Chemotherapy-Induced miRNA-29c/Catenin-δ Signaling Suppresses Metastasis in Gastric Cancer

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Abstract

Chemotherapy has improved the survival of patients with gastric cancer by unknown mechanisms. In this study, we showed that cisplatin and docetaxel used in gastric cancer treatment increase the expression of miRNA-29 (miR-29) family members and decrease the expression of their oncogenic targets, mediating a significant part of the efficacious benefits of these chemotherapy agents. In particular, patients with gastric cancer who experienced recurrences after chemotherapy tended to exhibit low levels of miR-29c expression in their tumors, suggesting that miR-29c activation may contribute to the chemotherapeutic efficacy.

Enforced expression of miR-29s in gastric cancer cells inhibited cell invasion in vitro and in vivo by directly targeting catenin-δ (CTNND1). Drug treatment suppressed gastric cancer cell invasion by restoring miR-29c-mediated suppression of catenin-δ and RhoA signaling. In parallel, drug treatment also activated several tumor-suppressive miRNAs, thereby decreasing expression of their oncogenic effector targets. Overall, our findings define a new mechanism for understanding the efficacious effects of cytotoxic chemotherapy in gastric cancer. Cancer Res; 75(7) April 1, 2015

Introduction

Gastric cancer is the fourth most common cancer, and its prognosis remains poor despite adequate surgery with radical lymphadenectomy (1). Most patients with gastric cancer demonstrate locally advanced disease at the time of diagnosis and may also require chemotherapy and/or radiotherapy (1–2). Recent studies have demonstrated an advantage for perioperative chemotherapy compared with surgery (3). However, the molecular mechanisms of chemotherapeutic drugs used for gastric cancer treatment are not well established.

MicroRNAs (miRNA) play critical roles in multiple biologic processes by regulating mRNAs via cleavage or translational repression (4). The loss of homeostasis in the miRNA/mRNA axis leads to relevant pathologic events, including gastric carcinogenesis (5). Therefore, miRNAs could serve as potential biomarkers for gastric cancer clinical diagnosis and prevention. The microRNA-29 (miR-29) family consists of three members (miR-29a, miR-29b, and miR-29c) and has been shown to suppress cancer cell growth (6–9). Our previous study indicated that miR-29s not only functioned as ts-miRs in gastric cancer cell metastasis and the corresponding mechanisms is not well established. Recently, Saito and colleagues reported that miR-29c could be activated by celecoxib, suggesting that selective COX2 inhibitors may hold promise for gastric cancer treatment by restoring miR-29c (10). These findings suggested that miR-29s not only functioned as ts-miRs in gastric cancer but also might serve as effective predictors for gastric cancer prevention.

Herein, we identify miR-29c as a potential biomarker for predicting the prognosis of patients with gastric cancer who receive...
We further find that miR-29s act as metastasis suppressors by directly targeting catenin-δ (CTNND1) in gastric cancer. Moreover, we demonstrate that chemotherapeutic drugs represent their suppressive impact on gastric cancer cell invasion via remodifying the miR-29c-mediated catenin-δ/GTP Rho axis.

Materials and Methods

Clinical specimens and cell lines

Tissues were collected as previously described (12). The patient characteristics are provided in Supplementary Table S1. The normal gastric epithelial cell line (GES-1) was a kind gift from Dr. Juan Shi, Peking Union Medical College, Beijing, China. 293T and gastric cancer cell line, HGC27, MGC803, BGC823, and MKN45, were obtained from the ATCC and grown in DMEM with 10% FBS (Hyclone) at 37°C and 5% CO2 cell culture incubator. Cell lines were tested 1 month before the experiment by methods of morphology check by microscopy, growth curve analysis, and mycoplasma detection according to the ATCC cell line verification test recommendations.

Quantification of RNA and protein

Total RNA was extracted from the cells and tissues with TRIzol reagent (Invitrogen). Quantitative real-time PCR (qRT-PCR) assay was conducted to detect the ts-miRs expression, and primers sequences are listed in Supplementary Table S3. Western blotting of proteins was performed as described previously (12). The antibodies included those against catenin-δ, PDK1, YWHAZ, GTP-Rho, Rho, coflin, phosphorylated-cofilin (p-Cofilin), and GAPDH (catenin-δ, PDK1, and GAPDH from Abcam, others from CST).

