

# Oncogenic miR-106b-5p promotes cisplatin resistance in triple-negative breast cancer by targeting GDF11

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**Summary.** Background. Cisplatin (CDDP) is a standard treatment for triple-negative breast cancer (TNBC), but patient resistance to CDDP limits its efficacy. A growing study confirms that microRNAs (miRNAs) are significantly important in breast cancer, especially TNBC. This research was carried out to examine the function of miR-106b-5p in CDDP resistance of TNBC as well as the downstream mechanism.

**Methods.** The miR-106b-5p and growth-differentiation factor 11 (GDF11) expressions in the tissues from TNBC patients and CDDP-treated TNBC cell lines were measured by RT-qPCR. Thereafter, cell proliferation and migration in the presence of CDDP treatment were evaluated via CCK-8 and Transwell assays in the TNBC cells. A xenograft mice model was also established to verify the miR-106b-5p silencing effect on the growth of CDDP resistance TNBC cells *in vivo*. Luciferase reporter experiments were performed to predict the relationship between miR-106b-5p and GDF11 expression.

**Results.** The results showed that miR-106b-5p was upregulated in the TNBC tumor cells and TNBC cells treated with CDDP and knockdown of this caused inhibition of the TNBC cell lines' proliferation, migration and suppressed the growth of the TNBC xenografted tumors, in the presence of CDDP treatment. In addition, it was observed that miR-106b-5p can bind to GDF11; as a result in the TNBC tissues and CDDP-treated TNBC cell lines the down-regulation of GDF11 was observed. Moreover, GDF11 silencing promoted CDDP-treated TNBC cell lines' proliferation and migration and reversed the interference effect of miR-106b-5p.

**Conclusions.** MiR-106b-5p was upregulated in TNBC and this upregulation may promote CDDP resistance of the TNBC cells by targeting GDF11 and inhibiting its expression.

**Key words:** TNBC (triple-negative breast cancer), Cisplatin, miR-106b-5p, GDF11

## Introduction

In women, breast cancer is the most commonly known cancer reported worldwide (Barzaman et al., 2020). As per the latest data from the World Health Organization (WHO), new cases of breast cancer exceeded those of lung cancer globally in 2020; thus, breast cancer has become the world's largest cancer (Sung et al., 2021). Triple-negative breast cancer (TNBC) is considered the most aggressive, with a poor prognosis, few treatment options, and a high recurrence rate (Tsang and Tse, 2020). Chemotherapy is a standard therapy for TNBC, but its effectiveness is limited by the development of drug resistance (Lyons, 2019). Cisplatin (CDDP) may be used alone or in conjunction with other medications to treat TNBC, although CDDP resistance can lead to TNBC treatment failure (Nedeljković and Damjanović, 2019). Therefore, identifying therapeutic targets for overcoming CDDP resistance in TNBC is critically needed.

MicroRNA (miRNA) is a type of short single-stranded RNA composed of 18-22 nucleotides. Their function is to act as an important regulator of post-translational gene expression through binding to target mRNA 3' untranslated regions (UTRs) (Mohr and Mott, 2015). miRNAs have been widely accepted to be involved in carcinogenesis, migration, metastasis, and drug resistance and exhibit distinct profiles in various cancers including breast cancer (Ali Syeda et al., 2020). Emerging evidence has shown that miRNA can regulate CDDP drug resistance or sensitivity in many types of cancers via modulating apoptosis, autophagy, and epithelial-mesenchymal transition (Robertson and Yigit, 2014; Dehghanzadeh et al., 2015; Wang et al., 2020). For example, miR-381 was decreased in CDDP-resistant breast cancer tissues and cells, and its overexpression improved CDDP sensitivity while its knockdown caused the opposite result in breast cancer cells (Yi et al., 2019).

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Upregulation of miR-133b inhibited the proliferation of CDDP-resistant TNBC cells and enhanced CDDP sensitivity (Lin et al., 2021). The miR-106b-5p has been discovered to be elevated in high-grade breast cancer tumors and to have a much greater expression in TNBC when compared to other breast cancer subtypes, (Moi et al., 2019), thus, miR-106b-5p has been shown to be significant in the development of TNBC tumorigenesis. Nevertheless, the contribution of miR-106b-5p in TNBC CDDP resistance has yet to be discovered.

In addition, via bioinformatics analysis, it was established that miR-106b-5p binds to growth-differentiation factor 11 (GDF11). GDF11 is a catabolic TGF- $\beta$  family myokines, secreted by muscle and reduced muscle mass (Karsenty and Olson, 2016). GDF11 has been reported to be associated with breast cancer progression, and its declining expression in high-grade breast adenocarcinoma was observed (Wallner et al., 2018); elimination of this substance has a deteriorating prognosis for patients with hereditary breast cancer (Alvarez et al., 2016). Furthermore, GDF11 was shown to be a tumor suppressor in TNBC (Bajkar et al., 2017). However, the impact of miR-106b-5p on the TNBC regulation of GDF11, particularly its impact on CDDP resistance, has not been studied.

The goal of this study was to determine the role of miR-106b-5p in TNBC CDDP resistance and whether miR-106b-5p has an impact via targeting GDF11. Our study may provide new TNBC CDDP-resistant receptors which may prove to be promising therapeutic targets.

