

# Targeted Inhibition of CCL22 by miR-130a-5p Can Enhance the Sensitivity of Cisplatin-Resistant Gastric Cancer Cells to Chemotherapy

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**Objective:** This study set out to explore the regulatory mechanism of miR-130a-5p in cisplatin (DDP)-resistant gastric cancer (GC) cells.

**Materials and Methods:** Forty cases of GC and paracancerous tissues were collected, and the miR-130a-5p and CCL22 levels were detected by qRT-PCR. DDP-resistant cell lines of GC cells were established. Cell viability, invasion, and apoptosis were measured by CCK-8, Transwell, and flow cytometry, respectively. The relationship between miR-130a-5p and CCL22 was verified by dual-luciferase reporter enzyme, and the protein levels of caspase-3, bax, bcl-2, and CCL22 were determined by Western blot.

**Results:** miR-130a-5p was low expressed in GC tissues and cells, while CCL22 showed marked negative correlation, and the area under the curve (AUC) for diagnosing GC was not less than 0.850. Up-regulating miR-130a-5p or knocking down CCL22 expression can inhibit the proliferation and invasion of GC cells and promote their apoptosis, reverse the resistance of NCI-N87/DDP to DDP, and also enhance the chemosensitivity of GC cells. Dual-luciferase reporter enzyme identified that there was a targeted relationship between miR-130a-5p and CCL22. At the same time, miR-130a-5p and CCL22 were up-regulated or down-regulated, and the malignant proliferation, invasion, apoptosis, and DDP chemotherapy resistance of the cells had no difference compared with miR-NC with transfection-unrelated sequences.

**Conclusion:** Up-regulating miR-130a-5p can enhance the sensitivity of DDP-resistant GC cells to chemotherapy and regulate their biological function by targeted inhibition of CCL22.

**Keywords:** miR-130a-5p, CCL22, cisplatin, gastric cancer, chemosensitivity

## Introduction

Gastric cancer (GC) is a highly heterogeneous and invasive malignant tumor. Its carcinogenic principle is related to the further deterioration of peptic ulcer caused by gastric environment inflammation.<sup>1,2</sup> Relevant statistics of GC reveal that there are about 95,1600 new cases and 72,3100 deaths worldwide.<sup>3</sup> GC is one of the major causes of human cancer death worldwide, with high metastasis rate and poor prognosis, but it can be cured by surgical resection in the early stage.<sup>4,5</sup> However, later period is the most diagnosis period of GC patients, and they can only be treated by chemotherapy frequently. Cisplatin (DDP) is a commonly used first-line chemotherapy drug for treating advanced GC, and its chemical resistance is a stumbling block preventing GC patients from getting better.<sup>6-8</sup> The anti-cancer

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mechanism of DDP is to interfere with DNA replication of tumor cells and promote their apoptosis. But, its drug resistance is complicated. The decrease of DDP absorption, change of molecular targets, decrease of tumor cell activator, and enhancement of DNA damage repair may all lead to the decrease of chemosensitivity.<sup>9</sup> Therefore, it is crucial to study the mechanism of DDP drug resistance for GC treatment.

MicroRNA (miRNA) is a molecular regulator that regulates gene expression at post-transcriptional and translational levels. In addition to being relevant to basic physiological processes, it also plays a part in regulating chemosensitivity of tumor cells.<sup>10,11</sup> Wu et al<sup>12</sup> reported that miR-204/ZEB1 axis could mediate the regulation of chemosensitivity of prostate cancer cells. Withers et al<sup>13</sup> pointed out that miRNA derived from adipose tissue, as a new type of adipogenic factor, participated in the chemoresistance mechanism of tumor cells. Selective regulation of miRNA might bring good news to obese tumor patients. As a member of miRNA family, miR-130a-5p has been reported as a tumor suppressor gene, which is tied to chemotherapy resistance of breast cancer, but the mechanism of chemotherapy resistance related to GC has not been clearly defined.<sup>14,15</sup> CCL22 is a chemokine derived from macrophages and belongs to CC chemokine family, which is associated with tumor immune infiltration of hepatocellular carcinoma.<sup>16,17</sup> Studies have shown that the CCL22 expression in tumor microenvironment can promote the immunosuppression of regulatory T cells, lead to the reduction of GC anti-tumor immunity, and mediate the chemotherapy resistance of colorectal cancer.<sup>18,19</sup>

We found that miR-130a-5p and CCL22 have targeted sites through the online target gene prediction website, and we suspect that there may be miR-130a-5p/CCL22 regulatory network relevant to the DDP chemotherapy sensitivity of GC. Therefore, we have carried out experiments to verify the conjecture.

## Materials and Methods

### Collection of Tissue Samples

Forty patients who underwent GC surgery in LONGHUA Hospital from June 2017 to June 2019 were collected. There were 23 males and 17 females. They were 35 to 75 years old, with an average age of (59.28±6.54). With their consent, 40 GC tissues (GC group) and 40 paracancerous tissues (normal group) were obtained, respectively, during the surgery. They were stored in liquid nitrogen tanks. Inclusion criteria were as below: patients diagnosed

as GC by pathological diagnosis;<sup>20</sup> patients diagnosed as GC for the first time; patients without communication barriers; patients with complete clinical data. Exclusion criteria were as below: patients who had received radiotherapy and chemotherapy; patients complicated with other malignant tumors; patients complicated with severe renal dysfunction; patients complicated with serious infectious diseases; patients with other gastric diseases; patients who refused to provide experimental specimens. All patients and their families agreed to participate in the experiment and sign an informed consent form. The test has been approved by the Hospital Ethics Committee.

### Cell Culture and Transfection

We purchased human GC cells MKN74, NCI-N87, SNU-1, KE-39 and human normal gastric mucosa cells GES-1 (Guandao Bioengineering Co., Ltd., Shanghai, China, C0888, GD-C0038632A64991, C1902, C7149, C0355) and cultured them in DMEM medium containing 10% PBS (Winter Song Boye Biotechnology Co. Ltd., Beijing, China, PM150312B) at 37°C, 5% CO<sub>2</sub>. When observing cell adhesion growth and fusion reached 85%, we supplemented 0.25% pancreatin (Taize Jiaye Technology Development Co., Ltd., Beijing, China, 25200056) for digestion. After that, the cells were placed into culture medium for further culture and passage.

