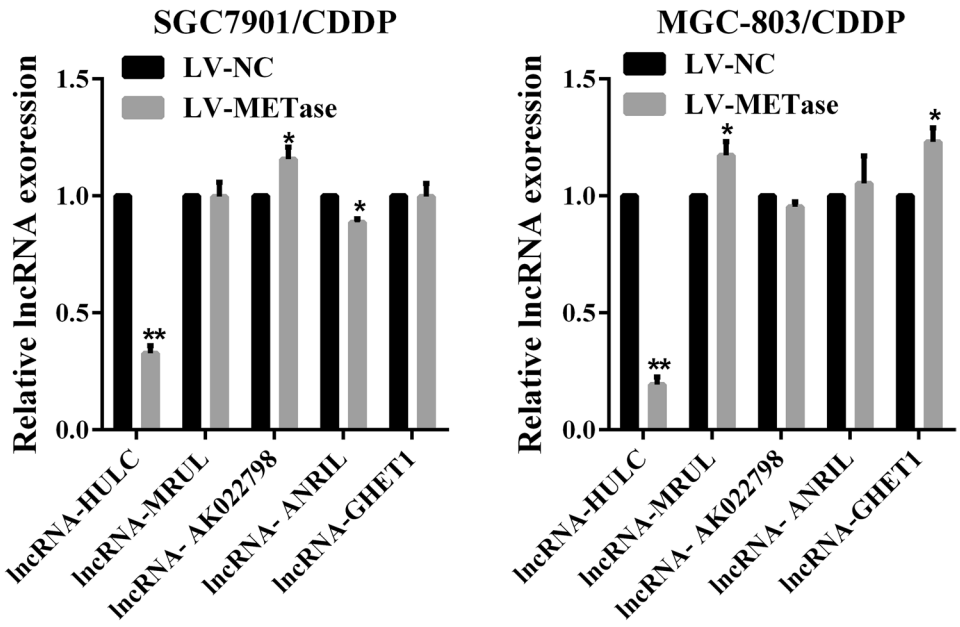

Fig. 2 Selection of abnormally expressed lncRNAs in drug-resistant gastric cancer cells by transfecting with LV-METase. SGC7901/CDDP and MGC-803/CDDP cells were transfected with LV-METase or LV-NC. LncRNA HULC, lncRNA MRUL, lncRNA AK022798, lncRNA ANRIL, and lncRNA GHET1 were detected using qRT-PCR. *$P < 0.05$ vs LV-NC

complex, and lncRNA HULC was accumulated in FoxM1 precipitate, indicating that lncRNA HULC could interact with FoxM1. pcDNA-HULC promoted FoxM1 protein level in SGC7901 and MGC-803 cells, and si-HULC reduced FoxM1 protein level in SGC7901/CDDP and MGC-803/CDDP cells (Fig. 3c), indicating lncRNA HULC positively regulated FoxM1 protein level in gastric cancer cells and drug-resistant gastric cancer cells. Under the treatment of CHX, FoxM1 protein level was gradually reduced with the increase of time in si-HULC group (Fig. 3d), indicating that lncRNA HULC stabilized the FoxM1 protein. Ubiquitination assay showed si-HULC promoted FoxM1 ubiquitination (Fig. 3e). These findings indicated that lncRNA HULC interacted with FoxM1 and regulated FoxM1 protein level through regulating its degradation.

METase downregulated FoxM1 protein level and suppressed autophagy to reduce CDDP resistance of drug-resistant gastric cancer cells through regulating HULC