Cell proliferation, migration, and invasion assays

The cellular proliferation rate was measured using CCK-8 (DOJINDO) as previously described (12). Scratch wound assay
was conducted to detect cell migration and performed as described (13). Invasion assay was evaluated by the ability of cells passing through Matrigel-coated membrane matrix (BD Biosciences) and performed as described (12).

Affinity precipitation of cellular GTP-Rho

Active Rho pull-down was conducted in HGC-27 cells transfected with miR-29s through Active Rho Pull-Down and Detection Kit (Pierce) as described (13).
Immunohistochemistry and light microscopy

Immunohistochemistry was conducted to measure the catenin-δ, Ki-67, and caspase-3 expression as described (14). The localization of catenin-δ in gastric cancer cells was detected using immunostaining as described (13).

Tumorigenicity and metastasis formation assay

All experimental procedures involving animals were performed in accordance with The Guide for the Care and Use of Laboratory Animals (NIH publications Nos. 80–23, revised 1996) and according to the institutional ethical guidelines of Peking Union Medical College for animal experiments. Tumorigenicity and metastasis formation assay were performed as described (14–15).

Detailed experimental procedures are provided in Supplementary Materials and Methods.

Statistical analysis

Each experiment was repeated at least 3 times. The Student t test (2-tailed) was performed and 3-group data were analyzed using
miRNA-29c Predicting Gastric Cancer Chemotherapy

Results

miR-29 expression is activated by cisplatin and docetaxel in gastric cancer cells

To elucidate the role of miR-29 in gastric cancer chemotherapy, we determined their expression in gastric cancer cells treated with cisplatin or docetaxel. qRT-PCR analysis was performed to effectively discriminate miR-29a, miR-29b, and miR-29c expression (Supplementary Fig. S1A–S1D) and then applied to evaluate their levels in gastric cancer cell lines, showing that miR-29 levels depicted significant reduction (Fig. 1A). We next treated MGC803 and HGC27 cells, which have low miR-29s expression, with cisplatin and docetaxel (0–10 μg/mL), and the estimated IC_{50} values in these cell lines were 0.9 and 0.09 mg/L for cisplatin in MGC803 and HGC27 cells, respectively, and 0.008 and 0.0009 mg/L for docetaxel in MGC803 and HGC27 cells, respectively (Fig. 1B and C). Further qRT-PCR analysis indicated that miR-29 expression was augmented following cisplatin and docetaxel treatment (Fig. 1D and E). Moreover, we observed that MCL1 and BCL2, which are validated miR-29s targets (6, 16), were decreased in drug-treated gastric cancer cells (Fig. 1F and G). These findings suggested that chemotherapy may hold clinical promise for gastric cancer treatment through regulating miR-29–mediated program.

Deregulated miR-29c expression is associated with gastric cancer progression and survival

We next investigated the correlation between miR-29 and gastric cancer development. qRT-PCR was used to assess miR-29 levels in tissues from 166 cases of gastric cancer, and we found that miR-29a, miR-29b, and miR-29c were significantly down-regulated in the majority of gastric cancer tissues examined when compared with matched normal gastric tissues (Fig. 2A). The association between miR-29 expression and histologic grade was explored, and no significant difference was found between well- and poorly differentiated gastric cancer tissues (Supplementary Fig. S2A–S2C). However, we observed that miR-29c down-regulation was correlated with a more extensive venous invasion (P = 0.02; Fig. 2B) and a more aggressive tumor phenotype (P = 0.05; Fig. 2C). No clear relationship was observed between decreased miR-29a or miR-29b expression and worse prognosis in patients with gastric cancer (Supplementary Fig. S2D and S2E).

We further investigated whether chemotherapy held better clinical promise for patients with gastric cancer with higher miR-29 expression in tumors. Follow-up studies were performed with 66 cases of patients with gastric cancer undergoing chemotherapy after resection, and these patients were divided into 2 groups based on recurrence 3 years after treatment. We observed that the tumors of patients with gastric cancer who underwent recurrence had lower miR-29c levels (Fig. 2G; Supplementary Fig. S2F).