## Materials and methods

### Cell culture

Two TNBC cell lines, MDA-MB-453 and MDA-MB-231, were acquired from ATCC (American Type Culture Collection). These cells were cultivated in DMEM (Sigma, United States) and further supplemented with fetal bovine serum (FBS) 10% from Thermo Fisher Scientific, USA, streptomycin (100  $\mu\text{g}/\text{mL}$ ), and penicillin (100 U/mL). All the cells were carefully incubated at a temperature of 37°C and 5% CO<sub>2</sub>. They were subcultured when the cell fusion degree reached more than 90%, and logarithmic stage cells were selected for subsequent experimental studies. For the CDDP (Cat#: P4394; Sigma, USA) treatment, different concentrations (0.25, 0.5, 1.0, 2.0, 4.0, and 8.0  $\mu\text{M}$ ) of CDDP were applied to the cells for 48h.

### Cell transfection

For cell transfection, both the cell lines (MDA-MB-231 and MDA-MB-453) were seeded in 6-well plates where each well had  $1 \times 10^6$  cells; they were transfected at 70% confluence with a miR-106b-5p inhibitor negative control (inhibitor-NC) and miR-106b-5p inhibitor, (both from SwitchGear Genomics, USA), GDF11-targeting small interfering RNA (siRNA)

duplexes, or si-NC (both from RiboBio, China). For the transfection, 5  $\mu\text{L}$  of vectors and 5  $\mu\text{L}$  of Lipofectamine 2000 (Cat#: 11668019; ThermoFisher, USA) were each diluted in 95  $\mu\text{L}$  Opti-MEM (Invitrogen, USA) and mixed for 15 min, and thereafter added to the cells' culture medium. Forty-eight hours after the transfusion, the efficacy of the transfection was measured via RT-qPCR.

### Collection of clinical samples

Clinical samples of paired noncancerous normal tissues and tumor tissues were collected from 45 TNBC patients in The Clinical Medical College & First Affiliated Hospital of Chengdu Medical College. None of the patients had received chemoradiotherapy prior to their surgeries, and all the tissues were confirmed as TNBC by at least three pathologists. All the TNBC patients provided written informed permission prior to the collection and utilization of their biological samples. The research was authorized by the Ethics Committee of The Clinical Medical College & First Affiliated Hospital of Chengdu Medical College in compliance with the Helsinki Declaration. The samples were kept at -80°C and used for RNA extraction as well as RT-qPCR analysis.

### CCK-8 assay

The CCK-8 Kit (Cat#: C0038; Beyotime, China) was used to assess the proliferation of the cells in the study. A total of  $4 \times 10^4$  cells/well were injected into 96-well plates containing the control cells (MDA-MB-231 and MDA-MB-453), or cells transfected with the appropriate vectors were injected into the plates. Approximately 24, 48, and 72 hours of culture with 2  $\mu\text{M}$  of CDDP were completed, and thereafter 10  $\mu\text{L}$  of CCK-8 solution was added to the plates, which were then incubated at 37°C for 2 hours in the dark. A microplate spectrophotometer (Bio-Rad Laboratories, Inc.) was used to measure the optical density (OD) value of each well in these plates, which were subjected to 450 nm wavelength radiation.

### RT-qPCR

From the cell and tissues, the total RNAs were extracted using a TRIZOL Kit (Cat#: 15596018, ThermoFisher, USA). cDNA was prepared using the TaqMan reverse transcription (Cat#: N8080234, Applied Biosystems, USA). PCR was performed using a CFX96 Real-time system from BIO-RAD, USA, and the SuperScript™ III Platinum™ SYBR™ Green One-step PCR Kit (Cat#: 11736059, Invitrogen, USA) to quantify the relative expression levels of the genes. The reactions were prepared and the final volume of each sample was 15  $\mu\text{L}$  (0.4  $\mu\text{L}$  of the SuperScript III RT/Platinum Taq Mix, ROX Dye to a final concentration of 50 nM, 0.3  $\mu\text{L}$  of each primer at 10  $\mu\text{M}$ , 5  $\mu\text{L}$  of RNA, 10  $\mu\text{L}$  of the 2X

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SYBR Green Reaction Mix, and water to complete the volume). Initial temperatures of 50°C for 3 min and 95°C for 5 min were applied in thermal cycling, followed by 95°C for 15 s, 60°C for 30 s for the next 40 cycles, and a final temperature of 40°C for 1 min. All the data have been normalized to represent reference genes using the Bio-Rad CFX manager software (version 3.1). The values were normalized against either U6 for miR-106b-5p or GAPDH for GDF11. The primer sequences are listed in Table 1.

### Cell migration detection

Cell migration was tested using a Transwell assay. The transfected cells (MDA-MB-231 and MDA-MB-453) were suspended in a serum-free medium ( $2 \times 10^5$  cells/mL), from which 100  $\mu$ L cells were taken and injected into a transwell insert (specifically to the upper chamber). In the bottom of the chambers, DMEM (500  $\mu$ L) containing 10 % FBS and 2  $\mu$ M CDDP were present. Following a 24-hour incubation period, the cells were transferred to the bottom chamber, 4% paraformaldehyde was added to fix them, and they were stained with crystal violet (Cat#: C0121; Beyotime, China) for 15 minutes each, after which the cell counts were counted using the Image J software.

