

## Explore the correlation between miR-338-5p and ZEB2 expression with clinical features as well as prognosis of Gastric Cancer (GC)

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### Abstract

Gastric cancer is the second leading cause of cancer-related death worldwide, with the highest incidence rate in Eastern Asia. While surgery is primarily used for the treatment of early-stage gastric cancer, many patients develop advanced-stage cancers, or experience relapse after surgery, and therefore, require chemotherapy. Currently, cisplatin is one of the first-line chemotherapy drugs for gastric cancer. Unfortunately, initial cisplatin treatment frequently leads to recurrence of cancer, which unrelentingly retaliates with fast spreading and drug resistance. Thus, the acquisition of cisplatin-resistance seriously hampers the effectiveness of chemotherapy, and is associated with poor patient prognosis and survival. Although some drugs have been applied in combination to cisplatin to gastric treatment cancer, the effectiveness of these regimens to counter chemoresistance is still limited. Therefore, it is imperative to elucidate how gastric cancer acquires cisplatin-resistance and develop therapeutic strategies to reverse chemoresistance.

Epithelial-to-mesenchymal transition (EMT) has been associated with chemoresistance in various cancers. During EMT, cells lose cell-cell adhesions and gain cell-matrix interaction, acquiring traits linked to enhanced invasion and migration abilities. EMT also generates cancer stem cells (CSC), which are capable of initiating metastases in secondary organs. Recent research, provided additional evidence about the association between HOXA13 upregulation and gastric cancer progression. Also, it showed that HOXA13 contributes to invasion and EMT of gastric cancer cells via the TGF- $\beta$  signaling pathway. The low expression of miR-195 played important roles in the pathogenesis and development of gastric cancer, possibly by influencing the proliferation and growth of gastric cancer cells. In clinical practice, the detection of miR-195 played a certain role in guiding the treatment and prognosis of patients with gastric cancer. Reportedly, miR-138 sensitized NSCLC cells to ADM through regulation of EMT regulator ZEB2, these findings provided new insight into the mechanism responsible for the chemoresistance in human NSCLC and implied that miR-138 may serve as a potential therapeutic candidate in drug-resistant NSCLC patients. Zinc finger E-box-binding homeobox 2 (ZEB2) is a transcription factor that intracellularly promotes EMT by inhibiting E-Cadherin expression. It has been reported that ZEB2 overexpression is clinically associated with the poor survival of patients with colorectal cancer, prostate cancer, pancreatic cancer, etc. It also maintains the stemness of cancer cells. Therefore, suppressing ZEB2 activation is a promising approach for suppressing cancer by inhibiting EMT. This possibly deprives cancer of chemoresistance. RNA interference using small interfering RNA (siRNA) is an effective method for gene silencing. These specifically designed double-stranded RNAs interfere the expression of target genes that possess a homologous sequence with the siRNAs. RNA interference with siRNA has been used to develop novel cancer therapies whereby conventional treatments lack efficacy. Previously, a lot of efforts have been devoted to silencing ZEB1 with siRNA, in which reversal of cancer EMT characteristics has been observed. This also holds promise to re-sensitize cancer cells to chemotherapy. Despite the functional role of ZEB2 has been revealed, few researches have focused on ZEB2 silencing in cancers, particularly gastric cancer. Herein we set force to explore the effectiveness of ZEB2 siRNA silencing to sensitize cisplatin-resistant human gastric cancer cells SGC7901/DDP. The silencing efficiency was evaluated and the effects on sensitivity to cisplatin and cell apoptosis were demonstrated *in vitro*. The results of this study justified the use of ZEB2 siRNA in combination with cisplatin to treat gastric cancer.

This study was specifically designed to confirm the hypothesis that microRNA338-5p (miR-338-5p) affects the development of cisplatin (DDP) resistance in human gastric cancer cells by targeting zinc finger E-box binding homeobox 2 (ZEB2). A total of 50 gastric cancer tissues and their corresponding normal adjacent tissue samples were collected. Then, the expression levels of miR-338-5p and ZEB2 in both gastric cancer specimens and cells were detected using the quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR) and immunohistochemical methods. A dual-luciferase reporter gene assay was conducted to evaluate the effect of miR-338-5p on the 3'-untranslated region (3'UTR) luciferase activity of ZEB2. SGC7901/DDP cells were transfected with miR-338-5p mimics and ZEB2 siRNA, respectively. Subsequently, changes in cellular proliferation and apoptosis were detected through the methyl thiazolyl tetrazolium assay and flow cytometric analysis, respectively. We also carried out a western blot analysis

assay in order to detect the expression of apoptosis-related genes and ZEB2. miR-338-5p was significantly downregulated and ZEB2 was significantly upregulated in both gastric cancer tissues and SGC7901/DDP cells when compared with those in normal tissues and SGC7901 cells ( $P < 0.01$ ). The dual luciferase reporter gene assay showed that miR-338-5p could specifically bind with the 3'UTR of ZEB2 and significantly suppress the luciferase activity by 42% ( $P < 0.01$ ). Upregulation of miR-338-5p or downregulation of ZEB2 enhanced the sensitivity of SGC7901/DDP cells to DDP. miR-338-5p was significantly downregulated in both gastric cancer tissues and cells, while the expression of ZEB2 exhibited the opposite trend. Our study further demonstrated that miR-338-5p could enhance the sensitivity of SGC7901/DDP cells to DDP through targeted regulation of ZEB2 expression in gastric cancer tissues.

**CONCLUSION:** The data further demonstrated that upregulation of miR-338-5p and downregulation of ZEB2 could increase the chemotherapeutic sensitivity of gastric cancer and chemotherapy-induced apoptosis. Collectively, all of these data suggest that miR-338-5p enhanced the sensitivity of gastric cancer to chemotherapy by directly targeting and regulating the expression of ZEB2. Thus, both miR-338-5p and ZEB2 exhibit great potential to serve as effective therapeutic targets for increasing the sensitivity of gastric cancer to DDP.

The findings illustrated that miR-338-5p expression was significantly downregulated in gastric cancer, while ZEB2 expression exhibited the opposite trend. Moreover, ZEB2 was found to be a direct target of miR-338-5p.

The upregulation of miR-338-5p or downregulation of ZEB2 could enhance the sensitivity of SGC7901/DDP cells to DDP-induced apoptosis and therefore miR-338-5p and ZEB2 can potentially regulate chemotherapy sensitivity through the apoptotic signaling pathway in gastric cancer patients.

**Key word:** 50 Gastric cancer tissue specimens and adjacent non-cancerous tissues, ZEB2 (Zinc finger E-box binding homeobox 2 ) Protein, immunohistochemistry method. The relative miR-338-5p expression in tissue will be detected by quantitative PCR method.

## INTRODUCTION

### Gastric Cancer

According to the World Health Organization (WHO), 723,000 cancer-related deaths are caused by stomach cancer each year worldwide. It is the fifth most common cancer worldwide, but the third leading cause of cancer-related deaths. In the United States, there are approximately 25,500 new cases of stomach cancer each year. It represents 2 percent of all new cancers diagnosed in the country. The majority of people diagnosed with stomach cancer either already have metastasis or eventually develop it. Metastasis occurs when the cancer spreads from the area in which it first developed. Around 90 to 95 percent of all stomach cancers are a type referred to as adenocarcinoma of the stomach. In this type, the cancer develops from the cells that form the mucosa, the most superficial lining of the stomach that produces mucus. Stomach cancer is the third leading cause of cancer-related deaths. The most common type of stomach cancer is adenocarcinoma of the stomach. Early symptoms include heartburn, persistent indigestion, and difficulty swallowing. There are several symptoms associated with stomach cancer. However, as they also exist in many other less serious conditions, gastric cancer may be difficult to recognize at first. It is for this reason that so many people with stomach cancer are not diagnosed until the disease is already advanced.<sup>1</sup>

Symptoms : Early symptoms of stomach cancer may include:

- ✓ a sensation of being very full during meals.
- ✓ swallowing difficulties, known as dysphagia.
- ✓ feeling bloated after meals.
- ✓ frequent burping.
- ✓ Heartburn.
- ✓ Indigestion that does not go away.
- ✓ stomachache, or pain in the breastbone.
- ✓ trapped wind.
- ✓ vomiting, which may contain blood.

When the stomach cancer becomes more advanced, the following signs and symptoms typically become more apparent: a buildup of fluid in the stomach, which may cause the stomach to feel "lumpy", anemia, black stools that contain blood, fatigue, loss of appetite, weight loss.<sup>2</sup>

Causes: Cancer starts when the structure of DNA changes. When this happens, it can disrupt the instructions that control cell growth.