miR-29 suppresses gastric cancer cell migration and invasion in vitro

Given the parallels between miR-29c and metastatic potential, we next investigated the role of miR-29 in gastric cancer cell movement. miR-29s were overexpressed in MGC803 and HGC27 cells (Supplementary Fig. S3), and wound-healing assays were performed to assess gastric cancer cell migration. Scramble-treated MGC803 cells completely sealed linear scratch wounds between 12 and 36 hours after injury, whereas miR-29s–treated MGC803 cells sealed only about 70% of the wound area after 36 hours (Fig. 3A). Similar results were observed in HGC27 cells (Fig. 3B). In vitro invasion assays were also conducted to evaluate gastric cancer cell metastasis and demonstrated that miR-29 significantly decreased invasive gastric cancer cells compared with scramble-treated cells (Fig. 3C and D).

miR-29c inhibits gastric cancer cell metastasis in vivo

To confirm the in vitro findings, we conducted in vivo metastasis assays. In doing so, 5 × 10^6 viable HGC27 cells infected with Lenti-mir-29c (Lenti-29c) or Lenti-scramble (Lenti-scr) were resuspended in 0.1-mL PBS and injected into the lateral tail veins of nude mice. Five weeks after injection, the animals were euthanized, and xenografts were harvested. Statistical analysis was performed using SPSS 16.0 software (SPSS Inc.). P < 0.05 was considered statistically significant.

Figure 4. Catenin-β1 is a bona fide target of miR-29s in gastric cancer. A, sequence present in the 3′UTR of catenin-β1 targeted by miR-29s. The sequences shaded in gray represent mutants of the miR-29–matched seed sequence. B, miR-29 expression decreased the luciferase activity when linked to the segment containing the target sequence within the 3′UTR in catenin-β1 mRNA. Mutation of the seed sequence abolished miR-29s–dependent repression. C, the protein level of catenin-β1 in HGC27 and MGC803 cells upon miR-29s mimic or scr treatment, as measured by immunoblotting. GAPDH served as a loading control and data were normalized by Scr-treated gastric cancer cells. The numbers below the panels represent the normalized protein expression levels (D, miR-29 inhibitors (anti-29a, anti-29b, and anti-29c) blocked the action of endogenous miR-29s in gastric cancer cells. The catenin-β1 protein level was measured by immunoblotting. GAPDH served as a loading control and data were normalized by Anti-con (negative control oligonucleotide)-treated gastric cancer cells. E and F, cisplatin and docetaxel treatment led to a decrease in catenin-β1 protein levels in HGC27 (E) and MGC803 (F) cells, as shown by immunoblotting analysis. The numbers below the panels represent the normalized protein expression levels. GAPDH served as a loading control and data were normalized by control treated gastric cancer cells. G, immunofluorescence microscopy of HGC27 cells stained with anti-catenin-β1 and DAPI (nuclei), showing a decrease in catenin-β1 staining after treatment with miR-29s mimics. Bar, 50 μm. H, pathologic analysis of tissue sections from recipient mice at 4 weeks postinjection. H&E staining and labeling with anti-catenin-β1, anti-Ki-67, and caspase-3 was performed. Bar, 50 μm. I, normalization of catenin-β1, Ki-67, and caspase-3 expression in the tissue sections. Data are mean ± SEM (n = 3) and are representative of three independent experiments. J, qRT-PCR analysis of miR-29 expression in 8 pairs of gastric cancer samples. U6 snRNA served as a loading control. \( \triangle \), C, gastric cancer tissues; \( \odot \), N, the matched normal gastric tissues. The numbers indicate specimens of different patients with gastric cancer. K, immunoblot analysis of catenin-β1 in 8 pairs of gastric cancer samples described as K. GAPDH served as a loading control. The catenin-β1 expression level was normalized to normal control and is shown in the histogram. n = 8. L, representative images of catenin-β1 immunohistochemical analysis in the same samples described in H. Magnification, ×20. Bar, 100 μm. The fold change was normalized to the normal control. \( * \), P < 0.05; \( ** \), P < 0.01.
sacrificed, and the lungs and liver were dissected for microscopic histology. The number of liver metastases in mice injected with Lenti-29c-infected HGC27 cells was significantly lower than that in mice injected with Lenti-scr-infected cells (Fig. 3E and F). Hema-
toxyanin and eosin (H&E) staining was also performed to assess the pathologic properties of the liver tissues, which showed more metastatic nodules in Lenti-scr–treated than in Lenti-29c–treated mice (Fig. 3G). Similar results were observed with H&E staining in the lung tissues, although we did not observe significant changes in outward appearance (Fig. 3H).