### Animal experiment

Ten 4-week-old BALB/c mice (18 $\pm$ 2 g) (Hunan SJA Laboratory Animal Company, China) were randomly divided into two groups of 5 mice. The animal experiments were approved by The Clinical Medical College & First Affiliated Hospital of Chengdu Medical College. A new type of miRNA mimic that has been chemically developed (MiR-106b-5p agomir) and negative control (agomir-NC) were both acquired by RiboBio in China. MDA-MB-231 cells were plated to a 30-50% confluence and stably transfected with 100 nM miR-122 agomir or agomir-NC. The transfected MDA-MB-231 cells were subcutaneously injected ( $4 \times 10^5$  cells/mouse) into the fourth pair of mammary fat pads of each mouse. The mice were then treated with 5 mg/kg of CDDP (intraperitoneal injection) once a week. The mice were examined every 4 days and subjected to euthanasia

28 days after tumor inoculation. Tumor growth was determined by monitoring the tumor volume, which was calculated with the following formula: volume=(length  $\times$  width<sup>2</sup>) $\times$ 0.5. The tumor weight was measured after sacrifice.

### Dual-luciferase reporter assay

The binding sites between miR-106b-5p and GDF11 were predicted by the Starbase database and verified with a dual-luciferase report assay using the Luciferase Reporter System (Cat#: E1910, Promega, USA). It was necessary to combine the GDF11 3'-UTR with the miR-106b-5p complementary sequences, which were synthesized and produced into pMIR-REPORT (Ambion, USA) to obtain the GDF11 wild-types (WT). On the other hand, the mutant (MUT) reporters of GDF11 were generated with the help of a QuickMutation Site-Directed Mutagenesis Kit (Beyotime, China). To co-transfect the MDA-MB-231 and MDA-MB-453 cells with the miR-106b-5p mimic or miR-NC cells, luciferase reporter vectors were constructed and used in conjunction with lipofectamine 2000 reagents (ThermoFisher, United States). At 48 hours after transfection, with the application of the dual-luciferase reporter system, the relative luciferase intensity was measured.

### Western blotting

Western blotting was used to assess the expression of the protein (GDF11). After 2  $\mu$ M of CDDP treatment, the control or transfected cells were lysed using the RIPA buffer (Merck KGaA) and thereafter centrifuged at 10000 RCF for 10 min at 4°C to obtain the total protein. The protein concentration of the supernatant was determined using a BCA Protein assay kit (Cat#: 23225; ThermoFisher, USA) according to the manufacturer's instructions. The proteins were separated using 10 % SDS-PAGE, which was carried out for 30 min at 225 V in a Tris-Glycine SDS Running Buffer, and were then transferred onto a nitrocellulose membrane. The membranes were probed with primary antibodies against GDF11 (Cat#: ab234647; 1:2000; Abcam, UK) at 37°C for an hour and GAPDH (Cat#: ab181602; 1:10000; Abcam, UK) at 4°C overnight. In the next step, the secondary HRP antibody (Cat#: ab6721; 1: 10000; Abcam, UK) was used to probe the membranes for 2 hours at room temperature. Following this, the BeyoECL Plus (Beyotime, USA) was added to the membranes for visualizing the protein blots.

### Statistical analysis

The mean  $\pm$  standard deviation was calculated using data from three different trials and GraphPad Prism 6 was used to conduct the statistical analysis. Statistical analyses were performed using the t-test to compare two groups. To compare distinct groups, Tukey's post hoc

**Table 1.** Real-time PCR Primer synthesis list.

Gene	Sequences	
miR-106b-5p	Forward	5'-TGCGGCAACACCAGTCGATGG-3'
	Reverse	5'-CCAGTGCAGGGTCCGAGGT-3'
GDF11	Forward	5'-AGTGGGTCACTACAAGCTGC-3'
	Reverse	5'-GATAAGGGGAGCTTCAGGGG-3'
U6	Forward	5'-CTCGCTTCGGCAGCAC-3'
	Reverse	5'-AACGCTTCACGAATTTGCGT-3'
GAPDH	Forward	5'-AGAAAAACCTGCCAAATATGATGAC-3'
	Reverse	5'-TGGGTGTCGCTGTTGAAGTC-3'

and one-way ANOVA tests were utilized. To analyze the correlation between two gene expressions in TNBC, the Pearson correlation analysis was used. For the statistical tests, a value of  $P < 0.05$  was considered statistically significant.