Cells that should die may not do so, and cells that should be newly created may be produced too rapidly or in an uncontrollable way.

Experts are not sure why some stomach cells mutate and become cancerous. It is not known why only a few people develop stomach cancer.<sup>3</sup>

Treatment: Treatment for stomach cancer depends on several factors, including the severity of the cancer and the individual's overall health and preferences. Treatments may include surgery, chemotherapy, radiation therapy, medications, and taking part in clinical trials.

Surgery: The surgeon's aim is to remove the stomach cancer from the body as well as a margin of healthy tissue. This is necessary to make sure no cancerous cells are left behind.

Examples include: Removing tumors from the stomach lining in early-stage cancer: The surgeon will use endoscopy to remove very small tumors that are confined to the inside lining of the stomach. This is called endoscopic mucosal resection.

Subtotal gastrectomy: A part of the stomach is surgically removed.

Total gastrectomy: The whole stomach is surgically removed.

Abdominal surgeries are significant procedures and may require prolonged recovery time. People may have to stay in hospital for 2 weeks after the procedure. This will be followed by several weeks of recovery at home.

Radiation therapy: In radiation therapy, energy rays are used to target and kill cancerous cells. This type of therapy is not commonly used to treat stomach cancer because of the risk of harming other nearby organs. However, if the cancer is advanced or causing serious symptoms, such as bleeding or severe pain, radiation therapy is an option.

Neoadjuvant radiation: Neoadjuvant radiation refers to the use of radiation therapy before surgery to make the tumors smaller, so that they can be removed more easily.

Adjuvant radiation: Adjuvant radiation is radiation therapy used after surgery. The aim is to kill off any remaining cancer cells around the stomach. People may experience indigestion, nausea, vomiting, and diarrhea as a result of undergoing radiation therapy.

Chemotherapy: Chemotherapy is a specialist treatment that uses drugs to stop rapidly-growing cancer cells from dividing and multiplying. These drugs are known as cytotoxic medicines. The medication travels throughout the patient's body and attacks cancer cells at the primary site of the cancer and any other regions it has metastasized to.

Neoadjuvant chemotherapy: Neoadjuvant chemotherapy is administered before surgery to shrink the tumor so that it can be removed more easily.

Adjuvant chemotherapy: Adjuvant chemotherapy is administered after surgery to destroy any cancerous cells that may be left behind. Chemotherapy may be the preferred treatment modality for certain types of gastric cancer, including gastrointestinal stromal tumors and gastric lymphoma.

Targeted medications: Examples of targeted medications include Sutent (sunitinib) and Gleevec (imatinib), which attack specific types of abnormalities in cancerous cells for people with gastrointestinal stromal tumors.

Clinical trials: These are experimental therapies which may be trying out new drugs or using existing therapies in novel ways. Patients may want to take part in some of the latest treatments. It is important to remember that clinical trials are experimental and in no way guarantee a cure for stomach cancer. Patients should discuss this option carefully with their doctors and family and bear in mind that such therapies have many unknowns, for example, the investigators may not be sure what side effects the participants might experience.<sup>4</sup>

Stages: There are several stages of stomach cancer. The higher the stage, the more advanced the cancer is, and the lower the chances of survival. Unlike some other cancers, these are also given a letter depending on whether the stomach cancer has spread to any nearby lymph nodes.

These include:

Stage 0: Highly abnormal precancerous cells are present in the mucosa but have not spread to other layers of the stomach or nearby lymph nodes.

Stage IA: The cancer has moved into one of the next layers of the stomach, such as the submucosa, but not nearby lymph nodes.

Stage IB: The cancer has moved into one of the next layers of the stomach and into one or two nearby lymph nodes.

Stage IIA: The cancer has developed into an even deeper layer, and may have spread to one or two lymph nodes. If the tumor has grown deep enough, it may not need to have spread to qualify as a stage IIA cancer.

Stage IIB: The tumor may not have necessarily spread as deep as a stage IIA stomach cancer but has spread to a greater number of lymph nodes, sometimes up to 15.

Stage IIIA: This stage sees the cancer spread to a deeper layer and up to 15 lymph nodes or start to grow through the stomach wall and spread to fewer lymph nodes. It has also started to reach nearby organs and structures.<sup>5</sup>

Stage IIIB: The cancer has not grown as deep as a stage IIIA stomach cancer but has spread to over 16 lymph nodes. It has started to reach nearby organs and structures.

Stage IIIC: The cancer has either grown through most layers of the stomach and spread to over 16 lymph nodes or spread to nearby organs and structures and up to 15 lymph nodes.

Stage IV: The cancer has spread to distant sites. However, it may or may not have spread to nearby lymph nodes.

While this is not an exhaustive list of the criteria used to categorize stomach cancers, it does provide a picture of how an oncologist will grade the development of a tumor.<sup>6</sup>

Diagnosis: Individuals with some of the signs and symptoms of stomach cancer should see their doctor as soon as possible. The physician will ask the patient about their symptoms, family history, and medical history, as well as lifestyle choices, such as eating habits or smoking. They will also carry out a physical examination to check for stomach tenderness or lumpiness. If the doctor suspects possible stomach cancer, the patient will be referred to a specialist for tests.

Diagnostic measures may include the following.

Gastrosopic exam: The specialist looks at the inside of the patient's stomach with a fiber optic camera. Some tissue samples may be taken if the doctor suspects cancer. This is called a biopsy.<sup>7</sup>

Ultrasound scan: If cancer is suspected in the top part of the stomach, the specialist may carry out this type of scan.

Barium meal X-ray: In a barium swallow study, the patient swallows a liquid which contains barium. This helps identify the stomach during an X-ray.

Laparoscopy: The specialist may want to look inside the abdomen in more detail to determine how much the cancer has spread. In a procedure called a laparoscopy, the patient is placed under a general anesthetic, and a laparoscope, a thin tube with a camera at the end, is inserted through a small incision in the lower part of the stomach.

CT scan or PET scan: These scans take a series of radiographic pictures of the inside of the body. The images help the specialist determine how advanced the cancer is, and where in the body it has spread to. These types of scans also help the doctor decide on the most appropriate treatment.<sup>8</sup>

Risk factors: Risk factors linked to stomach cancer include:

Certain medical conditions: These include esophagitis, gastroesophageal reflux disease (GERD), peptic stomach ulcers, Barrett's esophagus, chronic gastritis, and stomach polyps.<sup>9</sup>

Smoking: Regular, long-term smokers have twice the risk of developing stomach cancer compared to non-smokers.

Helicobacter pylori infection: This bacterium is harmless for most people. However, it can cause infection and stomach ulcers in some individuals. Chronic ulcers pose some risk in the development of gastric cancer

Family history: Having a close relative who has or has had stomach cancer can increase the risk.<sup>10</sup>

Consuming foods which contain aflatoxin fungus: These may be present in crude vegetable oils, cocoa beans, tree nuts, groundnuts, figs and other dried foods and spices.

Diet: People who regularly eat salted fish, salty foods, smoked meats, and pickled vegetables have a higher risk of developing gastric cancer.

Age: The risk of developing stomach cancer increases significantly after the age of 55 years.

Sex: Men have twice the risk of developing stomach cancer compared with women.<sup>7</sup>

Previous or existing cancers: People who have or have had cancer of the esophagus or non-Hodgkin's lymphoma are more likely to develop stomach cancer. Men with previous or current prostate, bladder, or testicular cancer are at higher risk, as are females with a history of cervical, ovarian, or breast cancer.

Some surgical procedures: Surgery to the stomach or a part of the body that affects the stomach, like the vagus nerve, can increase the risk of stomach cancer.

Prevented: Experts do not know exactly what causes stomach cancer, and there are no vaccines against it. Therefore, there is no way to prevent it. However, steps can be taken to reduce the risk of developing the disease; these include:

Fruit and vegetables: People who eat plenty of fruit and vegetables are usually less likely to develop stomach cancer, when compared with those who do not.

Salty and smoked foods: Reduce the quantity of these in the diet.

Smoking: If you smoke, quit. If you don't smoke, avoid it.