Targeted inhibition of CCL22 (si-CCL22), targeted overexpression of CCL22 (sh-CCL22), negative control RNA (si-NC), negative control RNA (Sh-NC), miR-130a-5p overexpression sequence (mimics), miR-130a-5p inhibition sequence (inhibitor), and miR negative control (miR-NC) were employed to transfect cells with Lipofectamine™ 2000 kit (BioMagbeads, Wuxi, China, 11668019), and the operation steps were strictly in line with the kit instructions.

### Establishment of Drug-Resistant Cell Lines

Drug-resistant cell lines were established by increasing the DDP concentration (Kerry-based Biotechnology Co., Ltd., Beijing, China, 80458). The logarithmic phase NCI-N87 cells were inoculated into a culture solution containing DDP, and its low concentration started at 0.5  $\mu$ m. After 48 h, the solution was discarded, and fresh solution was supplemented. After digestion, 1  $\mu$ m DDP was supplemented and treated for 48 h. Through this process of changing the solution and gradually increasing the DDP concentration, a cell

line that can tolerate 10  $\mu\text{M}$  DDP was obtained and named NCI-N87/DDP.

## Real-Time Quantitative PCR

Firstly, the total RNA in tissues and cells was extracted with Trizol reagent (Baiaolaibo Technology Co., Ltd., Beijing, China, QN-2070-ZOG). Then, 5  $\mu\text{g}$  of total RNA was taken, respectively, according to the instructions of kits (Beijing Huada Protein Research and Development Center Co., Ltd., China, BPI01030) for reverse transcription cDNA operation, and 1  $\mu\text{L}$  of synthesized cDNA was taken after transcription for amplification. Amplification system was as follows: cDNA 1  $\mu\text{L}$ , upstream and downstream primers 0.4  $\mu\text{L}$  each, 2X TransScript<sup>®</sup> Tip Green qPCR SuperMix 10  $\mu\text{L}$ , Passive Reference Dye (50X) 0.4  $\mu\text{L}$ , Nuclease-free Water made up to 20  $\mu\text{L}$ . CCL22 employed  $\beta$ -Actin as internal reference, miR-130a-5p employed U6 as internal reference, and the data were analyzed by  $2^{-\Delta\Delta\text{Ct}}$ .

## Cell Viability Detected by MTT Assay

Cells transfected for 24 h were collected, the cell density was adjusted to  $4 \times 10^3$  cells/well. They were inoculated on 96-well plates, incubated 0, 24, 48 and 72 h at 37°C, respectively, and 20  $\mu\text{L}$  MTT solution (5  $\mu\text{mg/mL}$ ) was supplemented at each time point (Kerry-based Biotechnology Co., Ltd., Beijing, China, 120752), and cultured 4 h at 37°C. A total of 200  $\mu\text{L}$  dimethyl sulfoxide was supplemented to each well, and then the OD value of cells in each group was measured at 450 nm wavelength via spectrophotometer (Jinda Sun Technology Co., Ltd., Beijing, China, 7415Naco). Besides, different concentrations of DDP (0  $\mu\text{mol/L}$ , 1  $\mu\text{mol/L}$ , 2  $\mu\text{mol/L}$ , 4  $\mu\text{mol/L}$ , 8  $\mu\text{mol/L}$ , 16  $\mu\text{mol/L}$ ) were added to NCI-N87 cells 24 h after transfection.

## Cell Invasion Detected by Transwell

Cells transfected for 24 h were collected, and the cell density was adjusted to  $3 \times 10^4$  cells/well. They were inoculated on a 24-well plate, digested with pancreatin, and transferred to an upper chamber. A total of 200  $\mu\text{L}$  of RPMI1640 culture solution (Huaxia Ocean Technology Co., Ltd., Beijing, China, KGM31800) was supplemented to the upper chamber, and 600  $\mu\text{L}$  of RPMI 1640 containing 10%FBS was supplemented to the lower chamber, and then cultured 48 h at 37°C. The substrates and cells in the upper chamber that did not pass through the membrane surface were wiped off, rinsed 3 times with PBS (Kerry-based Biotechnology Co., Ltd., Beijing, China, 120830), fixed 10 min with paraformaldehyde, washed 3 times with double distilled water, and

stained with 0.5% crystal violet after drying. The cell invasion was observed by a microscope.

## Apoptosis Detected by Flow Cytometry

Transfected cells were digested with 0.25% trypsin, washed twice with PBS after digestion, supplemented with 100  $\mu\text{L}$  of binding buffer, and prepared into  $1 \times 10^6/\text{mL}$  suspension. Next, they were sequentially supplemented with AnnexinV-FITC and PI 10  $\mu\text{L}$  each, incubated 5 min in dark at room temperature, and detected with flow cytometry (AMG0002051, Beijing, China, AMG). The test was repeated 3 times and the results were averaged.

## Resistance Assessment of GC Cells

Transfected cells were inoculated into 96-well plates at a density of  $1 \times 10^5$  per well, and 2 mL DDP of 0, 1, 2, 4, 8, and 16  $\mu\text{mol/L}$  was supplemented, respectively. Forty-eight hours after incubation, the fresh culture medium was replaced, and 10  $\mu\text{L}$  of MTT solution was supplemented into each well. Next, they were cultured 2 h in an incubator. The absorbance value of each well was measured at 450 nm via SpectraMax M5 microplate reader (IMAGE Trading Co., Ltd., Beijing, China, 21261000) to detect cell proliferation, and the test was repeated 3 times. IC50 of cells was calculated based on cell survival rate.