## Results

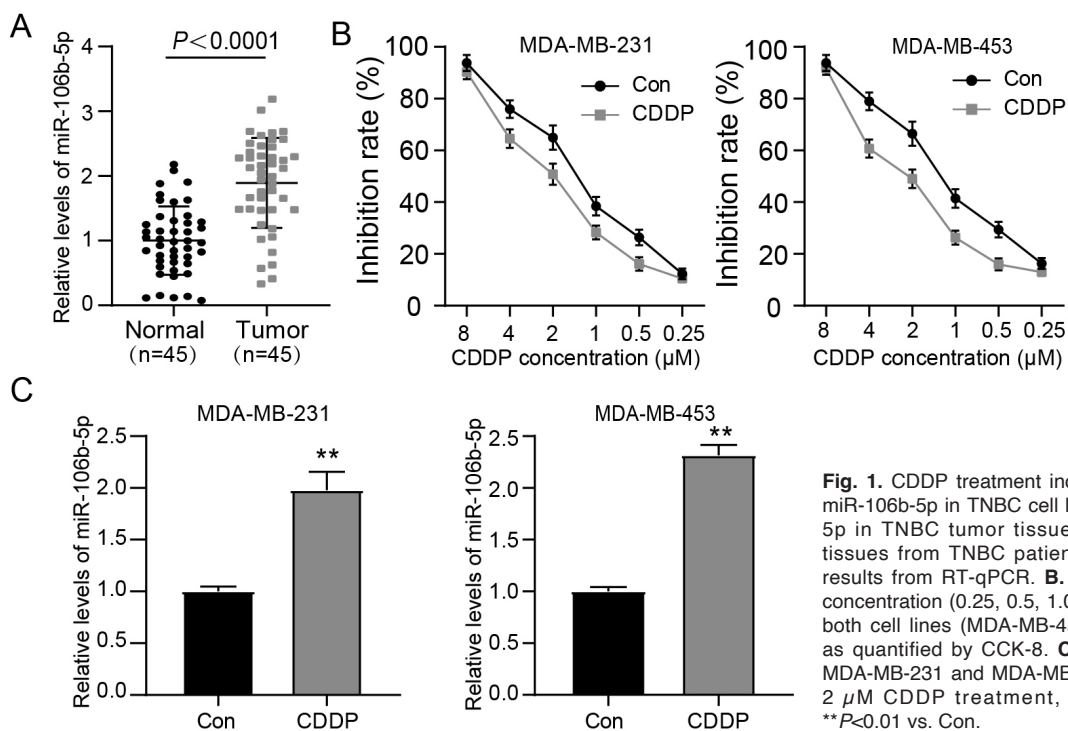
### CDDP treatment increases the expression levels of miR-106b-5p in TNBC cell lines

First, we examined the expression patterns of miR-106b-5p in TNBC to see how they changed. As shown in Figure 1A, compared to paired normal tissues, the TNBC tissues in patients had higher expression levels of miR-106b-5p. Following that, various concentrations of CDDP were administered to the MDA-MB-453 and MDA-MB-231 cell lines, and the rate at which cell viability was inhibited was determined. As demonstrated in Figure 1B, CDDP significantly reduced the viability of the MDA-MB-231 and MDA-MB-453 cells in a concentration-dependent manner. Moreover, 2  $\mu\text{M}$  of CDDP was chosen for the following experiments since CDDP at this concentration resulted in a nearly 50% inhibition of cell viability (Fig. 1B). In both the MDA-MB-231 and MDA-MB-453 cells, the levels of miR-106b-5p expression were measured before and after treatment with 2  $\mu\text{M}$  of CDDP. During CDDP treatment, the miR-106b-5p levels increased significantly (Fig. 1C). As a result of these observations, it was suggested that an increased expression of miR-106b-5p in TNBC might

be a contributor to CDDP resistance.

### Inhibition of miR-106b-5p suppresses CDDP-induced TNBC cell proliferation and migration in vitro and reduces CDDP-induced TNBC tumor cell growth in vivo

Next, we confirmed the function of miR-106b-5p in the CDDP resistance TNBC cells. As exhibited in Figure 2A, it was discovered that the expression levels of MDA-MB-231 and MDA-MB-453 were considerably lowered in the presence of a miR-106b-5p inhibitor. Subsequently, the proliferation of cells was detected in the 2  $\mu\text{M}$  CDDP therapy. The results showed that compared to the cells that were transfected with the NC, the cells that were transfected with the miR-106b-5p inhibitor exhibited decreased cell proliferation (Fig. 2B). Furthermore, cell migration was examined, and it was discovered that the miR-106b-5p inhibitor caused a reduction in cell migration of both the cell lines in the presence of 2  $\mu\text{M}$  of CDDP treatment, compared to the control (Fig. 2C). Based on these data, it seems that miR-106b-5p knockdown may enhance the sensitivity of TNBC cell lines to CDDP. In addition, an in vivo experiment using xenograft mice was performed to verify the in vitro findings. Consistently, it was observed in the CDDP-treated mice that the growth of the TNBC tumor in a xenograft was significantly reduced by the miR-106b-5p inhibitor (Fig. 3A-C). MiR-106b-5p interference was shown to reduce the malignant phenotype of the CDDP-resistant TNBC cell lines both in vivo and in vitro, according to the findings.



**Fig. 1.** CDDP treatment increases the expression level of miR-106b-5p in TNBC cell lines. **A.** The level of miR-106b-5p in TNBC tumor tissues and noncancerous normal tissues from TNBC patients (n=45), quantification data results from RT-qPCR. **B.** The inhibition rate of a series concentration (0.25, 0.5, 1.0, 2.0, 4.0, 8.0  $\mu\text{M}$ ) of CDDP on both cell lines (MDA-MB-453 and MDA-MB-231) viability, as quantified by CCK-8. **C.** The level of miR-106b-5p in MDA-MB-231 and MDA-MB-453 cell lines before and after 2  $\mu\text{M}$  CDDP treatment, as quantified by RT-qPCR. \*\* $P < 0.01$  vs. Con.