SGC7901/CDDP and MGC-803/CDDP cells were transfected with LV-METase and pcDNA-HULC. qRT-PCR analysis indicated that LV-METase decreased HULC expression, whereas pcDNA-HULC promoted HULC expression (Fig. 4a). Western blot analysis indicated that LV-METase reduced FoxM1 protein level, whereas pcDNA-HULC promoted FoxM1 protein level (Fig. 4a), indicating that METase downregulated FoxM1 protein level through regulating HULC. Then, 1 mM autophagy inhibitor 3-MA was added into cells. We found LV-METase reduced the expression of beclin 1 and the ratio of LC3-II/LC3-I, LV-METase + pcDNA-HULC promoted the expression of beclin 1 and the ratio of LC3-II/LC3-I, and 3-MA further reduced cell autophagy (Fig. 4b), indicating that METase suppressed autophagy through regulating HULC. In addition, LV-METase reduced cell viability, LV-METase + pcDNA-HULC increased cell viability, and 3-MA further reduced cell viability (Fig. 4c), indicating that METase reduce CDDP resistance of SGC7901/CDDP and MGC-803/CDDP cells through regulating HULC. The trend of MDR-1 protein level also indicated that METase reduced CDDP resistance of SGC7901/CDDP and MGC-803/CDDP cells through regulating HULC (Fig. 4c).

Interfering HULC suppressed autophagy to reduce CDDP resistance of drug-resistant gastric cancer cells through regulating FoxM1

As shown in Fig. 5a, si-HULC significantly decreased HULC expression and FoxM1 protein level, and pcDNA-FoxM1 further promoted FoxM1 protein level in SGC7901/CDDP and MGC-803/CDDP cells (Fig. 5a). Then, 1 mM autophagy inhibitor 3-MA was added into cells. We found that si-HULC reduced the expression of beclin 1 and the ratio of LC3-II/LC3-I, si-HULC + pcDNA-FoxM1 increased the expression of beclin 1 and the ratio of LC3-II/LC3-I, and 3-MA further reduced cell autophagy (Fig. 5b). In addition, si-HULC inhibited cell viability, si-HULC + pcDNA-FoxM1 promoted cell viability, and 3-MA further inhibited cell viability (Fig. 5c), indicating that HULC reduced CDDP resistance of SGC7901/CDDP and MGC-803/CDDP cells through FoxM1. The trend of MDR-1 protein level also indicated HULC reduced CDDP resistance of SGC7901/CDDP and MGC-803/CDDP cells through FoxM1 (Fig. 5c). The trend of MDR-1 protein level also indicated that HULC