## Western Blot Test

The cultured cells of each group were extracted for total protein by RIPA lysis method, and the protein concentration was detected by BCA kit (Kerry-based Biotechnology Co., Ltd., Beijing, China, 120982), adjusted to 4  $\mu\text{g}/\mu\text{L}$ , and separated by 12%SDS-PAGE (Luyuan Bode Biotechnology Co., Ltd., Beijing, China, 100915-12) electrophoresis. After ionization, the membrane was transferred to PVDF membrane (Kerry-based Biotechnology Co., Ltd., Beijing, China, ISEQ00011), dyed with ponceau S working fluid (Baiaolaibo Technology Co., Ltd., Beijing, China, RFT056-ZEX), soaked 5 min in PBST (Kerry-based Biotechnology Co., Ltd., Beijing, China, 120898), and closed 2 h by 5% defatted milk powder (Luyuan Bode Biotechnology Co., Ltd., Beijing, China, MP012). CCL22, caspase-3, bax, bcl-2, and  $\beta$ -Actin with a dilution ratio of 1:500 were supplemented and closed all night long at 4°C. After washing the membrane, the primary antibody was removed and horseradish peroxidase was supplemented to label goat anti-rabbit secondary antibody with a dilution ratio of 1:1000. The antibodies were all

purchased from Beijing Future Biotechnology Co., Ltd., incubated 1 h at 37°C, and rinsed 3 times with PBS, each time for 5 min. Excess liquid on the membrane was absorbed by filter paper. It gave out light under ECL and developed, and the gray value was analyzed.

## Dual-Luciferase Report Assay

miR-130a-5p downstream target genes were predicted by Targetscan7.2 ([http://www.targetscan.org/vert\\_72/](http://www.targetscan.org/vert_72/)). CCL22 3' UTR-Wt, CCL22 3' UTR-Mut, mimics, and miR-NC were transferred into GC cells by Lipofectamine™ 2000 kit, and luciferase activity was tested by dual-luciferase reporter gene assay kit (Baiaolaibo Technology Co., Ltd., Beijing, China, KFS303-LBV).

## Statistical Methods

In this research, data analysis and picture drawing were conducted by GraphPad 6. Comparison between the two groups was under independent-samples *t* test, and comparison among groups was determined by one-way analysis of variance and expressed as *F*. Post hoc pairwise comparison was under LSD-*t* test, and multi-time point expression was conducted by repeated measures analysis of variance and expressed as *F*. Backtesting was under Bonferroni. The diagnostic value of miR-130a-5p and CCL22 in GC was plotted using the receiver operating characteristic curve (ROC). The correlation between miR-130a-5p and CCL22 in tissues was analyzed by Pearson test.  $P < 0.05$  was seen as statistical difference.

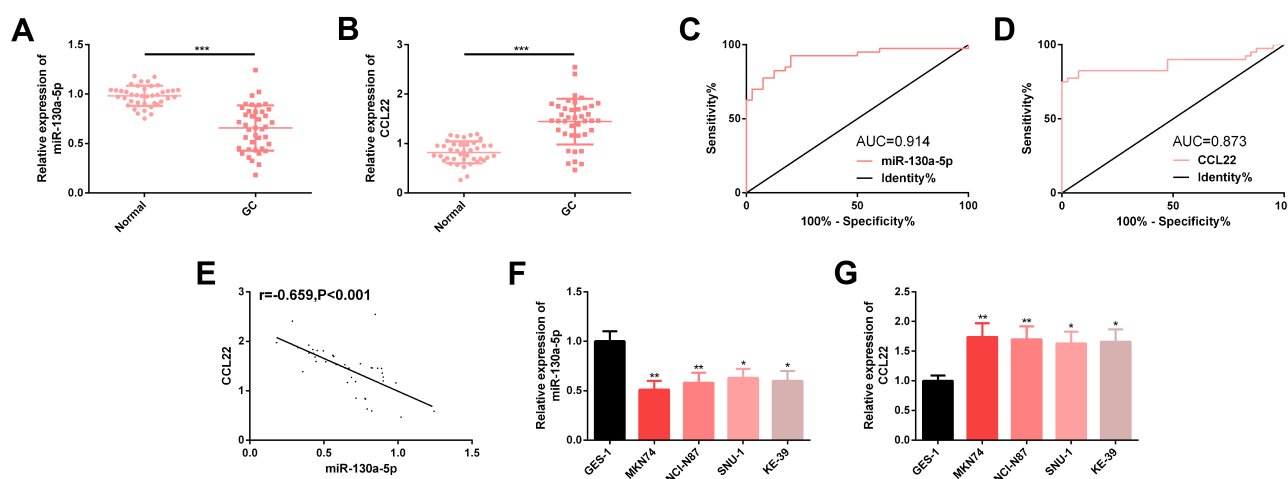
## Results

### miR-130a-5p Is Down-Regulated in GC Tissues and Cells, While CCL22 Is Up-Regulated

The miR-130a-5p expression level in GC tissues was dramatically lower and the CCL22 expression level in cells was dramatically higher. It suggested that both might be involved in the pathological process of GC. Further, their area under ROC curve (AUC) in diagnosing GC was 0.914 and 0.873, respectively, which suggested that both of them might be auxiliary biomarkers for GC diagnosis. The correlation between miR-130a-5p and CCL22 was evaluated by Pearson correlation coefficient, and it was found that miR-130a-5p was markedly negatively correlated with CCL22, indicating that they both might play an antagonistic role in the pathological process of GC as tumor suppressor and tumor promoter ( $r = -0.659$ ,  $P < 0.05$ ) (Figure 1).

### Up-Regulating miR-130a-5p Can Inhibit the Malignant Behavior of GC Cells

We further explored the pathological mechanism of miR-130a-5p in GC through cytological study. The results showed that the miR-130a-5p expression in cells transfected with mimics was not only markedly enhanced but also the cell proliferation, invasion, and bcl-2 protein levels were dramatically inhibited. The apoptosis level as well as caspase-3 and bax protein levels increased remarkably. However, the cell biological function and apoptosis-related protein levels of



**Figure 1** Expression of miR-130a-5p and CCL22 in tissues and cells. (A and B) Based on miR-130a-5p and CCL22 expression levels in GC tissue, the former is low expressed, while the latter is highly expressed. (C and D) According to ROC curves of miR-130a-5p and CCL22 in diagnosing GC, AUC of both was not less than 0.850. (E) miR-130a-5p has a remarkable negative correlation with CCL22 ( $r = -0.659$ ,  $P < 0.05$ ). (F and G) miR-130a-5p, CCL22 expression levels in GC cells are similar to those in tissues, and their expression difference in MKN74, NCI-N87 cells is more remarkable.