Catenin-δ is a direct target of miR-29

To identify miRNA targets of miR-29s relevant to gastric cancer cell invasion, we interrogated the TargetScan (17) and miRanda (18) miRNA target prediction programs. Catenin-δ was identified as a possible target of miR-29s and encodes a member of the armadillo protein family, which functions in adhesion between cells and signal transduction (19). The 3’UTR of catenin-δ houses a sequence that matches the seed sequence contained in miR-29 (Fig. 4A). Transfection of the catenin-δ-3’UTR-luciferase reporter in combination with miR-29 mimics in 293T cells revealed that miR-29 repressed the luciferase activity of this reporter. Moreover, mutation of miR-29 sites abrogated this reduction in luciferase activity (Fig. 4B). Furthermore, elevating miR-29s in HGC27 or MGC803 cells reduced the catenin-δ protein level (Fig. 4C), whereas diminishing miR-29 activity using specific inhibitors (anti-29) resulted in increased catenin-δ expression (Fig. 4D, Supplementary Fig. S4). Because cisplatin and docetaxel treatment led to an increase of miR-29 levels in gastric cancer cells and catenin-δ is a miR-29 target, catenin-δ level may be decreased in gastric cancer cells treated by these drugs. Immunoblotting analysis confirmed this hypothesis (Fig. 4E and F). Moreover, loss of the predominant membrane localization of catenin-δ and gain of cytosolic or nuclear catenin-δ labeling closely correlated with cancer invasion (20–21). Therefore, we further analyzed the levels of catenin-δ in the cytoplasm via immunostaining. Compared with scramble-treated cells, HGC27 cells transfected with miR-29 mimics demonstrated a significant decrease in catenin-δ expression (Fig. 4G).

We also validated the miR-29c–mediated catenin-δ axis in xenograft mouse tumors. miR-29c was overexpressed in HGC27 cells using a lentivirus and subsequent cell growth analysis indicated that miR-29C restoration suppressed HGC27 cell proliferation in vitro and inhibited gastric cancer tumor formation in vivo (Supplementary Fig. S5). Immunohistochemical analysis was performed in randomly selected xenograft mouse tumors, which showed that Lenti-29c–injected tumors expressed lower levels of catenin-δ than the controls. Ki-67 and caspase-3 expression analysis demonstrated that miR-29c retarded gastric cancer cell growth (Fig. 4H and I). These data indicated that catenin-δ was a functional target of miR-29 in gastric cancer.

Catenin-δ is upregulated in gastric cancer tissues with low levels of miR-29

We next investigated whether catenin-δ was expressed at a higher level in gastric cancer tissues with downregulated miR-29s. qRT-PCR and immunoblots were conducted for 20 pairs of gastric cancer tissues, demonstrating that the levels of miR-29 were decreased in these tissues (Fig. 4J). In addition, catenin-δ expression was significantly upregulated in the same specimens shown by immunoblots (Fig. 4K; Supple-

mRNA for catenin-δ was also increased in gastric cancer tissues shown by immunohistochemical analysis (Fig. 4L, Supplementary Fig. S7). These findings indicated a significant inverse correlation between miR-29 and catenin-δ expression.

Silencing catenin-δ decreases gastric cancer cell migration and invasion

Of note, loss of E-cadherin leads to catenin-δ mislocalization in the cytoplasm and disrupts its interaction with Rho GTPases and subsequent promotion of cell metastasis(22). Because the miR-29/catenin-δ axis was established in gastric cancer cells, we reasoned that silencing catenin-δ should retard the ability of gastric cancer cells to seal scratch wounds. As predicted, si_catenin-

δ–treated HGC27 cells sealed wounds at a slower rate compared with si_Con treatment (Fig. 5A). Furthermore, in vitro invasion assay was used to assess the effects of catenin-δ on HGC27 cell metastasis, demonstrating that si_catenin-δ treatment resulted in a significant decrease in invasive cells (Fig. 5B).