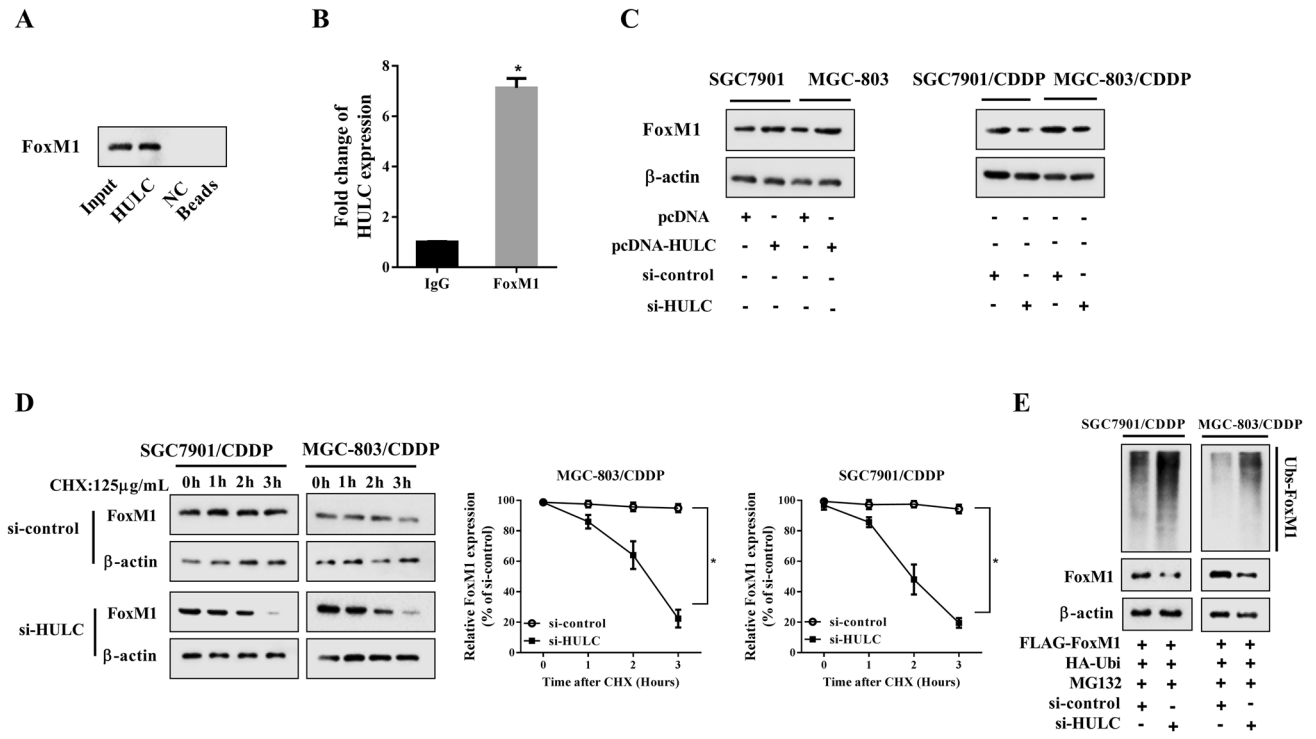

Fig. 3 LncRNA HULC interacted with FoxM1. **a** RNA pull-down assay showed that FoxM1 was detected in HULC pull-down complex. NC was used as negative control of HULC group. **b** RIP assay showed that lncRNA HULC was accumulated in FoxM1 precipitate. IgG was used as negative control. $^*P < 0.05$ vs IgG. **c** SGC7901 and MGC-803 cells were transfected with pcDNA or pcDNA-HULC. SGC7901/CDDP and MGC-803/CDDP cells were transfected with si-control or si-HULC. FoxM1 protein level was detected using western blot. **d** SGC7901/CDDP and MGC-803/CDDP cells were transfected with si-control or si-HULC, and then, 125 μg/ml CHX was used to treat cells for 0, 1, 2, and 3 h. FoxM1 protein level was detected using western blot. $^*P < 0.05$ vs si-control. **e** SGC7901/CDDP and MGC-803/CDDP cells were transfected with FLAG-FoxM1, HA-Ub, si-control, or si-HULC. Then, ubiquitination inhibitor MG132 was used to treat cells. Ubiquitination assay was used to detect FoxM1 ubiquitination, and Flag antibody was used to detect FoxM1 protein level

reduced CDDP resistance of SGC7901/CDDP and MGC-803/CDDP cells through FoxM1 (Fig. 5c).

Overexpressed HULC promoted autophagy to promote CDDP resistance of gastric cancer cells through regulating FoxM1

As shown in Fig. 6a, pcDNA-HULC significantly increased HULC expression and FoxM1 protein level, and si-FoxM1 did not change the expression of HULC expression in MGC-803 and SGC7901 cells. In addition, pcDNA-HULC increased the expression of beclin 1 and the ratio of LC3-II/LC3-I, pcDNA-HULC + si-FoxM1 decreased the expression of beclin 1 and the ratio of LC3-II/LC3-I, and autophagy antagonist RAPA further promoted cell autophagy (Fig. 6b). Besides, cell viability was significantly promoted by pcDNA-HULC, inhibited by pcDNA-HULC + si-FoxM1, and further increased by RAPA (Fig. 6c), indicating HULC promoted CDDP resistance of gastric cancer cells through regulating FoxM1. The trend of MDR-1 protein level also indicated HULC promoted CDDP resistance of gastric cancer cells through regulating FoxM1 (Fig. 6c).

Interfering HULC inhibited tumor growth in vivo

Compared with sh-NC group, the tumor volume was significantly reduced in sh-HULC group (Fig. 7a). Besides, HULC expression and FoxM1 protein level were significantly decreased in tumor tissues of sh-HULC group (Fig. 7b). Autophagy-related proteins beclin 1 and the ratio of LC3-II/LC3-I were also reduced in sh-HULC group, indicating interfering HULC suppressed autophagy.