**Notes:** \*Represents a comparison with GES-1 or between the two ( $P < 0.05$ ); \*\*Represents ( $P < 0.01$ ); \*\*\*Represents ( $P < 0.001$ ).

cells transfected with inhibitor were opposite to those of cells transfected with mimics, and the differences were statistically significant ( $P < 0.05$ ). The above results indicated that up-regulating miR-130a-5p was beneficial to inhibit the malignant behavior of GC cells, while down-regulating it aggravated the malignant expression of GC cells in proliferation, invasion, and apoptosis. Caspase-3 and Bax were pro-apoptotic factors, while Bcl-2 was a common anti-apoptotic factor. Low level of Bcl-2 and high level of Caspase-3 and Bax often reflect that cells are induced by apoptosis<sup>21</sup> (Figure 2).

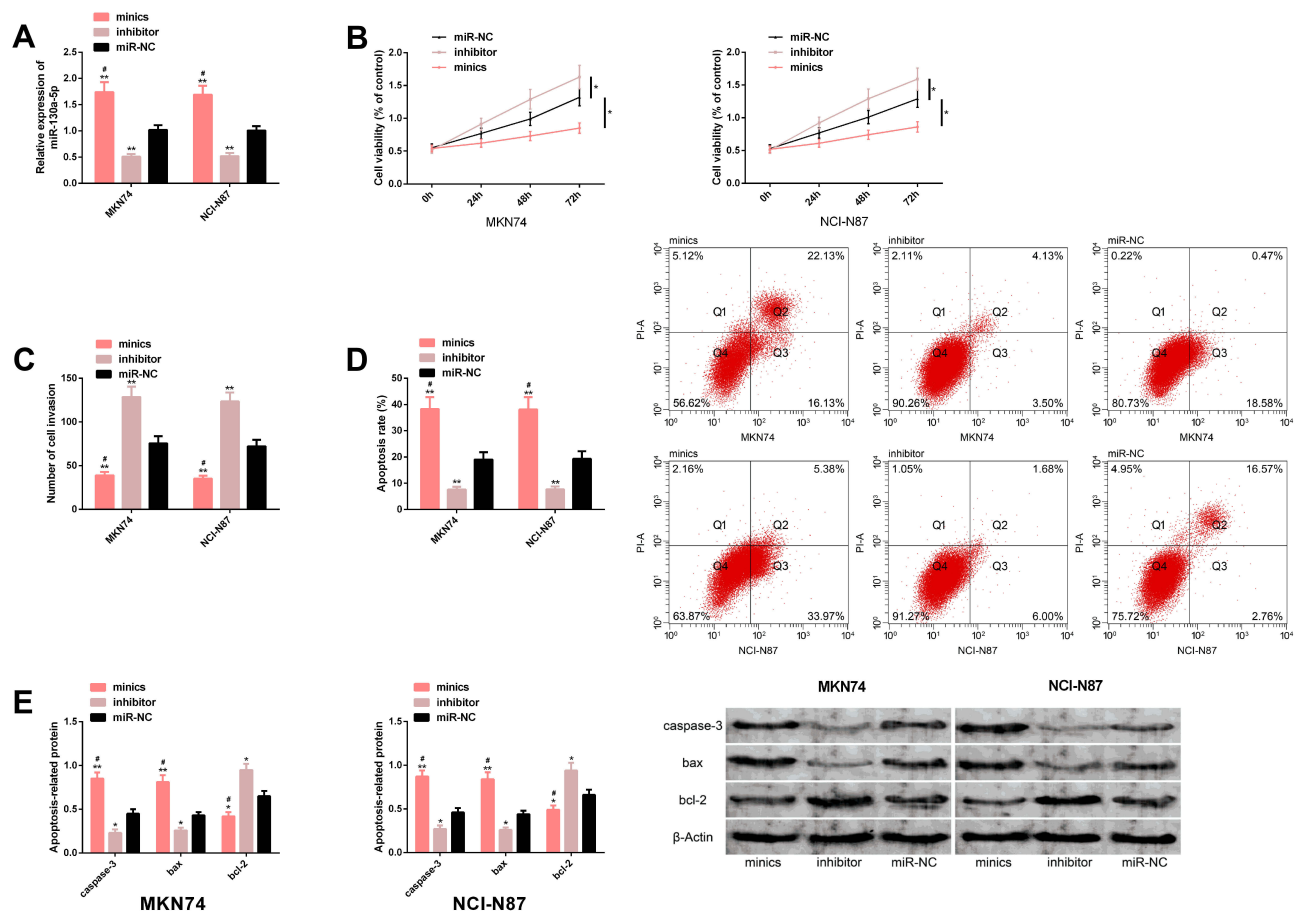
## Down-Regulating CCL22 Can Inhibit the Malignant Behavior of GC Cells

We also studied the biological expression of CCL22 in GC cells. The results manifested that the CCL22 expression in cells transfected with si-CCL22 was not only obviously inhibited but also the cell proliferation, invasion, and bcl-2 protein

level were obviously inhibited, and the apoptosis level as well as caspase-3 and bax protein levels increased obviously. Nevertheless, the cell biological function and apoptosis-related protein level of cells transfected with sh-CCL22 were contrary to those of cells transfected with si-CCL22, and the difference was statistically significant ( $p < 0.05$ ). These results indicated that CCL22 was likely to be carcinogenic in GC, and down-regulating CCL22 expression was helpful to inhibit the malignant behavior of GC cells (Figure 3).