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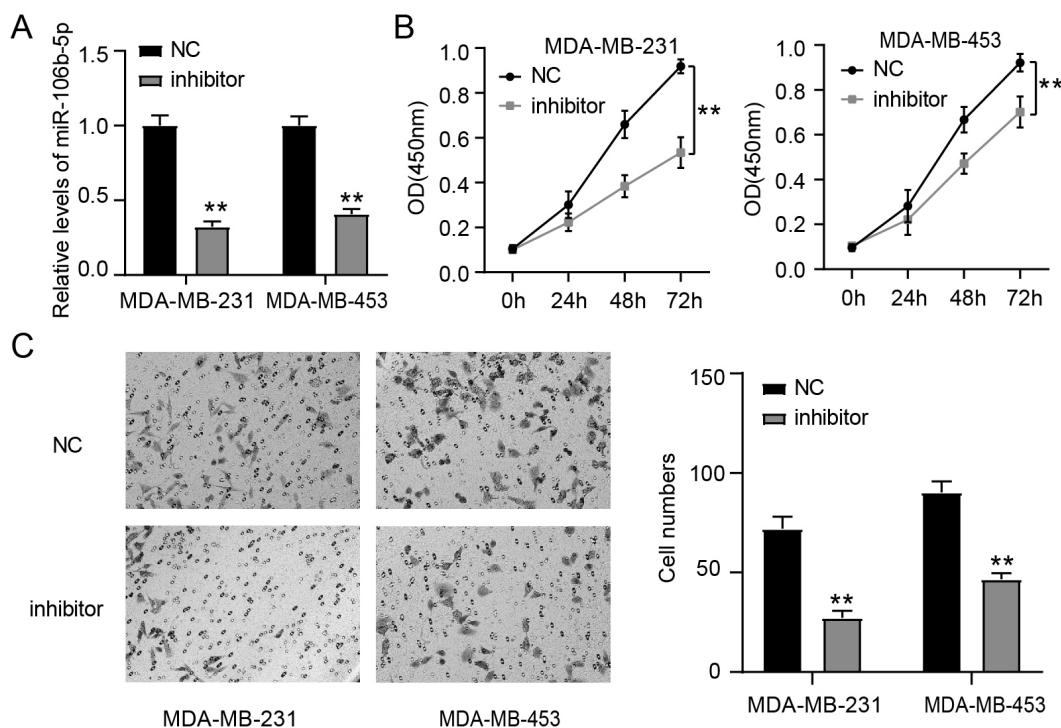
### miR-106b-5p directly targets GDF11

Starbase databases were used to search for downstream targets of miR-106b-5p and showed that GDF11 and miR-106b-5p could be combined. The binding site sequences of miR-106b-5p and GDF11 are presented in Figure 4A. To determine their binding properties, the dual-luciferase reporter assay was used. The findings revealed that the miR-106b-5p mimic significantly lowered luciferase activity in the WT GDF11 group in both cell lines. MUT in the binding site of GDF11, on the other hand, resulted in the retention of luciferase (Fig. 4B). In addition, GDF11 exhibited a significantly downregulated expression in the TNBC tissues when compared to the normal tissues (Fig. 4C).

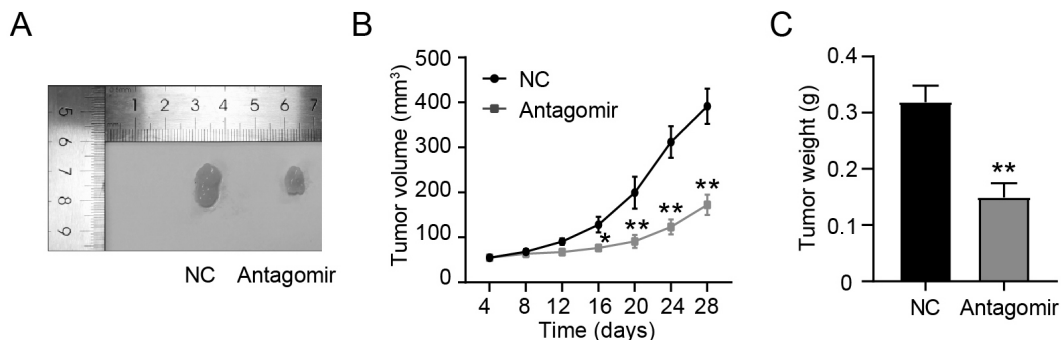
Furthermore, as denoted in Figure 4D, the miR-106b-5p and GDF11 expression levels in the TNBC tissues were negatively correlated. According to the evidence presented above, miR-106b-5p is shown to influence TNBC resistance to CDDP via targeting GDF11.