Discussion

Although the role of LV-METase in enhancing the sensitivity of drug-resistant gastric cancer cells to CDDP has been studied (Xin et al. 2018a), the underlying mechanism is not completely clear. In this study, we found that LV-METase suppressed autophagy and drug resistance of drug-resistant

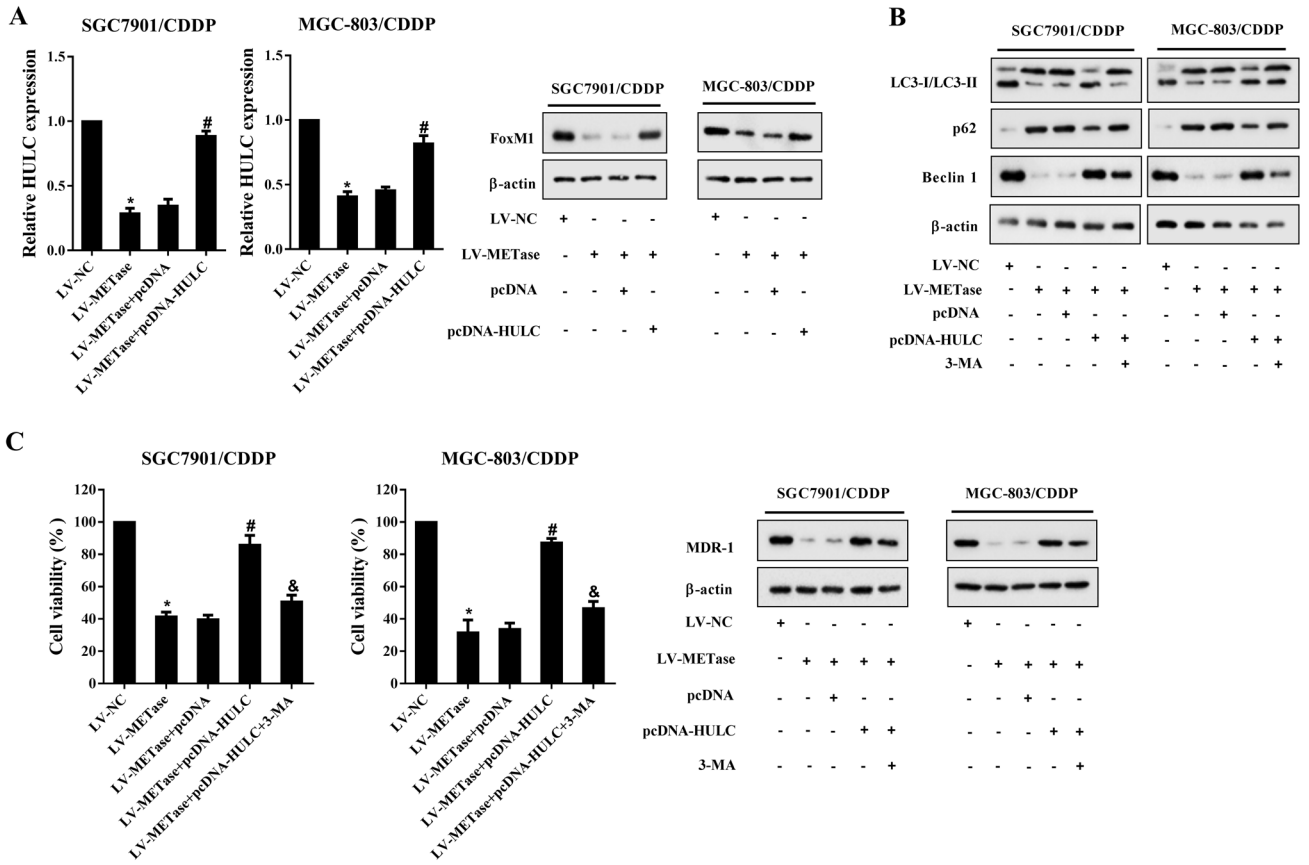

Fig. 4 METase downregulated FoxM1 protein level and suppressed autophagy to reduce CDDP resistance of drug-resistant gastric cancer cells through regulating HULC. SGC7901/CDDP and MGC-803/CDDP cells were divided into LV-NC, LV-METase, LV-METase + pcDNA, and LV-METase + pcDNA-HULC groups. **a** After 24 h, HULC expression and FoxM1 protein level were detected using qRT-PCR and western blot. After transfection, 1 mM autophagy inhibitor 3-MA was used to treat cells. SGC7901/CDDP and MGC-803/CDDP cells were divided into LV-NC, LV-METase, LV-METase + pcDNA, LV-METase + pcDNA-HULC, and LV-METase + pcDNA-HULC + 3-MA groups. **b** Autophagy-related proteins (LC3-I, LC3-II, p62, and beclin 1) were detected using western blot. **c** Cell viability was detected using CCK-8 assay after the stimulation of 1 μg/ml CDDP for 48 h. MDR-1 protein level was detected using western blot. $^{*}P < 0.05$ vs LV-NC, $^{\#}P < 0.05$ vs LV-METase + pcDNA, and $P < 0.05$ vs LV-METase + pcDNA-HULC