## Up-Regulating miR-130a-5p Can Reverse the Chemotherapy Sensitivity of DDP-Resistant GC Cells and Help to Improve the Chemotherapy Sensitivity of Non-Resistant GC Cells

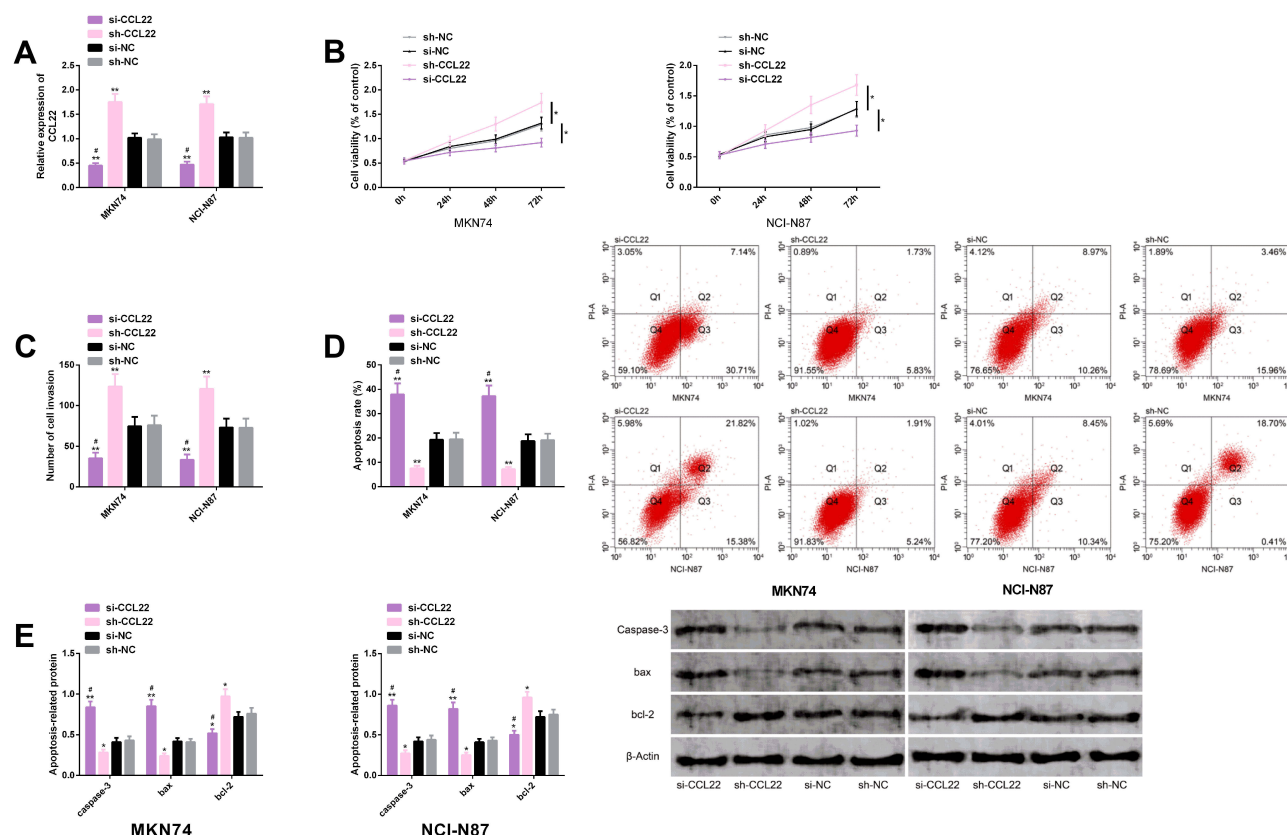
From dose-response Curve, we found that under DDP intervention, the cell proliferation rate decreased obviously



**Figure 2** Up-regulation of miR-130a-5p helps to inhibit malignant behavior of GC cells. (A) miR-130a-5p expression in GC cells MKN74 and NCI-N87. (B–D) Up-regulation of miR-130a-5p is helpful to inhibit the malignant proliferation and invasion behavior of GC cells, as well as to increase the apoptosis level, and flow cytometry. (E) Up-regulation of miR-130a-5p is helpful to increase the protein levels of Caspase-3 and bax and reduce the bcl-2 protein level, as well as the protein map.

**Notes:** \*Represents a comparison with miR-NC or between the two ( $P < 0.05$ ); \*\*Represents  $P < 0.01$ ; #Represents a comparison with inhibitor ( $P < 0.05$ ).