To further probe the correlation between cell phenotypic alterations and the miR-29/catenin-δ axis, we performed a rescue assay that increased and then decreased the level of catenin-δ via anti-

miR-29 in combination with si_catenin-δ, as described (12). Immunoblotting was used to evaluate catenin-δ expression and demonstrated that the level of catenin-δ was altered under different co-transfection conditions in HGC27 cells (Fig. 5C). Furthermore, in vitro invasion assay was conducted to better determine alterations in cell motility corresponding with cate-

nin-δ protein level variations, and we observed that silencing catenin-δ to prevent induction by anti-29 treatment led to an increase in invasive cells (Fig. 5D and E).

Downregulation of catenin-δ decreases F-actin via Rho GTP activation

We next investigated the role of catenin-δ in the formation of filamentous actin (F-actin). HGC27 cells were transfected with either si_catenin-δ or si_Con for 48 hours, and immunostaining was performed. As expected, silencing catenin-δ markedly suppressed actin organization, which was demonstrated by phalloi-
din staining compared with si_Con-treated cells (Fig. 5F and G). The above findings prompted us to explore the possible mechanisms controlling this process. Because cytosolic catenin-

δ dampens the activity of small Rho family GTPases and evokes cell migration, we examined the status of activated Rho in HGC27 cells treated with si_catenin-δ using a GST fusion protein contain-
ing the Rho-binding domain of mouse Rhotekin. Immunoblotting revealed that si_catenin-δ resulted in a significant decrease in catenin-δ protein, and the level of activated RhoA was increased in si_catenin-δ–treated HGC27 cells compared with cells treated with si_Con (Fig. 5H). We further evaluated the expression of cofilin, a major downstream target of RhoA (23) and observed an increase in p-Cofilin following 48-hour treatment of HGC27 cells with si_catenin-δ, although no significant change was observed in total cofilin levels (Fig. 5H).

miR-29s increase F-actin and p-Cofilin via activated Rho

We next used an alternative approach to downregulate catenin-

δ, based on the fact that catenin-δ is a direct target of miR-29s in gastric cancer cells. Immunoblotting revealed that miR-29 treatment resulted in a decrease in catenin-δ protein levels as well as an increase in activated RhoA and p-Cofilin compared with the scr treatment (Supplementary Fig. S5I). We also explored whether the
miR-29s suppress gastric cancer cell migration by negatively regulating the catenin-δ pathway. A, HGC27 cells were treated with si_catenin-δ (siRNA for catenin-δ) or si_Con (control siRNA). Scratch wounds were made in confluent cells, and cells were allowed to migrate for 36 hours in the presence of si_catenin-δ or si_Con. Bar, 250 μm. B, representative images of in vitro invasion assay. Bar, 100 μm. In vitro invasion assay was conducted in HGC27 cells with indicated treatment. n = 3. C, immunoblots of catenin-δ in HGC27 cells 48 hours after treatment with anti-29s or anti-con and subsequently treated for an additional 72 hours with different combinations of si_catenin-δ or si_Con and anti-miR-29s or anti-con. The signal in each lane was quantified using Kodak Imaging software, and the expression ratio of catenin-δ to GAPDH was determined and shown. D, in vitro invasion assay was conducted as described in Materials and Methods. Migration magnification, ×200. Bar, 100 μm. E, normalized ratio in the in vitro invasion assay is shown in the bars. F, immunofluorescence microscopy of HGC27 cells stained for phalloidin, showing a marked decrease in F-actin 48 hours after treatment with si_catenin-δ. DAPI staining was used to show the nuclei. Bar, 50 μm. G, percentage of cells with stress fibers is shown in the bars. H, immunoblotting analysis in HGC27 cells showed a decrease in catenin-δ expression and an increase in GTP-Rho and p-Cofilin expression 48 hours after treatment with si_catenin-δ. GAPDH served as a loading control. I, treatment with miR-29 mimics resulted in a significant decrease in catenin-δ protein levels and a dramatic increase in GTP-Rho and p-Cofilin expression in HGC27 cells. GAPDH served as a loading control. J, immunofluorescence microscopy of HGC27 cells stained for phalloidin, showing a marked decrease in F-actin 48 hours after treatment with miR-29 mimics or scramble. DAPI staining was used to reveal the nuclei. K, percentage of cells with stress fibers is shown in the bars. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
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A

B

C

D

E

F

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upregulation of miR-29 had a similar effect on actin organization as silencing catenin-δ in gastric cancer cells. Immunostaining analysis was conducted and showed that miR-29 restoration resulted in decreased actin organization in HGC27 cells (Fig. 5I and K).