gastric cancer cells through regulating lncRNA HULC/FoxM1 pathway. Hence, we first determined the role of LV-METase in suppressing autophagy to inhibit CDDP resistance of drug-resistant gastric cancer cells, and identified the mechanism by which LV-METase downregulated lncRNA HULC expression. Therefore, this study will be helpful for further studies on the regulation of METase in autophagy-related CDDP resistance of gastric cancer.

LncRNA HULC is widely expressed in many cancer tissues and cells, and can act as an oncogene to accelerate the progression of cancers (Liu et al. 2019; Su et al. 2019). Studies also shown that lncRNA HULC plays an important role in regulating autophagy in cancers (Chen et al. 2017). For example, lncRNA HULC increased the expression of autophagy marker LC3 II and beclin 1 in liver cancer cells, and HULC promoted liver cancer via autophagy (Xin et al. 2018b). Moreover, it has been reported that lncRNA HULC may involve in autophagy-mediated drug resistance of liver cancer cells (Xiong et al. 2017). Previous have reported that lncRNA HULC was highly expressed in drug-resistant gastric cancer cells and indicated lower survival rate (Zhang et al. 2016). Our results further showed that lncRNA HULC was remarkably downregulated by LV-METase in drug-resistant gastric cancer cells, and HULC overexpression increased LC3 II/LC3 I and beclin 1 expressions, indicating that HULC promoted autophagy of drug-resistant gastric cancer cells. Besides, HULC up-regulated MDR-1 expression, indicating that HULC enhanced CDDP resistance of drug-resistant gastric cancer cells. Of course, it needs to clarify the underlying mechanism of HULC in regulating autophagy and CDDP resistance of drug-resistant gastric cancer cells.

A plenty of studies have shown that lncRNA HULC can regulate the expression or stability of proteins through directing binding to these proteins (Chen et al. 2017; Xiong et al. 2017). According to the bioinformatics