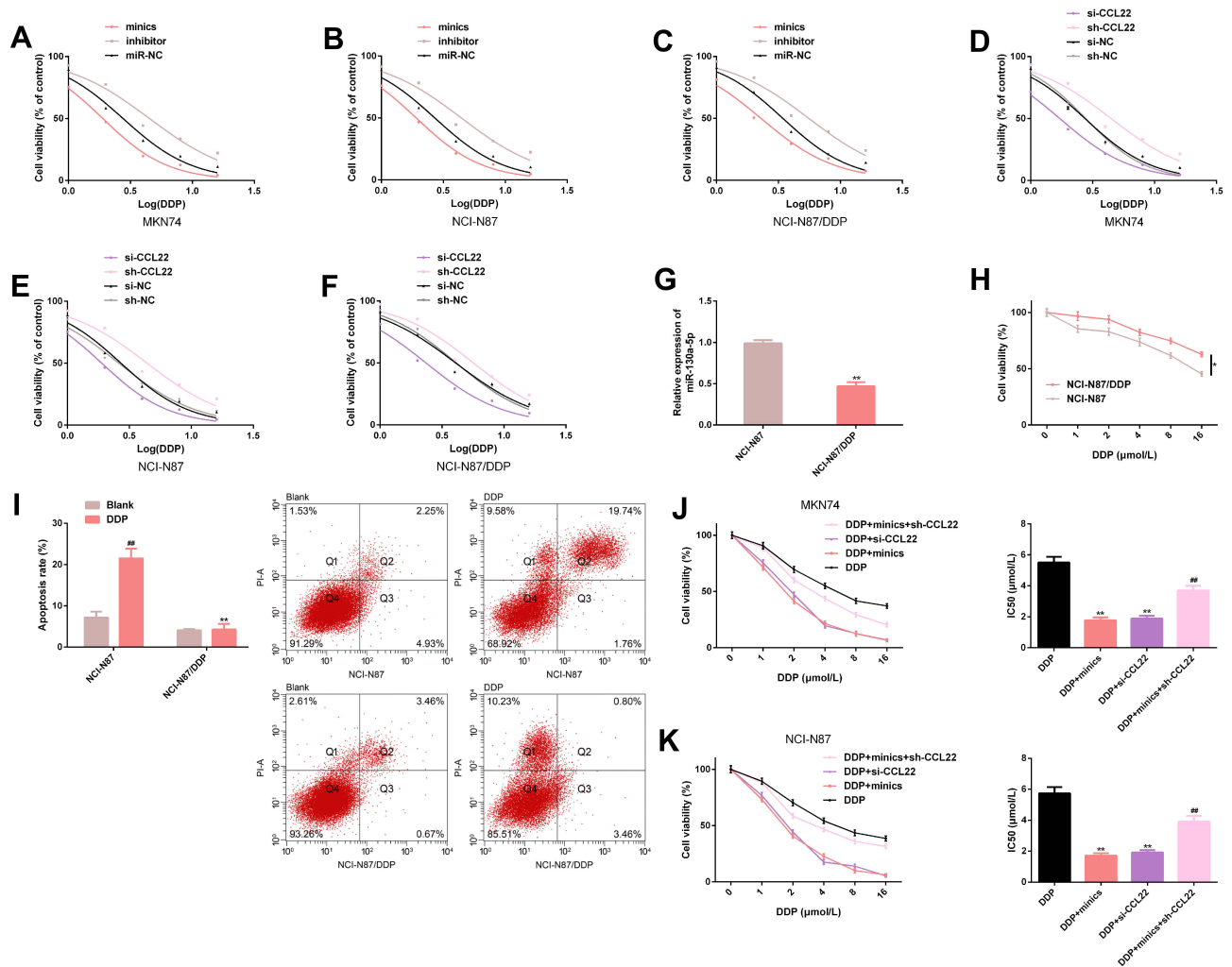
**Figure 3** Down-regulation of CCL22 helps to inhibit the malignant behavior of GC cells. (A) CCL22 expression in GC cells MKN74 and NCI-N87. (B–D) Down-regulation of CCL22 is conducive to inhibit the malignant proliferation and invasion behavior of GC cells, as well as to increase the level of apoptosis, and flow cytometry. (E) Down-regulation of CCL22 is conducive to increase the protein levels of Caspase-3 and bax, and decrease the bcl-2 protein level, as well as the protein map. **Notes:** \*Represents a comparison with Si-NC or between the two ( $P < 0.05$ ); \*\*Represents  $P < 0.01$ ; #Represents a comparison with Si-CCL22 ( $P < 0.05$ ).

with the increase of inhibitory concentration. When miR-130a-5p was up-regulated or CCL22 was down-regulated, the inhibitory effect of DDP on the proliferation of GC cells or DDP-resistant GC cells could be dramatically promoted, and the IC<sub>50</sub> of GC cells or DDP-resistant GC cells under the influence of DDP could be reduced. However, when we treated miR-130a-5p or CCL22 reversely, we obtained opposite results as described above. The above results showed that the proliferation of GC cells or DDP-resistant GC cells and IC<sub>50</sub> responded to miR-130a-5p and CCL22. The up-regulated miR-130a-5p or down-regulated CCL22 was conducive to the lower level of proliferation and more sensitive chemotherapy response of the above cells. Compared with non-resistant GC cells, DDP-resistant GC cells had lower expression level of miR-130a-5p, significantly higher proliferation, significantly lower apoptosis rate, and significantly higher IC<sub>50</sub>, suggesting that the two kinds of cells had different biological manifestations, while the malignant biological behavior of DDP-resistant GC cells was more remarkable, and the chemotherapy

sensitivity was significantly rough. What's more, the proliferation of GC cells decreased with the increase of DDP concentration. In the GC cells with miR-130a-5p up-regulated or CCL22 down-regulated, the proliferation was significantly inhibited, and IC<sub>50</sub> reduced markedly. This suggested that up-regulating miR-130a-5p or down-regulating CCL22 might help GC cells respond more sensitively to DDP (Figure 4).

## miR-130a-5p Has a Target Control Relationship with CCL22

We found a targeted binding site between miR-130a-5p and CCL22 through Targetscan7.2. The dual-luciferase activity assay results manifested that CCL22 3'UTR-Wt luciferase activity decreased dramatically after miR-130a-5p was up-regulated ( $P < 0.05$ ), but it had no effect on CCL22 3'UTR-Mut luciferase activity ( $P > 0.05$ ). This showed that there was a targeted relationship between miR-130a-5p and CCL22, and CCL22 was the direct target of miR-130a-5p. Western blot analysis signified that the



**Figure 4** Up-regulating miR-130a-5p conduces to reverse the chemosensitivity of DDP-resistant GC cells and improve the chemosensitivity of non-resistant GC cells. (A and B) Effect of miR-130a-5p on DDP sensitivity of GC cells. (C) Effect of miR-130a-5p on chemosensitivity of DDP-resistant GC cells. (D and E) Effect of CCL22 on DDP sensitivity of GC cells. (F) Effect of CCL22 on chemosensitivity of DDP-resistant GC cells. (G) Expression of miR-130a-5p in DDP-resistant GC cells and non-resistant GC cells. (H) Proliferation of DDP-resistant GC cells and non-resistant GC cells. (I) Apoptosis rate and flow cytometry of DDP-resistant GC cells and non-resistant GC cells. (J and K) Proliferation of GC cells exposed to DDP and IC50.

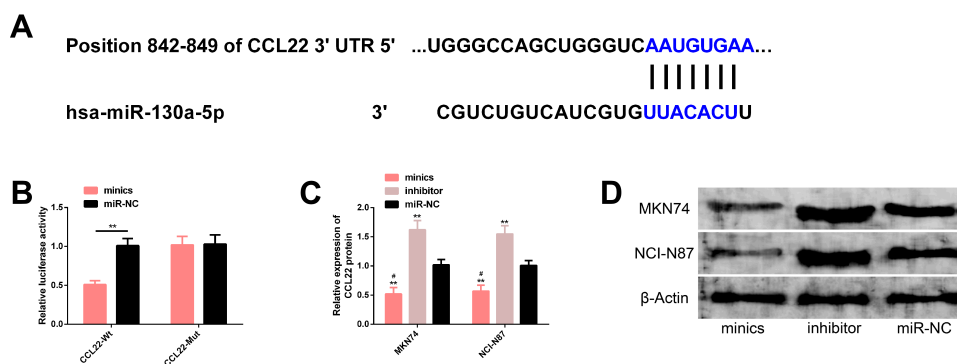
**Notes:** \*Represents a comparison with NCI-N87 or between the two groups ( $P < 0.05$ ); \*\*Represents  $P < 0.01$ ; ###Represents a comparison with blank or DDP+mimics ( $P < 0.01$ ).