### GDF11 silencing reverses the inhibitory effect of the miR-106b-5p inhibitor on CDDP resistance in TNBC cells

Finally, to validate the miR-106b-5p effects on TNBC resistance to CDDP via targeting GDF11, the expression of GDF11 in both the MDA-MB-231 and MDA-MB-453 cells before and after 2  $\mu$ M of CDDP treatment was measured. As illustrated in Figure 5A, CDDP treatment markedly reduced the expression of



**Fig. 2.** MiR-106b-5p inhibitor enhances the sensitivity of TNBC cells to CDDP. Cell lines (MDA-MB-231 and MDA-MB-453) transfected with NC or miR-106b-5p inhibitor, then treated with 2  $\mu$ M CDDP, then RT-qPCR results show miR-106b-5p level in cells (A). Cell proliferation at 24, 48 and 72h after treatment was measured by CCK-8 assay (B). Transwell assay measurements for cell migration value (C). \*\* $P < 0.01$  vs. NC.



**Fig. 3.** MiR-106b-5p inhibitor enhances the sensitivity of xenograft TNBC tumors to CDDP. MDA-MB-231 cells that transfection with NC or Antagomir were injected into mice, then Tumors were photographed at 28 days after injection (A). Tumor volume was read every four days (B). Tumor weight measured at 28 days after injection (C). \* $P < 0.05$  and \*\* $P < 0.01$  vs. Con.

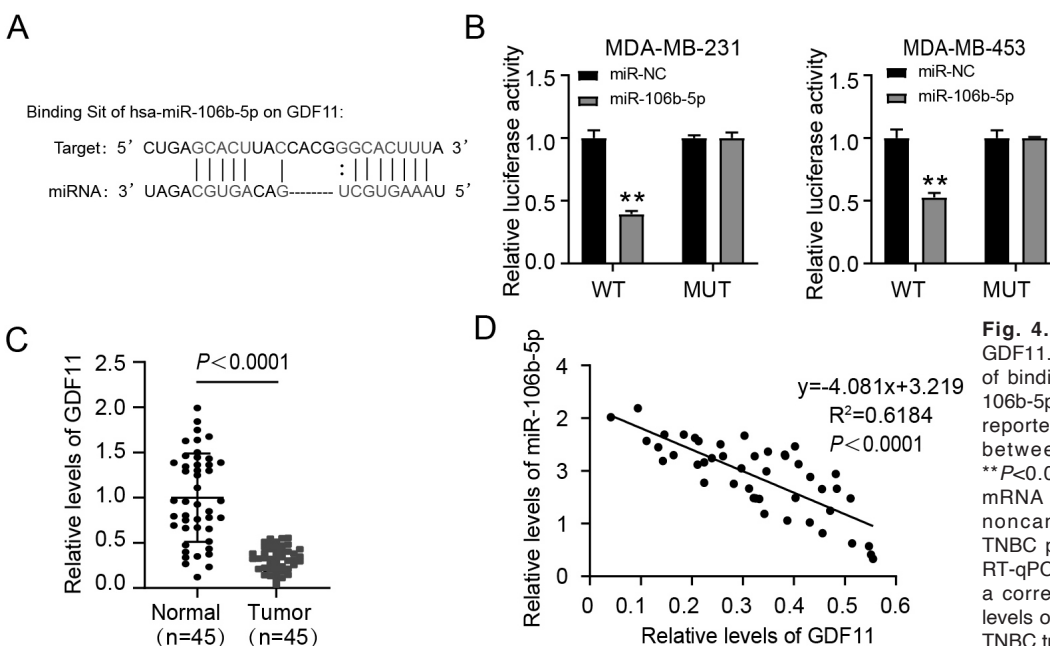
GDF11 in the two TNBC cell lines. Following that, GDF11 was silenced in both the MDA-MB-231 and MDA-MB-453 cells by the transfection of si-GDF11 into the cells. Co-transfection of inhibitors of GDF11 and miR-106b-5p into the cells resulted in the GDF11 and miR-106b-5p expression levels being in harmony with one another. As shown in Figure 5B, the expression of GDF11 was effectively inhibited in the CDDP-resistant MDA-MB-453 cells as well as in the MDA-MB-231 cells transfected with si-GDF11, while the miR-106b-5p inhibitor alleviated the low expression of GDF11 caused by GDF11 knockdown. Moreover, following treatment with 2  $\mu$ M of CDDP, GDF11 knockdown stimulated cell proliferation, whereas miR-106b-5p silencing significantly reversed the pro-proliferation effect of GDF11 knockdown (Fig. 5C). Additionally, in the presence of 2  $\mu$ M of CDDP, the low GDF11 expression significantly enhanced cell migration, and this enhancement was partially eliminated by the miR-106b-5p inhibitor (Fig. 5D). These data suggest that the low expression of GDF11 promotes the malignant phenotype of CDDP-resistant TNBC cells, and GDF11 mediates the regulation of miR-106b-5p on TNBC.

## Discussion

CDDP-based therapies are essential for the clinical treatment of TNBC; however, CDDP resistance has limited the therapeutic effectiveness in TNBC patients (Hill et al., 2019). Increased evidence indicates that miRNA is significant in the acquisition of TNBC cell chemical resistance (Xi et al., 2019; Lin et al., 2021). According to the findings of the current study, miR-106b-5p was elevated in the TNBC tissues and CDDP-

treated TNBC cells, and inhibiting it effectively lowered TNBC CDDP resistance both in vivo and in vitro. In addition, we identified that miR-106b-5p mediated its influence on TNBC by targeting GDF11 and negatively regulating GDF11 expression.