Check with a doctor: Individuals can ask whether they have any medical conditions that might increase the risk of developing stomach cancer. Those who do might consider having periodic screening.<sup>11</sup>

## MATERIALS AND METHOD

### 1. Materials

#### 1.1.1 Research and Grouping Objects

Before embarking upon the study, our proposal was submitted to the governing body of the hospital hand which is the Research Ethics Committee of First Affiliated Hospital of Anhui Medical University Oncology Department Laboratory for Cancer Research. where the ethical clearance was taken on 10th of October 2015. After which we started to collect data and look for our subjects. The process of finding our subject which was to look for 50 Cases of Gastric cancer, both male and female, selected from November 2015 in First Affiliated Hospital of Anhui Medical University.

**1.1.2 Case situation:** Tissue samples. Fifty gastric cancer tissues and paired normal adjacent tissues were acquired from patients who underwent surgical treatment in our hospital from 2015 to 2017. There were 28 males and 22 females, with an average age of 55.6 years (ranging from 33 to 68 years). Clinical TNM stages were determined according to the newly revised standards of TNM staging for gastric cancer from the 2010 American Joint Committee on Cancer (AJCC). No patients received radiotherapy, chemotherapy or hormone therapy before surgery. Tissue samples were immediately frozen in liquid nitrogen at the time of surgery, then stored at -80°C until the extraction of RNA. Another group of tumor tissues was fixed with 10% formalin, embedded in paraffin and immunohistochemistry was performed. Informed consent for this study was collected from every patient.

#### 1.2 Diagnostic Criteria

1.2.1 SGC7901, a human gastric cancer cell line, was obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China).

1.2.2 The SGC7901/DDP cells with DDP resistance were purchased from KeyGen Biotech Co.,Ltd. (Nanjing, China).

1.2.3 We transfected miR-338-5p mimics and ZEB2 siRNA (purchased from GenePharma, Shanghai, China) into gastric cancer cells in order to assess the effect of miR-338-5p and ZEB2 on both cell apoptosis and chemosensitivity.

1.2.4 Annexin V-fluorescein isothiocyanate (Annexin V -FITC) and propidium iodide (PI) apoptosis detection kit (Becton-Dickinson, Franklin Lakes, NJ, USA) was used to evaluate the apoptosis of SGC7901/DDP cells.

1.2.5 Statistical analyses were implemented using the SPSS 19.0 software.

### 2, Method and Materials

**2.2.1 Tissue samples.** Fifty gastric cancer tissues and paired normal adjacent tissues were acquired from patients who underwent surgical treatment in our hospital from 2015 to 2017. There were 28 males and 22 females, with an average age of 55.6 years (ranging from 33 to 68 years). Clinical TNM stages were determined according to the newly revised standards of TNM staging for gastric cancer from the 2010 American Joint Committee on Cancer (AJCC). No patients received radiotherapy, chemotherapy or hormone therapy before surgery. Tissue samples were immediately frozen in liquid nitrogen at the time of surgery, then stored at -80°C until the extraction of RNA. Another group of tumor tissues was fixed with 10% formalin, embedded in paraffin and immunohistochemistry was performed. Informed consent for this study was collected from every patient, and this study was approved by the Research Ethics Committee of First Affiliated Hospital of Anhui Medical University Oncology Department Laboratory for Cancer Research.

**2.2.2 Cell culture.** SGC7901, a human gastric cancer cell line, was obtained from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The SGC7901/DDP cells with DDP resistance were purchased from KeyGen Biotech Co.,Ltd. (Nanjing, China). Cells were maintained in RPMI-1640 medium containing 10% fetal bovine serum (FBS), streptomycin (100 µg/ml) and penicillin (100 U/ml) (all from Gibco, Grand Island, NY, USA) in a 37°C incubator with 5% CO<sub>2</sub>. In order to maintain the DDP resistant phenotype, DDP (at a final concentration of 1 µg/ml) was added to the culture medium for the SGC7901/DDP cells. SGC7901/DDP cells were cultured for one week in medium without DDP before experimentation.

**2.2.3 Cell transfection.** We transfected miR-338-5p mimics and ZEB2 siRNA (purchased from GenePharma, Shanghai, China) into gastric cancer cells in order to assess the effect of miR-338-5p and



ZEB2 on both cell apoptosis and chemosensitivity. The scramble group transfected with scramble miRNA mimics was synthesized by GenePharma and was used as the negative control. Cells were plated in 6-well plates at  $1 \times 10^5$  cells in each well in an antibiotic-free RPMI-1640 medium with 10% FBS. After 12 h, Opti-MEM medium (Gibco) without antibiotics and serum was used to replace the aforementioned medium. When cells grew to about 50% confluence, they were transfected with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) and cultured in a 37°C incubator with 5% CO<sub>2</sub>. Cells continued to culture after the complete medium was replaced after 6-8 h. Cells were harvested after a 48-h transfection and used for western blot analysis and RT-PCR.

**2.2.4 Quantitative** real time reverse transcription-polymerase chain reaction (qRT-PCR) analysis. Total RNA extraction from human gastric cancer tissues and cells was conducted using TRIzol reagent (Invitrogen) based on the manufacturer's instructions. Complementary DNA (cDNA) was acquired using the Omniscript reverse transcription kit (Qiagen, Hilden, Germany). After the reaction of reverse transcription, a real-time quantitative RT-PCR assay was conducted using an ABI7500 quantitative PCR instrument (Applied Biosystems) and the relative expression levels of miR-338-5p and ZEB2 mRNA were detected. The primers for miR-338-5p and ZEB2 (Invitrogen) are shown in Table I. The relative expression of miR-338-5p and ZEB2 mRNA was calculated using the  $2^{-\Delta\Delta Ct}$  method and normalized to the expression of U6 snRNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). All the aforementioned assays were replicated three times.

**2.2.5 Immunohistochemistry.** ZEB2 protein expression in gastric cancer specimens was detected using the universal PV-9000 two-step immunohistochemical method. In brief, formalin-fixed and paraffin-embedded tissues from gastric cancer patients were cut into 4- $\mu$ m slices. Then, conventional dewaxing, graded ethanol dehydration, antigen retrieval and the addition of 3% hydrogen peroxide were performed on tissue slices in order to block endogenous peroxidase. Primary antibodies (rabbit anti-human ZEB2 polyclonal antibody; BLOSS, Beijing, China) were applied to tissue slices at 4°C overnight. Then, tissues were incubated for 20 min at room temperature after the addition of polymerase adjuvants. Secondary antibodies labeled with horseradish peroxidase (HRP; BLOSS) were also applied and tissues were incubated for another 30 min at room temperature. Staining was performed using diaminobenzidine (DAB) and slices were counterstained using hemalum. Phosphate-buffered solution (PBS) instead of a primary antibody was considered as the negative control and a known positive antibody was set as the positive control. The immunohistochemical score of ZEB2 was calculated by multiplying the intensity of the staining (0, colorless; 1, light yellow; 2, yellow; and 3, brown) and the positive cell percentage (0,  $\leq 5\%$ ; 1, 6-25%; 2, 26-50%; 3, 51-75%; and 4,  $>75\%$ ). Cells were randomly selected from 5 high-over fields ( $\times 200$ ) in each slice and 100 cells were counted in each field. As suggested by the final scores, the integral levels of ZEB2 were evaluated as: negative (-),  $\leq 4$  points and positive (+),  $>4$  points. Two independent pathologists were responsible for analyzing these slices.

**2.2.6 Dual-luciferase** reporter assay. miRNA targeted genes were predicted using TargetScan. Then, the wild-type and mutant-type of ZEB2 3'UTR luciferase reporter vectors were constructed. miR-338-5p mimics were co-transfected with constructed wild-type or mutant-type luciferase reporter vectors into SGC7901/DDP cells using Lipofectamine 2000 (Invitrogen). The pGL3-control vector (Promega, Madison, WI, USA) was transfected as a control. The dual-luciferase reporter assay system (Promega) was used to examine the luciferase activity after the cells had been transfected for 48 h.

**2.2.7 Cell viability** assay. Cell viability was evaluated using the methyl thiazolyl tetrazolium (MTT) assay. Briefly, transfected cells were seeded in a 96-well plate at  $5 \times 10^3$  cells in each well for 48 h. After 12 h, the cells were treated with various concentrations of DDP (Qilu Pharmaceutical Co., Ltd., Shandong, China) with final concentrations at 0.01, 0.1, 1 and 10 times those of the human peak serum doses for DDP, as previously suggested. The peak plasma concentration of anticancer drugs is 2.0  $\mu$ g/ml for DDP. Approximately 48 h after the addition of DDP, MTT (20  $\mu$ l, 5 mg/ml; Sigma-ldrich, St. Louis, MO, USA) was added into each well, and the culture was sustained for 4 h in a 37°C incubator with 5% CO<sub>2</sub>. Subsequently, dimethyl sulphoxide (DMSO; 150  $\mu$ l) was added into each well and the cells were shaken lightly for 10 min to dissolve the crystals. Samples were read on a microplate reader (SpectraMax Plus; Molecular Devices, Sunnyvale, CA, USA) at 490 nm. The 50% inhibition concentration (IC<sub>50</sub>) of DDP was estimated.