CCL22 protein expression in MKN74 and NCI-N87 decreased markedly after mimics transfection ( $P < 0.05$ ), which indicated that miR-130a-5p could negatively regulate the protein level of CCL22 (Figure 5).

## Up-Regulating CCL22 Can Eliminate the Effect of Up-Regulation of miR-130a-5p on the Malignant Behavior of GC Cells

After transfecting mimics+sh-CCL22 and inhibitor+si-CCL22 into MKN74 and NCI-N87, the proliferation, invasion, apoptosis, and apoptosis-related protein levels of the cells of the two were not dramatically different from those of miR-NC ( $P > 0.05$ ), and the proliferation and invasion

were obviously improved compared with the cells transfected mimics. Apoptosis, bax, and caspase-3 protein levels were remarkably inhibited, and bcl-2 protein levels increased dramatically. Compared with inhibitor, the results of proliferation, invasion, apoptosis, and apoptosis-related protein levels were opposite, and the differences were statistically significant ( $P < 0.05$ ). The above results further supported the role of miR-130a-5p-ccl22 axis in the molecular mechanism of GC. Up-regulating CCL22 can eliminate the inhibition of up-regulation of miR-130a-5p on the malignant behavior of non-resistant GC cells and DDP-resistant GC cells. Similarly, down-regulating CCL22 can also eliminate the promotion effect



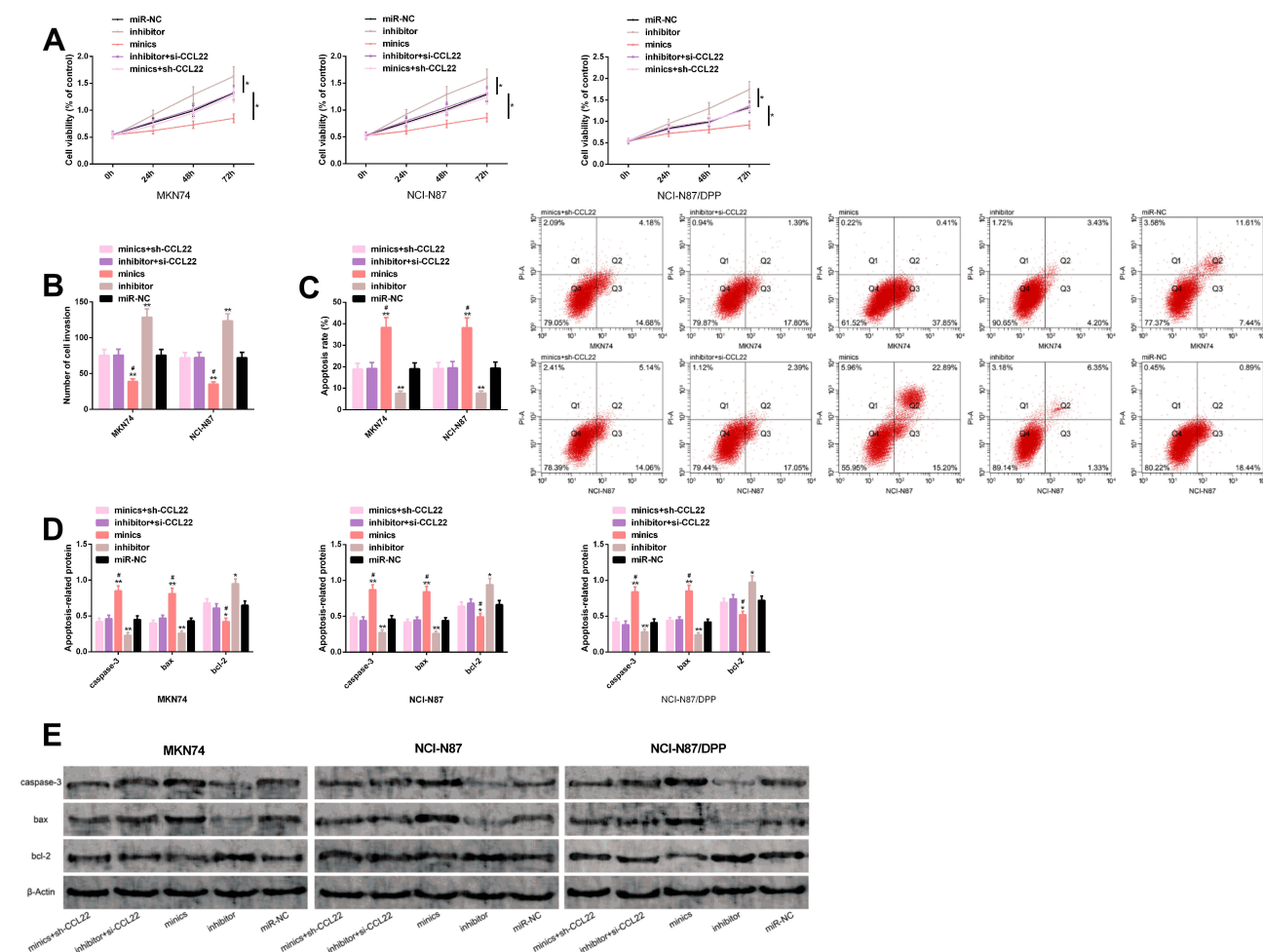
**Figure 5** Dual-luciferase activity assay. **(A)** There is a targeted binding site between miR-130a-5p and CCL22. **(B)** Relative luciferase activity-dual luciferase reporter assay. **(C)** CCL22 protein expression in MKN74 and NCI-N87 cells after transfection. **(D)** Protein map.