These results demonstrate that miR-29 regulates gastric cancer cell movement by suppressing the catenin-δ pathway.

Cytotoxic drug treatment suppresses gastric cancer cell movement via regulating miR-29c-mediated catenin-δ axis

To investigate whether cytotoxic drugs represented their suppressive effect on gastric cancer cell movement, a series of rescue assays was conducted. In doing so, miR-29c inhibitor was transfected into gastric cancer cells after cisplatin and docetaxel treatment to re-modify the miR-29c-mediated catenin-δ axis. qRT-PCR analysis revealed that cisplatin and docetaxel treatment led to an increase of miR-29c expression and this upregulation was dampened by anti-29c (Fig. 6A and B). We also observed that catenin-δ protein was decreased upon drugs treatment and increased with anti-29c transfection showed by immunoblots (Fig. 6C and D). The following wound-healing assay and in vitro invasion assay indicated that miR-29c inhibition to prevent its induction by cisplatin or docetaxel treatment led to increased cell movement (Fig. 6E–G). These findings suggest that the miR-29c-mediated catenin-δ axis is a target of cytotoxic drugs in gastric cancer cells.

Clinical correlation among miR-29c, catenin-δ, and tumor progression in patients with gastric cancer

We further probed the clinical correlations among miR-29c, catenin-δ, and the TNM stage of patients with gastric cancer. Expression analyses of miR-29c and catenin-δ were conducted with formalin-fixed, paraffin-embedded (FFPE) tissues from the 66 cases of gastric cancer mentioned above. qRT-PCR analysis indicated that miR-29c level was significantly decreased in majority of the examined gastric cancer samples (Supplementary Fig. S8A–S8C). Catenin-δ protein in these FFPE tissues was evaluated by immunohistochemical staining, demonstrating a significant inverse correlation between miR-29c and catenin-δ (Supplementary Fig. S8F and Supplementary Table S4; miR-29c, R² = 0.3382, P < 0.0001). We also observed that patients with gastric cancer with venous metastasis tended to express high levels of catenin-δ (Fig. 7A and B). Moreover, gastric cancer tissues with low levels of miR-29c demonstrated increased catenin-δ protein levels (Fig. 7C and D). Furthermore, in-depth statistical analysis indicated an inverse association between miR-29c and catenin-δ and the TNM stage (Fig. 7E).

Together, these findings indicate that miR-29c is an independent prognostic factor of gastric cancer development.

Drug-activating ts-miR represents a crucial mechanism for gastric cancer chemotherapy

The above findings prompted us to further investigate the effect of chemotherapy on other ts-miR validated in gastric carcinogenesis. HGC27 and MGC803 cells were treated with docetaxel and cisplatin, and qRT-PCR was performed to measure the expression of selected ts-miR in these cells. The results indicated that most ts-miR were upregulated in gastric cancer cells upon docetaxel or cisplatin treatment (Fig. 7F). miR-375, which was validated as a ts-miR in gastric cancer by negatively regulating PDK1 and YWHAZ (24), was the most significant of the increased miRNA. We also demonstrated that cisplatin and docetaxel treatment led to a marked decrease in PDK1 and YWHAZ protein in gastric cancer cells (Fig. 7G and H). These data suggest that chemotherapeutic drugs may contribute to gastric cancer treatment via miR-375-mediated axes.

Altogether, our findings suggest that multiple gastric carcinogenesis–related programs, including the ts-miR–mediated axis, are involved in the response to chemotherapy.

Discussion

Our data revealed that miR-29 suppressed gastric cancer cell invasion, suggesting that miR-29s levels might be correlated with gastric cancer progression. To test this hypothesis, miR-29 expression analysis was conducted in 166 cases of gastric cancer tissues, demonstrating that gastric cancer tissues with poor differentiation tended to have low miR-29 levels, albeit statistically insignificant (miR-29a, P = 0.84; miR-29b, P = 0.27; miR-29c, P = 0.90; Supplementary Fig. S2A–S2C). These data are not in agreement with Saito’s report of 23 cases of gastric cancer tissues (14 differentiated and 9 undifferentiated; ref. 11). However, we observed that miR-29c levels were dramatically decreased in patients with gastric cancer with more aggressive tumor phenotypes, indicating that miR-29c may represent the most vital tumor suppressor in this family.