**2.2.9 Apoptosis** assay. Annexin V-fluorescein isothiocyanate (Annexin V -FITC) and propidium iodide (PI) apoptosis detection kit (Becton-Dickinson, Franklin Lakes, NJ, USA) was used to evaluate the apoptosis of SGC7901/DDP cells. In brief, SGC7901/DDP cells were treated with DDP at a final concentration of 5  $\mu$ g/ml after transfection for 24 h. Cells following a 48-h treatment were washed two times using cold PBS. Then the cells were re-suspended in binding buffer and maintained at a concentration of 0.5- $1 \times 10^6$ /ml. The suspension (100  $\mu$ l) was incubated with 5  $\mu$ l of Annexin V -FITC and PI for 15 min at room temperature in

the dark. After the addition of 400 µl binding buffer into each tube, the cells were assessed using flow cytometry (Beckman FC 500 MCL/MPL; Beckman Coulter, Brea, CA, USA).

**2.2.10 Western blot analysis** assay. After transfection was sustained for 48 h, cells were collected and homogenized using RIPA buffer (Beyotime, China). Cellular proteins were extracted and the protein concentrations were assessed using a bicinchoninic acid (BCA) protein assay kit (Boster Biotechnology Co., Ltd., Wuhan, China). Equal amounts of proteins for each group were loaded and isolated with sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), after which they were transferred onto polyvinylidene fluoride membranes and blocked with 5% nonfat milk. Membranes were incubated with the ZEB2, Bcl-2, Bax, caspase-3 primary antibodies or the GAPDH antibody [Cell Signaling Technology (CST), Beverly, MA, USA] respectively, at 4°C overnight. Membranes were washed three times using Tris hydroxymethyl aminoethane (TBST; 10 min each) and then HRP-linked secondary antibodies were added followed by incubation at room temperature for 1 h. Membranes were washed again with TBST three times (10 min each) and signal detection was performed using a Super ECL Plus Detection reagent (Applygen Technologies, Inc., Beijing, China).

**2.2.11 Statistical analysis.** Statistical analyses were implemented using the SPSS 19.0 software. Differences in continuous variables among groups (mean ± SD) were compared using the procedure of analysis of variance (ANOVA) or the Student's t-test. Immunohistochemical results of the ZEB2 protein were analyzed by the Chi-squared test. A P-value <0.05 was defined as statistically significant.

## RESULT

Our results also confirmed that the upregulation of miR-338-5p or downregulation of ZEB2 could enhance the sensitivity of SGC7901/DDP cells to DDP-induced apoptosis and therefore miR-338-5p and ZEB2 can potentially regulate chemotherapy sensitivity through the apoptotic signaling pathway in gastric cancer patients.

Our findings illustrated that miR-338-5p expression was significantly downregulated in gastric cancer, while ZEB2 expression exhibited the opposite trend. Moreover, ZEB2 was found to be a direct target of miR-338-5p.

Our data further demonstrated that upregulation of miR-338-5p and downregulation of ZEB2 could increase the chemotherapeutic sensitivity of gastric cancer and chemotherapy-induced apoptosis. Collectively, all of these data suggest that miR-338-5p enhanced the sensitivity of gastric cancer to chemotherapy by directly targeting and regulating the expression of ZEB2. Thus, both miR-338-5p and ZEB2 exhibit great potential to serve as effective therapeutic targets for increasing the sensitivity of gastric cancer to DDP. Expression levels of miR-338-5p and ZEB2 in gastric cancer specimens. miR-338-5p expression in 50 gastric cancer tissues and matched normal adjacent tissues was tested by quantitative real-time RT-PCR. Gastric cancer tissues showed a significantly downregulated miR-338-5p expression level when compared to that noted in the normal adjacent tissues ( $P < 0.01$ ; Fig.1). Furthermore, we analyzed the correlation between the expression of miR-338-5p and the pathological characteristics of gastric cancer patients. As shown in Table II, patients with lymph node metastasis were associated with a marked lower expression level of miR-338-5p on average. In addition, patients at TNM III /IV stage had significantly lower miR-338-5p expression compared with patients at TNM I/II stage (all  $P < 0.01$ ). No significant correlation was found between miR-338-5p expression and age or gender (both  $P > 0.05$ ). The protein expression of ZEB2 in gastric cancer specimens was detected by immunohistochemical method (Fig.2). The association between the expression of ZEB2 and the clinical characteristics of the gastric cancer patients is shown in Table III. The expression of ZEB2 in patients with lymph node metastasis was significantly higher than that of patients without lymph node metastasis; the expression of ZEB2 in patients at a TNM III /IV stage was also significantly higher than that of patients at a TNM I/II stage (both  $P < 0.05$ ). No significant association between the expression of ZEB2 and age/gender was suggested (both  $P > 0.05$ ). Expression of miR-338-5p and ZEB2 in gastric cancer SGC7901 and SGC7901/DDP cells. Results from RT-PCR revealed that the expression of miR-338-5p was markedly downregulated in SGC7901/DDP cells with DDP resistance compared with SGC7901 cells ( $P < 0.01$ ). Meanwhile, the expression of ZEB2 in SGC7901/DDP cells was increased in comparison to SGC7901 cells ( $P < 0.01$ ; Fig.3). Therefore, we concluded that there is a potential relationship between miR-338-5p and ZEB2. ZEB2 is a target gene of miR-338-5p. For the purpose of clarifying the potential relationship between miR-338-5p and ZEB2, a putative conserved binding site of miR-338-5p at nucleotide position 392-398 of human ZEB2 3'UTR was predicted by the TargetScan database. Perfect base pairing is shown between the seed sequence of mature miR-338-5p and the 3'UTR of ZEB2 mRNA (Fig. 4A). The results of the dual luciferase reporter gene assays revealed that miR-338-5p decreased the luciferase activity of ZEB2 wild-type by 42% ( $P < 0.01$ ). However, the effect of miR-338-5p on the luciferase activity of ZEB2 with mutant-type 3'UTR was not significant (Fig.4B). As suggested by RT-PCR and western blot analysis

assays, the expression levels of both ZEB2 mRNA and protein were inhibited by the miR-338-5p mimics compared to the scramble group ( $P < 0.01$ ; Fig. 4C and D). The aforementioned findings indicate that ZEB2 is a direct target of miR-338-5p. Upregulation of miR-338-5p and knockdown of ZEB2 enhance the sensitivity of SGC7901/DDP cells to DDP. To explore the association between miR-338-5p and DDP resistance in SGC7901/DDP cells, the impact of miR-338-5p overexpression and downregulation of ZEB2 on the DDP sensitivity of cells was assessed. The data from qRT-PCR showed that miR-338-5p mimics significantly increased the expression level of miR-338-5p, suggesting that miR-338-5p was efficiently transfected into the SGC7901/DDP cells (Fig. 5A). Fig. 5B shows that ZEB2 was successfully knocked down by the transfection of ZEB2 siRNA. MTT assay revealed that the SGC7901/DDP cells transfected with miR-338-5p mimics exhibited a significantly lower survival status than the scramble group ( $IC_{50}, 8.14 \pm 0.59$  vs.  $11.97 \pm 0.71 \mu\text{g/ml}$ ,  $P < 0.01$ ). The sensitivity of DDP was significantly enhanced in cells transfected with ZEB2 siRNA compared to the scramble group, with the  $IC_{50}$  of DDP at  $8.78 \pm 0.39 \mu\text{g/ml}$  in the ZEB2 siRNA group ( $P < 0.01$ ; Fig. 6). Overexpression of miR-338-5p and downregulation of ZEB2 sensitize cells to DDP-induced apoptosis. The results of flow cytometry showed that the SGC7901/DDP cells transfected with miR-338-5p mimics had a significantly higher apoptosis rate ( $22.35 \pm 1.16$ ,  $P < 0.01$ ) compared with the scramble group ( $9.58 \pm 0.86$ ). The apoptosis rate of cells transfected with ZEB2 siRNA was also significantly increased ( $20.03 \pm 2.36$ ,  $P < 0.01$ ), while any differences between the miR-338-5p mimics and the ZEB2 siRNA group were insignificant ( $P > 0.05$ ; Fig. 7). These findings suggest that overexpression of miR-338-5p and knockdown of ZEB2 enhanced cell sensitivity to DDP and increased cell apoptosis. Expression of apoptosis-related molecules in SGC7901/DDP cells. Western blot analysis was carried out in SGC7901/DDP cells to further clarify the mechanism of miR-338-5p and ZEB2 in regulating DDP resistance in gastric cancer. The expression levels of Bax and caspase-3 were increased in the miR-338-5p mimic- and the ZEB2 siRNA-transfected cells, while the expression level of Bcl-2 was markedly decreased compared to the scramble group ( $P < 0.01$ ; Fig. 8).