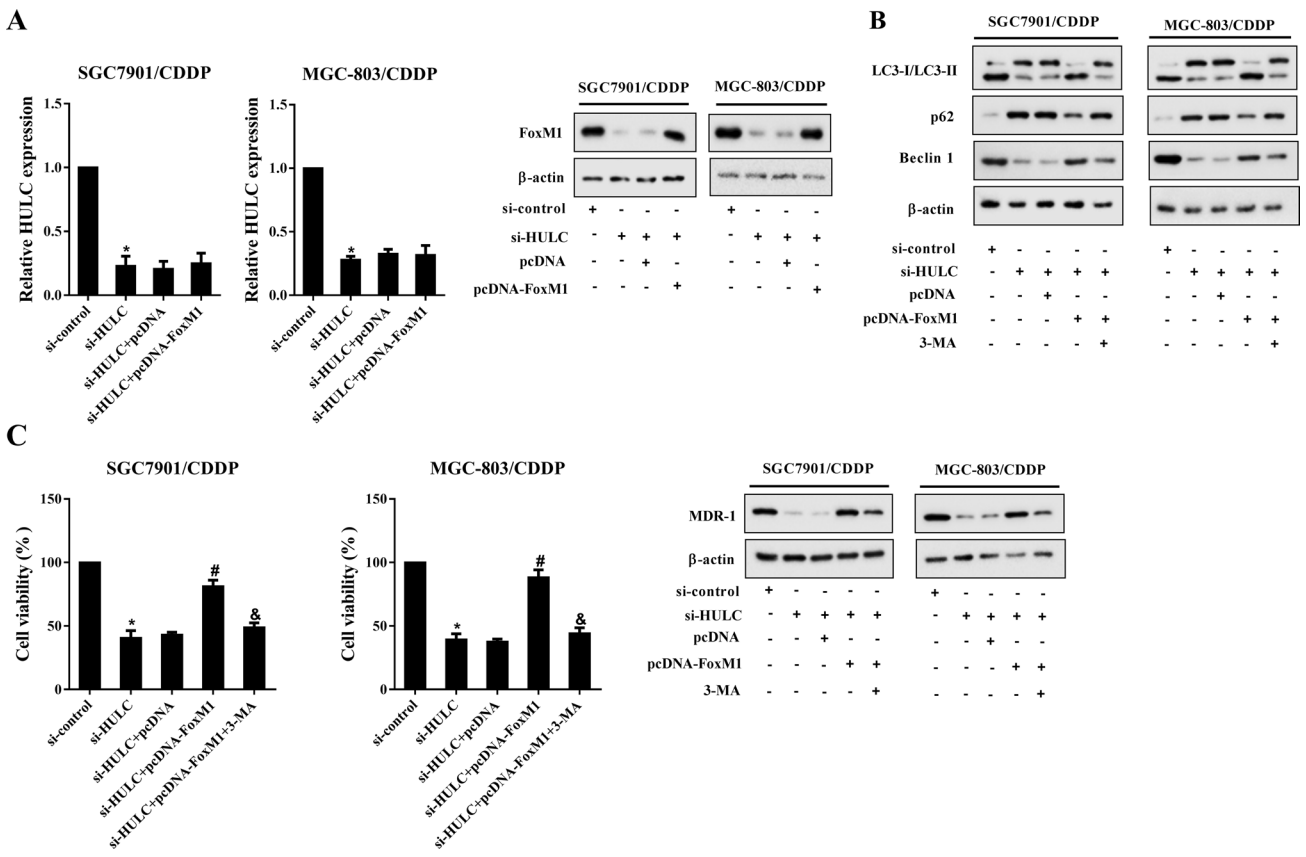

Fig. 5 Interfering HULC suppressed autophagy to reduce CDDP resistance of drug-resistant gastric cancer cells through regulating FoxM1. SGC7901/CDDP and MGC-803/CDDP cells were divided into si-control, si-HULC, si-HULC + pcDNA, and si-HULC + pcDNA-FoxM1 groups. **a** After 24 h, HULC expression and FoxM1 protein level were detected using qRT-PCR and western blot. After transfection, 1 mM autophagy inhibitor 3-MA was used to treat cells. **b** Autophagy-related proteins (LC3-I, LC3-II, p62, and beclin 1) were detected using western blot. **c** Cell viability was detected using CCK-8 assay after the stimulation of 1 μg/ml CDDP for 48 h. MDR-1 protein level was detected using western blot. $^{*}P < 0.05$ vs si-control, $^{\#}P < 0.05$ vs si-HULC + pcDNA, and $P < 0.05$ vs si-HULC + pcDNA-FoxM1

software (RNA–Protein Interaction Prediction), lncRNA HULC could directly bind to FoxM1 protein. As previously reported, FoxM1 plays a critical role in drug resistance of many cancers. FoxM1 was up-regulated in drug-resistant gastric cancer cell SGC7901/CDDP, and silencing FoxM1 enhanced the sensitivity of gastric cancer cells to CDDP (Li et al. 2016). Moreover, overexpressed FoxM1 promoted autophagy and increased the expression of LC3 II and beclin 1 expressions in drug-resistant gastric cancer cells (Tian et al. 2017). These studies indicated that FoxM1 is involved in autophagy-related drug resistance of gastric cancer. In this study, we found that lncRNA HULC could bind to FoxM1 protein, and observed FoxM1 protein level increased upon HULC overexpression and FoxM1 protein level decreased upon HULC knockdown. Furthermore, previous report showed that RNF168 mediated the ubiquitination and degradation of FoxM1 protein in breast cancer cells (Kongsema et al. 2016). Our results showed that silencing HULC resulted in a decrease in FoxM1 protein level in SGC7901/CDDP and MGC-803/CDDP cells treated with CHX, indicating lncRNA HULC stabilized the FoxM1 protein. Our study first determines that lncRNA HULC regulates the ubiquitination and degradation of FoxM1 protein, which will enrich the literature.