So far, several miRNAs have been demonstrated to regulate CDDP resistance in TNBC. For example, miR-1296 overexpression promotes CDDP sensitivity in TNBC cell lines (Albaker et al., 2021). Upregulation of miR-133b enhances the proliferation and DDP sensitivity of TNBC cells or CDDP-resistant TNBC cells (Lin et al., 2021). The abnormal regulation of miRNAs in breast cancer tissues can provide insights into the understanding and prediction of breast cancer initiation, progression, and maintenance (McGuire et al., 2015). The results of an investigation revealed that miR-106b-5p has a carcinogenic impact on hepatocellular carcinoma cells (Gu et al., 2019), glioblastoma (Shi et al., 2020), osteosarcoma (He et al., 2020) and other tumors. Furthermore, TNBC metastasis has been connected to miR-106b-5p, which has been shown to be highly expressed in TNBC (Moi et al., 2019; Banerjee et al., 2020). In this work, we discovered the effect of elevated miR-106b-5p in TNBC tissues compared to normal tissues; the results were with previous studies and suggest that miR-106b-5p may play a role in TNBC tumors. Furthermore, miR-106b-5p is related to the ability of cancer cells to tolerate CDDP. Francesca et al. (Mastropasqua et al., 2017) revealed that reducing miR-106b-5p levels might increase the chemosensitivity of renal cell carcinoma and colorectal cancer-derived cells to certain anti-tumor drugs. Similarly, we found that following CDDP therapy, miR-106b-5p expression was increased in the TNBC cell lines. Further studies



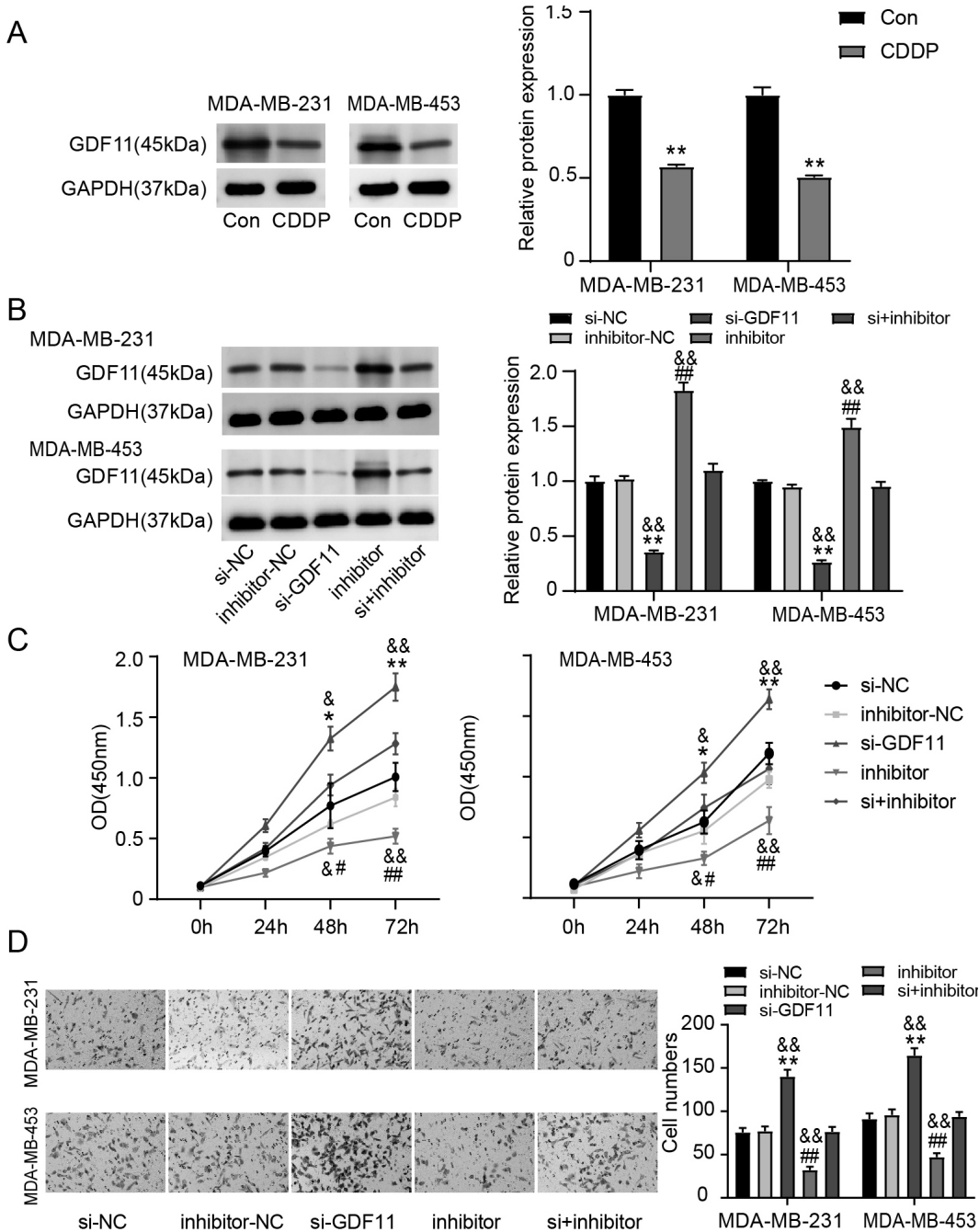
**Fig. 4.** miR-106b-5p directly targets GDF11. **A.** Starbase database prediction of binding site sequences among miR-106b-5p and GDF11. **B.** Dual-luciferase reporter assay data show the binding between miR-106b-5p and GDF11. **C.** The level of mRNA (GDF11) in tumor tissues and noncancerous normal tissues from TNBC patients (n=45), as quantified by RT-qPCR. **D.** Pearson's analysis showed a correlation between the expression levels of the miR-106b-5p and GDF11 of TNBC tumor tissue.