Table I. Sequence of the primers used for quantitative RT-PCR.

Genes	Primer pair sequences
miR-338-5p F: 5'-AGCGGTAATACTGCCGGGTA-3'	R: 5'-GTGCAGGGTCCGAGGT-3'
U6 F: 5'-GGGTGCTCGCTTCGGCAGC-3'	R: 5'-CAGTGCAGGGTCCGAGGT-3'
ZEB2 F: 5'-TGTCATTAGAAGAGGCGTAA-3'	R: 5'-GCAGAGCAGGTTAGAACT-3'
GAPDH F: 5'-CATCAGCAATGCCTCCTGCAC-3'	R: 5'-TGAGTCCTTCCACGATACCAAAGTT-3'

RT-PCR, reverse transcription-polymerase chain reaction; miR-338-5p, microRNA-338-5p; ZEB2, zinc finger E-box binding homeobox 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; F, forward; R, reverse.

Table II . Expression of miR-338-5p in gastric cancer specimens and the correlation with the clinicopathological features.

Clinicopathological factors	N	miR-200c	P-value
Age (years)			
≥55	26	0.29±0.03	0.245
<55	24	0.28±0.03	
Gender			
Male	28	0.28±0.02	0.254
Female	22	0.29±0.04	
Lymph node metastasis			
Yes	34	0.27±0.02	<0.001
No	16	0.31±0.02	
TNM stage			
I/II	19	0.30±0.03	<0.001
III/IV	31	0.27±0.02	

Bold indicates a significant difference. miR-338-5p, microRNA-338-5p



Table III . Correlation between the expression of ZEB2 protein and the clinicopathological features of gastric cancer.

Clinicopathological factors	N	ZEB2		P-value
		Positive	Negative	
Age (years)				
≥55	26	17	9	0.825
<55	24	14	10	
Gender				
Male	28	18	10	0.803
Female	22	14	8	
Lymph node metastasis				
Yes	34	23	11	0.035
No	16	5	11	
TNM stage				
I/II	19	7	12	0.020
III/IV	31	23	8	

Bold indicates a significant difference. ZEB2, zinc finger E-box binding homeobox 2.

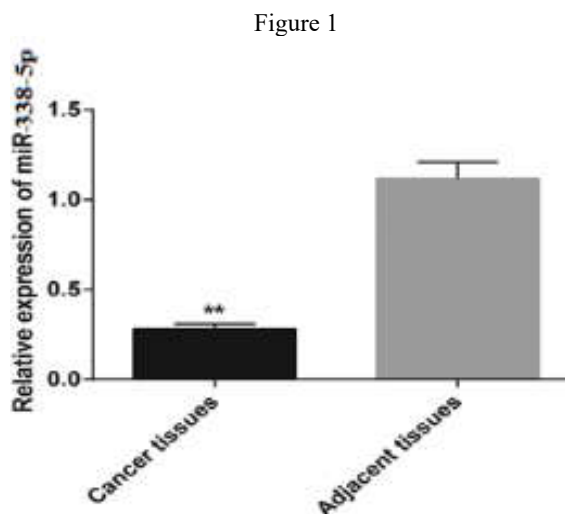


Figure 1. Relative expression of miR-338-5p in gastric cancer clinical specimens was detected by quantitative real-time RT-PCR. Data are expressed as the mean ± SD. \*\*P<0.01, compared with the corresponding control group. miR-338-5p, microRNA-338-5p

Figure 2

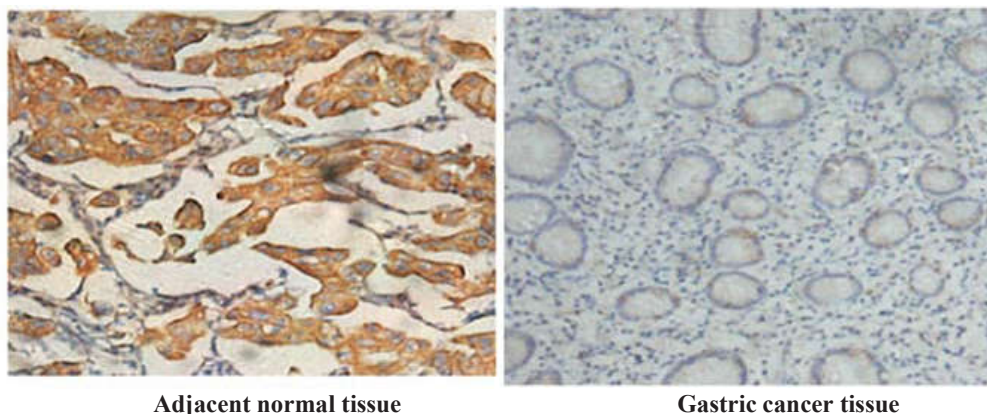


Figure 2. ZEB2 expression in cancer tissues and adjacent tissues was detected by immunohistochemistry. The results were obtained from 3 independent experiments. Data are expressed as the mean  $\pm$  SD. **\*\*P<0.01**, compared with the corresponding control group. ZEB2, zinc finger E-box binding homeobox 2.

Figure 3

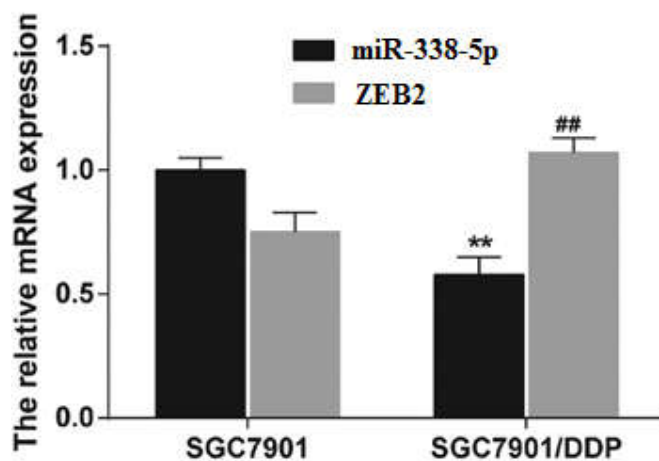


Figure 3. Relative expression of miR-338-5p and ZEB2 is detected by quantitative real-time RT-PCR in gastric cancer SGC7901 and SGC7901/DDP cells. The results were obtained from three independent experiments. Data are expressed as the mean  $\pm$  SD. **\*\*P<0.01**, **##P<0.01**, compared with the SGC7901 group. miR-338-5p, microRNA-338-5p; ZEB2, zinc finger E-box binding homeobox 2.

Figure 4

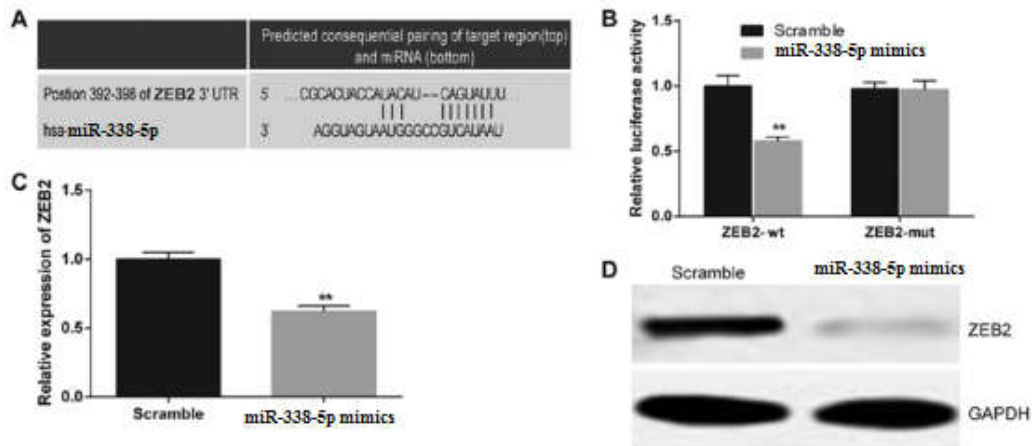


Figure 4. ZEB2 is a target gene of miR-338-5p. (A) Binding of miR-338-5p with ZEB2 3'-UTR as predicted by TargetScan. (B) Dual-luciferase reporter gene assay revealed that miR-338-5p significantly decreased the luciferase activity of ZEB2 wild-type (wt) 3'UTR. (C) ZEB2 mRNA levels in SGC7901/DDP cells transfected with miR-338-5p mimics or scramble sequence were examined by qRT-PCR. (D) The expression of ZEB2 protein was detected by western blot analysis using GAPDH as a loading control. The results were obtained from three experiments. Data are expressed as the mean  $\pm$  SD. \*\* $P < 0.01$  vs. the corresponding control. ZEB2, zinc finger E-box binding homeobox 2; miR-338-5p, microRNA-338-5p.