In conclusion, we revealed that METase decreased the expression of lncRNA HULC and the level of FoxM1 protein, which ultimately suppressed the autophagy of drug-resistant gastric cancer cells. In addition, METase/lncRNA HULC/FoxM1 pathway could regulate autophagy-mediated CDDP resistance of drug-resistant gastric cancer cells in vitro, and knockdown of HULC reduced tumor volume and autophagy of gastric cancer in vivo, which illustrated that 'METase/lncRNA HULC/FoxM1' pathway might be

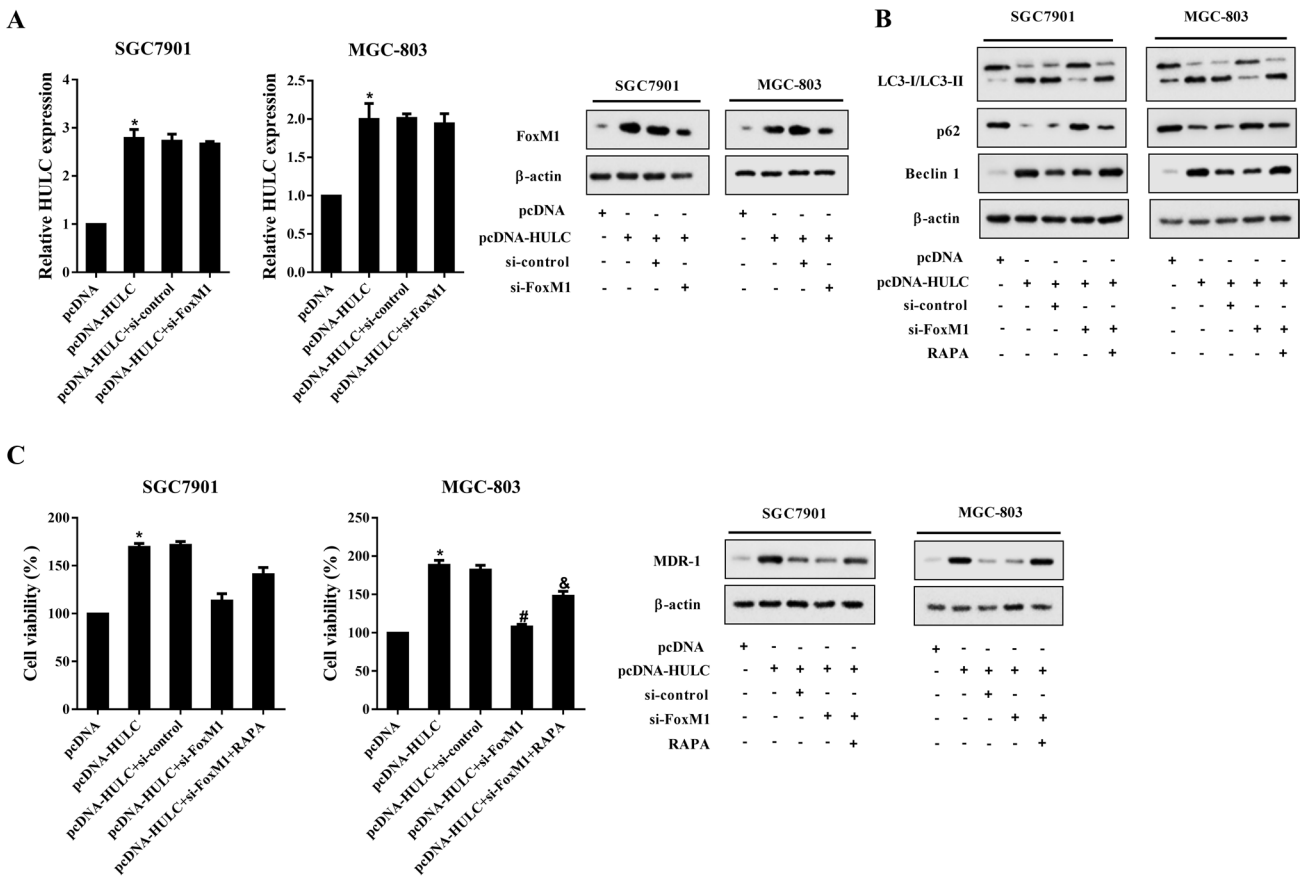

Fig. 6 Overexpressed HULC promoted autophagy to promote drug resistance of gastric cancer cells through regulating FoxM1. SGC7901 and MGC-803 cells were divided into pcDNA, pcDNA-HULC, pcDNA-HULC + si-control, and pcDNA-HULC + si-FoxM1 groups. **a** After 24 h, HULC expression and FoxM1 protein level were detected using qRT-PCR and western blot. **b** After transfection, 100 nM autophagy agonist RAPA was used to treat cells. Autophagy-related proteins (LC3-I, LC3-II, p62 and beclin 1) were detected using western blot. **c** Cell viability was detected using CCK-8 assay after the stimulation of 1 μg/ml CDDP for 48 h. MDR-1 protein level was detected using western blot. $^{*}P < 0.05$ vs pcDNA, $^{\#}P < 0.05$ vs pcDNA-HULC + si-control, and $P < 0.05$ vs pcDNA-HULC + si-FoxM1

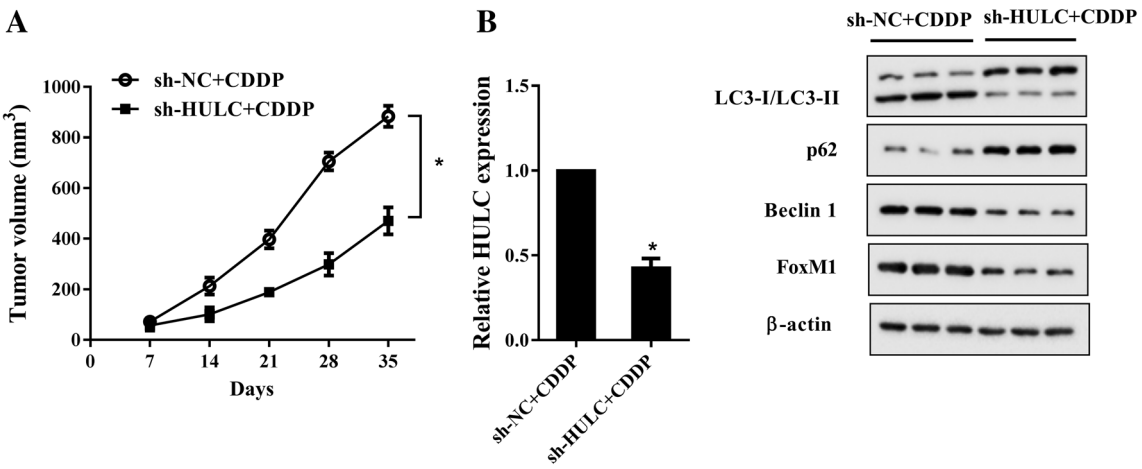

Fig. 7 Interfering HULC inhibited tumor growth in vivo. SGC7901/DDP cells (2×10^6) stably transfected with sh-HULC or sh-NC were subcutaneously injected into the left posterior ventral side of the mice. Therefore, the nude mice were divided into sh-NC and sh-HULC groups, with six mice in each group. Seven days after injection of SGC7901/DDP cells, 3.0 mg/kg CDDP was intraperitoneally injected every 3 days. The mice were sacrificed 35 days after SGC7901/DDP cells injection. **a** Tumor size was measured using a caliper and the volume was calculated using the formula: volume = length × width^2/2. **b** HULC expression, FoxM1 protein level, and autophagy-related proteins were detected using qRT-PCR and western blot. $^{*}P < 0.05$ vs sh-NC

a potential target for reducing CDDP resistance of gastric cancer.

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Compliance with ethical standards

Conflict of interest The authors have no actual or potential conflicts of interest to declare.

Ethical approval All animal experiments were approved by the Ethics Committee of The Second Affiliated Hospital of Nanchang University, and were conducted in accordance with the Declaration of Helsinki.

Consent for publication All authors are agreed for the publication.

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