Our data also elucidated that catenin-δ is a novel target of miR-29s and loss of miR-29c/Catenin-δ homeostasis contribut- ed to gastric cancer progression. Moreover, the miR-29c–mediated catenin-δ axis is chemotherapeutic target of cytotoxic drugs in gastric cancer cells. Actually, catenin-δ is a complicated protein and its function as tumor suppressor or oncogene depends on the subcellular localization(25). Accumulating reports demonstrate that catenin-δ is essential for adhesiveness in epithelial tissues, as well as increased protein stability and a reduction in E-cadherin turnover at the plasma membrane thereby functioning as tumor suppressor (26). Recent studies in vitro or in vivo confirm this idea (27–29). Because cisplatin and docetaxel treatment led to a decrease of catenin-δ proteins via modulating miR-29 expression, we next investigated whether these drugs held impact on E-cadherin levels. Our immunoblotting analysis indicated a significant increase of E-cadherin proteins in cell surface and cytoplasm but did not detect the expression of E-cadherin in nucleus of gastric cancer cells with drugs treatment (Supplementary Fig. S9A and S9B). Meanwhile, miR-29c treatment resulted in a moderated increase of E-cadherin proteins in cell surface and cytoplasm (Supplementary

Figure 6. Cisplatin and docetaxel treatment suppresses gastric cancer cell movement via regulating the miR-29c-mediated catenin-δ axis. A and B, miR-29c expression was evaluated in gastric cancer cells treated by cisplatin (A) and docetaxel (B) with or without miR-29c inhibition. C and D, catenin-δ protein level was assessed in gastric cancer cells treated by cisplatin (C) and docetaxel (D) with or without miR-29c inhibition. E and F, wound-healing assay was conducted in the rescue assay (MGC803; E, HGC27; F) and phase-contrast images were obtained immediately after wounding and at 12-hours intervals up to 36 hours. G, in vitro invasion assay was performed in the rescue assay. Representative images are shown (magnification, ×200). The normalized ratio of invasive cells is shown in the bottom panels. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
Fig. S9C and S9D). These data suggest that chemotherapeutic drugs lead to an increase of E-cadherin on cell surface probably via other unknown mechanism except via the miR-29c/catenin-δ axis through comparing the effect of cytotoxic drugs to miR-29c treatment. As stated above, mislocalization of catenin-δ in the cytoplasm or nucleus resulted in the promotion of migration and invasion via regulating Rho GTPase activity and growth factor receptor signaling, implying its role as an oncogene (30, 31). Interestingly, our immunostaining analysis indicated a dramatic decrease of catenin-δ protein levels in the cytoplasm of gastric cancer cells treated with miR-29 mimics, suggesting that mislocalized catenin-δ functions as an oncogene.

The importance and mechanism of cancer chemotherapy have been extensively investigated. With the emergence of miRNA research, many groups have attempted to identify miRNA signatures associated with chemosensitivity or chemoresistance. In this study, miR-29s were validated as responsive ts-miR in gastric cancer chemotherapy. These data promoted us to further...
investigate whether cytotoxic drugs held clinical promise through modulating a group of ts-miR or oncomiR in gastric cancer cells. To expand the scope of our findings, 22 known ts-miRs were selected and measured by qRT-PCR in cisplatin- and docetaxel-treated gastric cancer cells. As expected, most of these miRNAs were upregulated, suggesting that chemotherapeutic drugs achieve clinical promise for gastric cancer prevention through activating ts-miR, which may represent a global mechanism for gastric cancer chemotherapy. In addition, a previous study reported that the miR-29c levels were increased in gastric cancer cells following celecoxib treatment (11). In contrast to this study, another blocking agent for gastrointestinal tumors treatment, imatinib, was used to treat gastric cancer cells and led to a significant increase in miR-29c expression and a marked decrease in catenin-8 protein expression (Supplementary Fig. S10).suggesting that selective inhibitors may exert similar effects on gastric cancer treatment through the miR-29c-mediated axis. These findings demonstrate that miR-29c acts as not only a critical predictor of gastric cancer chemotherapy but also a powerful ts-miR for gastric cancer.

In conclusion, our findings indicate that loss of miR-29c/catenin-8 homeostasis contributes to gastric cancer progression. Re-modification of the miR-29c-mediated axis may represent a realistic approach for gastric cancer prevention.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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