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discovered that the inhibition of miR-106b-5p enhanced the sensitivity of the TNBC cell lines and TNBC xenograft tumor to CDDP both *in vitro* as well as *in vivo*, indicating that miR-106b-5p may promote CDDP resistance in TNBC. All these findings indicate that miR-106b-5p can be a promising therapeutic target for drug resistance in cancers.

The downstream targets of miR-106b-5p were predicted using the Starbase database to examine the

molecular mechanism by which miR-106b-5p induces CDDP resistance in TNBC. GDF11 inhibits the proliferation of cancer cells and promotes apoptosis in various types of cancer, including liver cancer (Frohlich et al., 2021), pancreatic cancer (Liu et al., 2018), and esophageal cancer (Gu et al., 2018). In addition, GDF11 was found to have a tumor suppression pattern and predict a poor prognosis in multiple breast cancer subtypes, including TNBC (Alvarez et al., 2016; Bajjkar



**Fig. 5.** GDF11 silencing reverses the inhibitory effect of miR-106b-5p inhibitor on CDDP resistance in TNBC cells. **A.** Western blotting results of GDF11 protein expression in MDA-MB-453 and MDA-MB-231 cells before and after treatment of 2  $\mu$ M CDDP. \*\* $P$ <0.01 vs. Con. **B.** Western blotting results of GDF11 in MDA-MB-231 and MDA-MB-453 cells treated with CDDP that transfected with si-NC, inhibitor-NC, si-GDF11, inhibitor and si + inhibitor. **C.** CCK-8 assay measurements for the proliferation of CDDP-treated MDA-MB-231 and MDA-MB-453 cells transfected with si-NC, inhibitor-NC, si-GDF11, inhibitor and si + inhibitor. **D.** CDDP-treated cells (MDA-MB-231 and MDA-MB-453) migration results, transfected with inhibitor-NC, si-NC, si-GDF11, inhibitor and si + inhibitor, determined by Transwell assay. \* $P$ <0.05 and \*\* $P$ <0.01 vs. si-NC; # $P$ <0.05 and ## $P$ <0.01 vs. inhibitor-NC; & $P$ <0.05 and && $P$ <0.01 vs. si + inhibitor.

et al., 2017; Wallner et al., 2018). Consistently, this study found that GDF11 was down-regulated in TNBC compared to normal tissues. Interestingly, Kieren et al. (Marini et al., 2018) reported that GDF11 inhibits CDDP resistance in lung adenocarcinoma. Our results in TNBC were similar to those in lung adenocarcinoma, which were that the down-regulation of GDF11 significantly reduced the sensitivity of the CDDP-resistant TNBC cell lines to CDDP, and promoted cell proliferation and migration. Combined with the targeting outcome of miR-106b-5p on GDF11, we suggested that GDF11 inhibits CDDP tolerance in TNBC cells via miR-106b-5p mediation.

However, it is worth mentioning that there are several limitations in this study. The expression of miR-106b-5p and its relationship with survival and prognosis need to be further explored. In addition, the upstream and downstream molecular mechanisms of the miR-106b-5p/GDF11 axis need to be further elucidated.

### Conclusion

Overall, the current study is the first to demonstrate that miR-106b-5p may have a role in the development of CDDP resistance in TNBC. Importantly, targeting GDF11 in TNBC may be required to inhibit miR-106b-5p for CDDP sensitivity. As a result, the miR-106b-5p/GDF11 axis might be a suitable target for treating CDDP resistance in TNBC. Whether this axis regulates the drug resistance of other chemotherapy drugs used for the treatment of TNBC or breast cancer needs to be elucidated.

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*Conflicts of interest.* No conflict of interests was declared by the authors.

*Ethical approval.* Ethics Committee of The Clinical Medical College & First Affiliated Hospital of Chengdu Medical College (Chengdu, China) approved this work. All clinical samples were processed in strict concurrence with the Declaration of Helsinki's ethical standards. All patients signed written informed consent.

The procedures executed in the animal study were authorized by the Ethics Committee of The Clinical Medical College & First Affiliated Hospital of Chengdu Medical College. All experiments on animals comply with the ARRIVE guidelines.

*Consent to participate.* All participants signed a written informed consent form.

*Publication Consent.* The participant received the consent of the publication.

*Data and material availability.* The corresponding author could provide access to the data and materials used and analyzed in this study upon request.

*Code availability.* Not available.

*Authors' contributions.* QZ and QLH performed the experiments and the analysis of the data. QZ designed and devised the study. QLH obtained the data. QZ and QLH processed and interpreted the data. The manuscript has been read and approved by all authors.

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