**Notes:** \*\*Represents a comparison with miR-NC or between the two ( $P < 0.01$ ); #Represents a comparison with inhibitor ( $P < 0.05$ ).

of down-regulation of miR-130a-5p on the malignant behavior of non-resistant GC cells and DDP-resistant GC cells (Figure 6).

## Discussion

Chemotherapy is still one of the main treatment methods for GC, but chemotherapy resistance is one of the difficult



**Figure 6** Co-transfection experiment. **(A–C)** Effects of mimics+sh-CCL22, inhibitor+si-CCL22 transfection on proliferation, invasion, and apoptosis of GC cells, and flow cytometry. **(D)** Effect of transfection of mimics+sh-CCL22, inhibitor+si-CCL22 on apoptosis-related proteins in GC cells. **(E)** Protein map.

**Notes:** \*Represents a comparison with miR-NC or between the two ( $P < 0.05$ ); \*\*Represents  $P < 0.01$ ; #Represents a comparison with inhibitor ( $P < 0.05$ ).



problems in clinical cancer treatment at present.<sup>22</sup> Nowadays, more and more miRNA play a vital role in tumor chemoresistance. For example, miR-296-3p can inhibit chemoresistance of nasopharyngeal carcinoma by targeting oncogenic proteins and mediating Ras/Braf/Erk/Mek/c-Myc and PI3K/Akt/c-Myc signal transduction pathways to form feedback loops.<sup>23</sup> Besides, miR-210-3p is a negative regulator of tumor, and its low expression will increase the carcinogenic ABCC1 expression, which promotes multidrug resistance of renal cell carcinoma.<sup>24</sup> However, we found that CCL22 targeted inhibition by miR-130a-5p could enhance the sensitivity of DDP-resistant GC cells to chemotherapy.

miR-130a-5p is an endogenous molecular regulator relevant to various human disease phenotypes. It has been reported that it has potential therapeutic value in congenital diaphragmatic hernia by regulating Shh/Gli1 signaling pathway. It can also be regulated by long non-coding RNA HOTAIR and mediate invasion and epithelial-mesenchymal transformation of esophageal squamous cell carcinoma.<sup>25,26</sup> Other studies have verified the influence of miR-130a-5p in GC. For example, Xie et al<sup>27</sup> reported that miR-130a-5p could inhibit the activation of Wnt/ $\beta$ -catenin pathway through targeted inhibition of CB1R, thus exerting tumor resistance and inhibiting malignant progression of GC cells. However, there are few reports on its sensitivity in GC chemotherapy. There are many reports about CCL22 in GC pathological mechanism and chemotherapy resistance. For instance, Wei et al mentioned that CCL22 could be used for serological diagnosis of GC patients to predict recurrence within one year.<sup>28</sup> Wu et al also pointed out that CCL22 was helpful to predict the prognosis of stages II/III GC patients treated with 5-fluorouracil and the benefits of chemotherapy.<sup>29</sup> In this study, miR-130a-5p and CCL22 had abnormal disorders in GC tissues and cells. miR-130a-5p might have a negative regulatory effect on CCL22, and both of them had certain value for diagnosing GC. Research showed that the exploration of early specific recognition markers of GC was helpful to provide new insights on precancerous lesions.<sup>30</sup>

We also explored the role of the two in the pathological mechanism of GC and chemotherapy sensitivity through cytological analysis, in which IC50 was used to measure chemosensitivity. IC50 is the half of the maximum inhibitory concentration of chemical anticancer agents, which can be used to evaluate the chemosensitivity of anticancer agents. Its value is negatively related to the chemosensitivity.<sup>31</sup> Our data suggested that up-regulating miR-130a-5p or down-regulating CCL22 might inhibit the malignant behavior of GC cells. Jin et al<sup>32</sup> found that down-regulating CCL22 could also inhibit

the proliferation and metastasis of RCC cells. Also, it has been reported that CCL22 can be targeted inhibited by miR-34c-5p in COPD, which contributes to the inflammatory response and the maintenance of protease antiprotein balance.<sup>33</sup> All the above studies showed the potential efficacy of CCL22 in various diseases. In our drug resistance study, DDP-resistant GC cells showed a high proliferation, and the apoptosis was not significantly improved under DDP intervention. DDP has concentration-dependent inhibitory effect on the proliferation of GC cells or DDP-resistant GC cells, and up-regulating miR-130a-5p or down-regulating CCL22 can dramatically enhance this inhibitory effect and improve chemotherapy sensitivity. We believe that developing miR-130a-5p promoter or CCL22 inhibitor has a potential clinical value for the inhibition of GC malignant progression and the improvement of chemosensitivity. Many scholars are interested in the chemotherapy sensitivity of GC to DDP and focus on targeted research. For example, Lei et al<sup>34</sup> pointed out that MGMT could regulate the chemotherapy sensitivity of GC cells through autophagy program, suggesting that inducing autophagy might be helpful to improve the chemosensitivity of GC cells. In addition, Kuai et al<sup>35</sup> reported that Trop2 could mediate the Notch1 signaling pathway to affect the multidrug resistance of GC cells. From the identification results of dual-luciferase reporter enzyme and the co-transfection experiment, miR-130a-5p and CCL22 had a definite target relationship; miR-130a-5p could negatively regulate the protein level of CCL22, and up-regulating CCL22 could eliminate the inhibition of up-regulating miR-130a-5p on the malignant expression of GC cells. The regulation mechanism of miR-130a-5p-CCL22 axis has shown its positive effect on GC malignant behavior and GC resistance, but the potential clinical application needs further experimental verification.

Overall, up-regulating miR-130a-5p can target CCL22 to inhibit the malignant progression of GC cells and enhance the chemotherapy sensitivity of DDP, thus becoming the conquering direction of GC chemotherapy drug resistance. However, there is still room for improvement. For instance, we should increase the studies on multidrug resistance of miR-130a-5p and CCL22 in GC, or supplement the diagnostic value of the two in metastatic GC phenotype, or further explore whether the two mediate the potential signaling pathway to affect its drug resistance mechanism and pathological regulation process, and also supplement the potential effect of miR-130a-5p on normal cells.

## Disclosure

The authors report no conflicts of interest in this work.

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