Figure 5

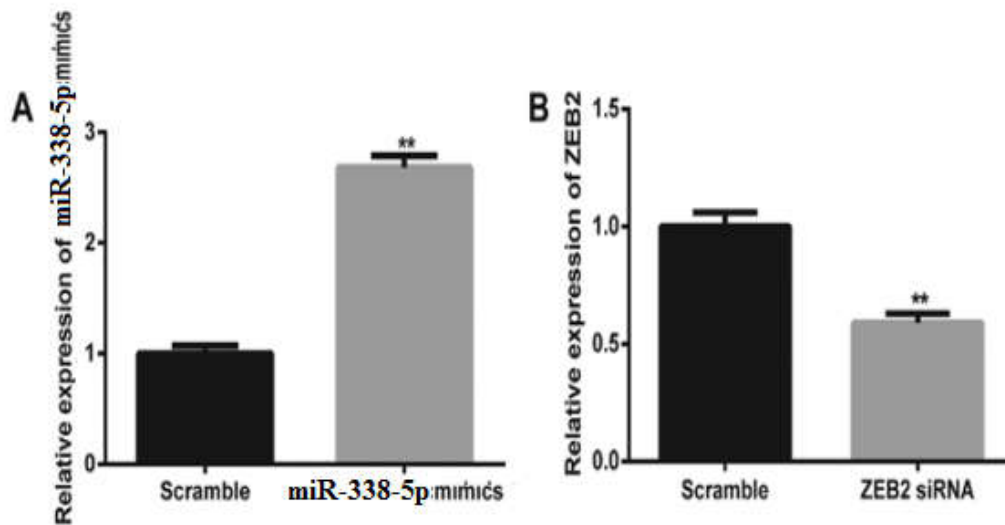


Figure 5. Expression levels of miR-338-5p and ZEB2 in gastric cancer SGC7901/DDP cells transfected with miR-338-5p mimics or ZEB2 siRNA were detected respectively by quantitative real-time RT-PCR. (A) The expression of miR-338-5p was significantly increased in cells transfected with miR-338-5p mimics. (B) The expression of ZEB2 was significantly decreased in cells transfected with ZEB2 siRNA. The results were obtained from 3 independent experiments. Data are expressed as the mean  $\pm$  SD. \*\* $P < 0.01$ , compared with the corresponding control group. miR-338-5p, microRNA-338-5p; ZEB2, zinc finger E-box binding homeobox 2.

Figure 6

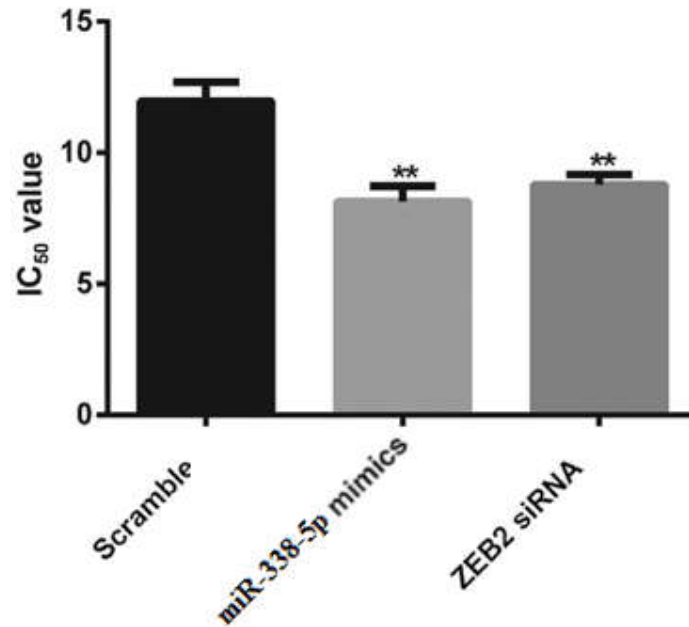


Figure 6. Regulation of miR-338-5p and ZEB2 expression alters the sensitivity of gastric cancer cells to DDP. SGC7901/DDP cells were transfected with miR-338-5p mimics or ZEB2 siRNA, after incubation with DDP for 48 h. Cell viability was detected using the MTT method and the IC<sub>50</sub> value of DDP was calculated. The data were obtained from 3 independent experiments. \*\*P<0.01 compared to the scramble group. miR-338-5p, microRNA-338-5p; ZEB2, zinc finger E-box binding homeobox 2; DDP, cisplatin.

Figure 7

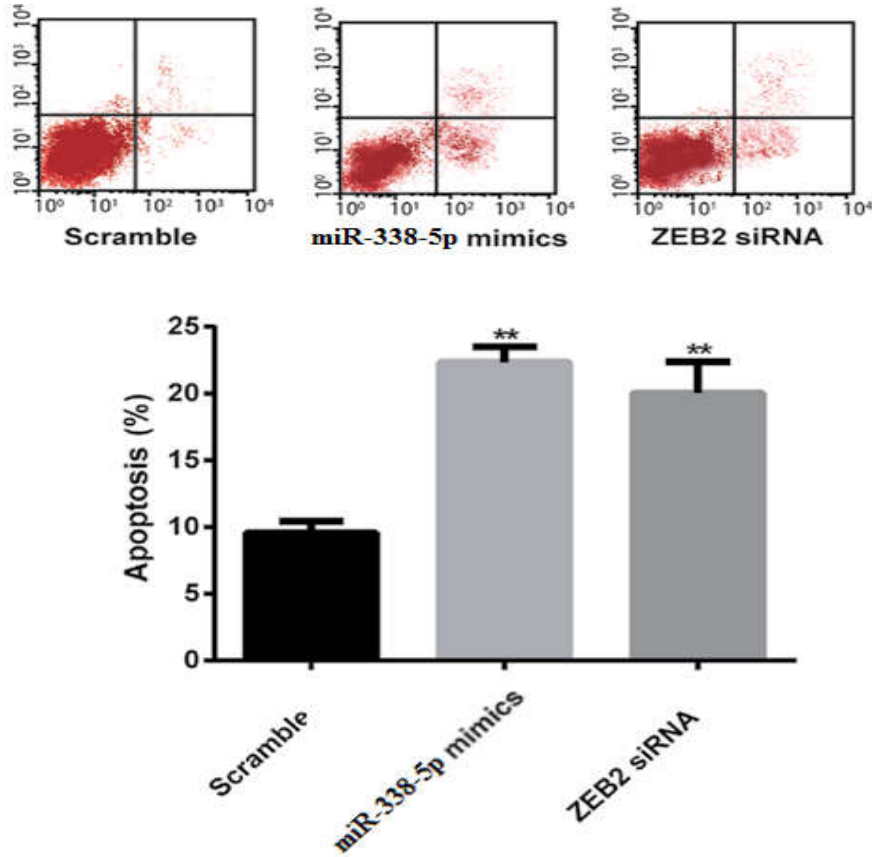


Figure 7. Overexpression of miR-338-5p and downregulation of ZEB2 sensitize SGC7901/DDP cells to DDP-induced apoptosis, respectively. In SGC7901/DDP cells, apoptosis assessed by flow cytometry showed a marked increase in the miR-338-5p mimic- and ZEB2 siRNA-transfected cells, compared with the scramble group. The data were obtained from 3 independent experiments. \*\*P<0.01 compared to the scramble group. miR-338-5p, microRNA-338-5p; ZEB2, zinc-finger E-box binding homeobox 2; DDP,cisplatin.



Figure 8

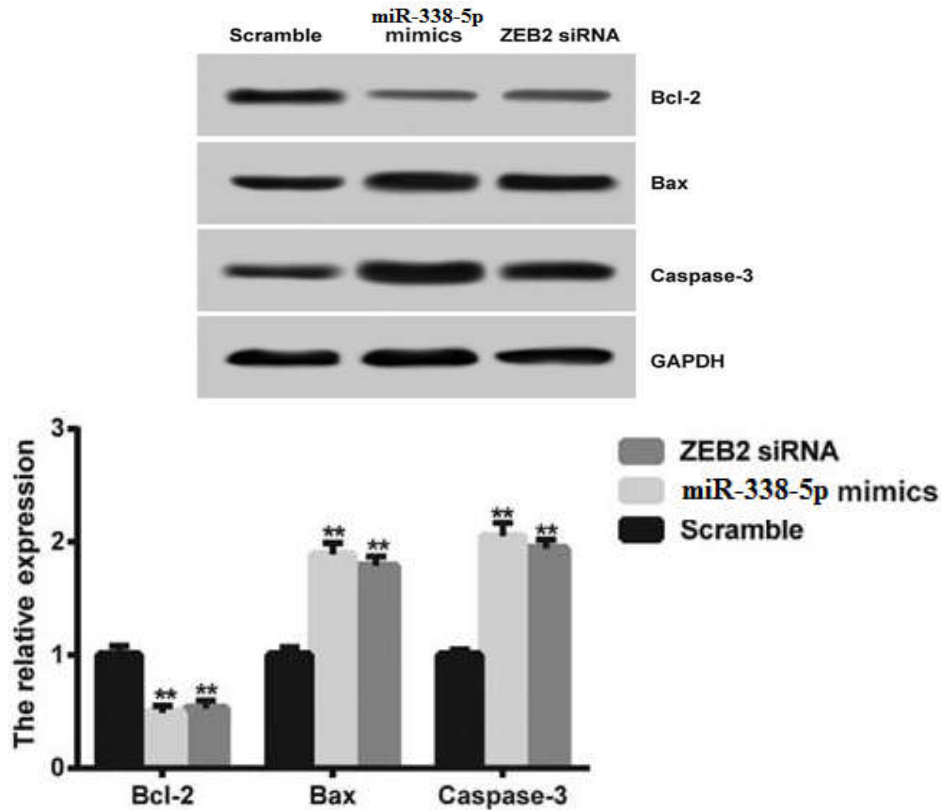


Figure 8. Alteration in the expression of Bcl-2, Bax and caspase-3 proteins in SGC7901/DDP cells after transfection with miR-338-5p mimics or ZEB2 siRNA. Results from western blot analysis showed that overexpression of miR-338-5p and downregulation of ZEB2 were associated with increased Bax and caspase-3 expression and suppressed Bcl-2 expression. The data were obtained from 3 independent experiments. \*\*P<0.01 compared to the scramble group. miR-338-5p, microRNA-338-5p; ZEB2, zinc finger E-box binding homeobox 2; DDP, cisplatin.

**DISCUSSION**

Gastric cancer is one of the most common malignant tumors and remains the second largest threat to individuals in the world 12. The incidence of gastric cancer is higher in Eastern countries, including China, Korea, and Japan 24. Gastric cancer is often diagnosed at late stages in which surgical procedures may not be effective. In 2015 Chen Y1, Chen J1,et al. Recently, studied about the increasing research evidence indicates that miRNA plays important roles in oncogenesis of hepatocellular carcinoma (HCC). There the objective of this study was to investigate the potential of plasma miRNAs as biomarkers for HCC determination. Material and Methods: This trial included 4 phases: (i) miRNAs in tumor tissues were screened with a miRNA array for determining candidate miRNAs. (ii) Candidate miRNAs were measured by RT-qPCR in plasma of 10 HCC patients before and after surgery (7-10 days) for target miRNAs that displayed a pattern of postoperative decrease. (iii) Plasma levels of target miRNAs in 37 HCC patients, 29 cirrhosis patients, and 31 healthy controls were measured by RT-qPCR for determining potential biomarkers. (iv) The powers of biomarkers for differentiating HCC were validated and the correlations with clinicopathological variables of HCC patients were analyzed. RESULTS: miRNA array demonstrated an abnormal expression of 92 miRNAs in tumor tissues compared to adjacent non-tumor tissues. Of those molecules with an over-expressed level in tumor tissues and preoperative plasmas, a decrease in postoperative plasma was observed in miR-15b-5p, miR-338-5p, and miR-764. Plasma levels of these miRNAs in HCC patients were higher than in the other 2 groups (P<0.05). Receiver-operator characteristic

(ROC) curve analyses suggested these plasma miRNAs could be useful biomarkers for determining HCC. miR-338-5p yielded an area under the ROC curve (AUC) of 0.799 (74.5% sensitivity and 82.8% specificity) and 0.909 (72.3% sensitivity and 99.68% specificity) to discriminate HCC patients from cirrhosis patients and healthy controls, respectively. The expression level of miR-338-5p was negatively correlated with the level of AFP ( $r=-0.306$ ,  $P=0.036$ ), and the expression level of miR-764 was positively correlated with the tumor size ( $r=0.371$ ,  $P=0.01$ ). CONCLUSIONS: Circulating miR-15b-5p, miR-338-5p, and miR-764 may be biomarkers for diagnosis of HCC.<sup>12</sup>

In 2016 Xibao, Yu Fen, et al, studied about the miR-338-5p modulates B cell biological functions by targeting NF- $\kappa$ B1, their the Objective To explore the effects of miR-338-5p on the nuclear factor  $\kappa$ B1 (NF- $\kappa$ B1) expression and the IgG-producing ability of B cells. Methods Dual-luciferase reporter assay was used to test the target gene of miR-338-5p. The purified CD20+ B cells were transfected with miR-338-5p agomiR, miR-338-5p antagomiR, NF- $\kappa$ B1 siRNA (siNF- $\kappa$ B1) and their corresponding negative control reagents, and then cultured with anti-IgM antibody and/or recombinant human B cell activating factor (rhBAFF). Real-time RCR and Western blotting were applied to determine the mRNA and protein levels of NF- $\kappa$ B1. IgG level in the supernatant was detected by ELISA. Results Compared with the control group, the hRluc/hLuc relative luciferase activity was significantly elevated in miR-338-5p mimic and NF- $\kappa$ B1-3'-UTR reporter co-transfected group. In the co-culture system with anti-IgM antibody and rhBAFF, the NF- $\kappa$ B1 mRNA, p105, p50 and IgG levels in B cells transfected with miR-338-5p agomiR were significantly increased, while the NF- $\kappa$ B1 mRNA and IgG levels in B cells transfected with miR-338-5p antagomiR were significantly decreased. The effect of siNF- $\kappa$ B1 on B cells was opposite to that of miR-338-5p agomiR. Correlation analysis suggested that NF- $\kappa$ B1 mRNA level was significantly positively correlated with IgG concentration. Conclusion miR-338-5p regulates the biological functions of B cells by positively regulating NF- $\kappa$ B1 expression and indirectly regulating BAFF signal.<sup>13</sup>

In 2015 Yang Z1, Sun B2, et al, carried out a study about the ZEB2 promotes vasculogenic mimicry by TGF- $\beta$ 1 induced epithelial-to-mesenchymal transition in hepatocellular carcinoma, their the Aims: Zinc finger E-box binding homeobox 2 (ZEB2), an epithelial-mesenchymal transition (EMT) regulator, has been involved in invasion and metastasis of human tumor. Although EMT may be involved in vasculogenic mimicry (VM) formation, no reports describing the relation between ZEB2 and VM are available. They hypothesize that ZEB2 may promote VM formation in hepatocellular carcinoma (HCC). Methods and Results: Paraffin-embedded tumor tissue samples from 92 patients were immunostained with anti-ZEB2 antibody. They found that the ZEB2 nuclear expression was significantly associated with VM formation and metastasis. Patients with VM and ZEB2 nuclear expression had a shorter survival period than those without expression. In vitro, ZEB2 overexpression significantly enhanced cell motility, invasiveness, and VM formation of HepG2 cells. ZEB2 upregulation also increased VE-cadherin, Flt-1, and Flk-1 expression and activated MMPs. ZEB2 knockdown inhibited cell motility, invasiveness, and VM formation in Bel7402 cells. ZEB2 knockdown also decreased VE-cadherin, Flt-1, and Flk-1 expression and MMP activity. In addition, EMT in HepG2 cells was induced by TGF- $\beta$ 1 treatment, and the kinetics of expression of EMT markers and regulators were assessed by Western blot analysis. The expression of ZEB2 increased significantly, and VM formation was promoted. Conclusion: ZEB2 can promote VM formation through the EMT pathway. Their findings may represent a novel therapeutic target in HCC.<sup>14</sup>

In 2015 Hegarty SV1, et al, carried out a study about the Zeb2: A multifunctional regulator of nervous system development, Zinc finger E-box binding homeobox (Zeb) 2 is a transcription factor, identified due its ability to bind Smad proteins, and consists of multiple functional domains which interact with a variety of transcriptional co-effectors. The complex nature of the Zeb2, both at its genetic and protein levels, underlie its multifunctional properties, with Zeb2 capable of acting individually or as part of a transcriptional complex to repress, and occasionally activate, target gene expression. This review introduces Zeb2 as an essential regulator of nervous system development. Zeb2 is expressed in the nervous system throughout its development, indicating its importance in neurogenic and gliogenic processes. Indeed, mutation of Zeb2 has dramatic neurological consequences both in animal models, and in humans with Mowat-Wilson syndrome, which results from heterozygous ZEB2 mutations. The mechanisms by which Zeb2 regulates the induction of the neuroectoderm (CNS primordium) and the neural crest (PNS primordium) are reviewed herein. We then describe how Zeb2 acts to direct the formation, delamination, migration and specification of neural crest cells. Zeb2 regulation of the development of a number of cerebral regions, including the neocortex and hippocampus, are then described. The diverse molecular mechanisms mediating Zeb2-directed development of various neuronal and glial populations are reviewed. The role of Zeb2 in spinal cord and enteric nervous system development is outlined, while its essential function in CNS myelination is also described. Finally, this review discusses how the neurodevelopmental defects

of Zeb2 mutant mice delineate the developmental dysfunctions underpinning the multiple neurological defects observed in Mowat-Wilson syndrome patients.<sup>15</sup>

In 2015 Prislei S1, Martinelli E1, et al, studied about the Role and prognostic significance of the epithelial-mesenchymal transition factor ZEB2 in ovarian cancer, ZEB2 is a key factor in epithelial-mesenchymal transition (EMT), a program controlling cell migration in embryonic development and adult tissue homeostasis. They demonstrated a role of ZEB2 in migration and anchorage-independent cell growth in ovarian cancer, as shown by ZEB2 silencing. They found that the RNA-binding protein HuR bound the 3'UTR of ZEB2 mRNA, acting as a positive regulator of ZEB2 protein expression. In Hey ovarian cell line, HuR silencing decreased ZEB2 and ZEB1 nuclear expression and impaired migration. In hypoglycemic conditions ZEB2 expression decreased, along with ZEB1, vimentin and cytoplasmic HuR, and a reduced cellular migration ability was observed. Analysis of ZEB2 and HuR expression in ovarian cancers revealed that nuclear ZEB2 is localized in tumor leading edge and co-localizes with cytoplasmic HuR. In a series of 143 ovarian cancer patients high expression of ZEB2 mRNA significantly correlated with a poor prognosis in term of both overall survival and progression-free survival. Moreover, at immunohistochemical evaluation, we found that prognostic significance of ZEB2 protein relies on its nuclear expression and co-localization with cytoplasmic HuR. In conclusion they findings indicated that nuclear ZEB2 may enhance progression of EMT transition and acquisition of an aggressive phenotype in ovarian cancer.<sup>16</sup>

In 2015 Sun DK, Wang JM, et al, researched about the .MicroRNA-138 Regulates Metastatic Potential of Bladder Cancer Through ZEB2, their the Aims: The cases of bladder cancer (BC) with poor prognosis largely result from the distal metastases of the primary tumor. Since microRNAs (miRNAs) play critical roles during cancer metastases, determination of the involved miRNAs in the regulation of the metastases of BC may provide novel therapeutic targets for BC treatment. Here, they aimed to study the role of miR-138 in regulation of BC cell invasion and metastases. Methods: They analyzed the levels of miR-138 and ZEB2, a key factor that regulates cancer cell invasion, in the BC specimens from the patients. they also studied the correlation between miR-138 and ZEB2. We performed bioinformatics analyses on the binding of miR-138 to the 3'-UTR of ZEB2 mRNA, and verified the biological effects of this binding through promoter luciferase reporter assay. The effects of miR-138-modification on BC cell invasion were evaluated in a transwell cell invasion assay and a scratch wound healing assay. Results: They found that the levels of miR-138 were significantly decreased and the levels of ZEB2 were significantly increased in BC specimens, compared to the paired normal bladder tissue. Metastatic BC appeared to contained lower levels of miR-138. Moreover, miR-138 and ZEB2 inversely correlated in BC specimens. Bioinformatics analyses showed that miR-138 targeted the 3'-UTR of ZEB2 mRNA to inhibit its translation. Furthermore, miR-138 overexpression inhibited ZEB2-mediated cell invasion and metastases, while miR-138 depletion increased ZEB2-mediated cell invasion and metastases in BC cells. Conclusion: Suppression of miR-138 in BC cells may promote ZEB2-mediated cancer invasion and metastases. Thus, miR-138 appears to be an intriguing therapeutic target to prevent metastases of BC.<sup>17</sup> On the other hand, resistance to chemotherapy is another challenge in clinical Practice.<sup>13</sup> DDP is a popular chemotherapeutic medication which induces tumor cell death by DNA damage . Therefore, finding the related factors that may affect the resistance to DDP may improve the survival status of gastric cancer patients. miRNAs are a class of endogenous ~22-nucleotide single strand and highly conserved non-coding RNAs, which are forecasted to modulate about 30% of gene expression through the interference with mRNA translation.<sup>18</sup> It has been reported that miRNAs play a multifunctional role in many biological processes including cell differentiation, apoptosis, proliferation, tumorigenesis, tumor development and tumor chemoresistance. miR-338-5p, which belongs to the miR-338-5p family, is involved in the inhibition of EMT, tumor invasion, and metastasis.<sup>19</sup> Many studies have shown that miR-338-5p can increase the sensitivity of cells to antitumor medications in a variety of cancers, including breast<sup>24</sup>, ovarian and non-small cell lung cancer. In this study, miR-338-5p was downregulated in gastric cancer and human gastric cancer cell line SGC7901 with DDP-resistance (SGC7901/DDP cells), which is consistent with the corresponding results from a previous stud .<sup>20</sup> Therefore, all of this evidence suggests that miR-338-5p may be involved in regulating the chemoresistance of gastric cancer patients. Generally, miRNAs mediate a series of biological processes through different target sites and they also regulate the expression of their downstream target mRNAs 18 .Previous studies have identified many downstream target mRNAs of miR-338-5p, such a RhoE and ZEB1/2.<sup>21</sup> Accordingly, the dual luciferase reporter gene assay in our experiments suggests that miR-338-5p could specifically bind with the 3'UTR of ZEB2 and significantly suppress the luciferase activity, implying that ZEB2 is a direct downstream target gene of miR-338-5p in SGC7901/DDP cells. ZEB2, as a key member of the Snail gene family, is closely associated with the biological processes of numerous tumors 48. Additionally, it has been reported that ZEB2 plays a major role in EMT by combining the E-box sequence of E-cadherin and then suppressing the transcription of numerous genes (e.g., bridge grain protein, cytokeratin and E-calcium sticky

protein). In our study, ZEB2 was markedly upregulated in gastric cancer tissues and SGC7901/DDP cells whereas miR-338-5p exhibited the opposite trend. Furthermore,<sup>22</sup> our results showed that the upregulation of miR-338-5p or downregulation of ZEB2 could increase the sensitivity of SGC7901/DDP cells to DDP. Previous studies also suggested that upregulated miR-338-5p enhanced the sensitivity to chemotherapy in patients with gastric cancer. Meanwhile, downregulation of ZEB2 also enhanced the sensitivity to chemotherapy in small-cell lung cancer.<sup>23</sup> Our results also confirmed that the upregulation of miR-338-5p or downregulation of ZEB2 could enhance the sensitivity of SGC7901/DDP cells to DDP-induced apoptosis and therefore miR-338-5p and ZEB2 can potentially regulate chemotherapy sensitivity through the apoptotic signaling pathway in gastric cancer patients. Accordingly, it has been reported that miR-338-5p not only regulates the induction of apoptosis by targeting FAP-1, but can also mediate the resistance to breast cancer medications by regulating a series of apoptosis-related genes. Moreover, knockdown of ZEB2 enhanced the sensitivity of lung cancer patients to chemotherapy since chemotherapy-induced apoptosis is potentially stimulated. In the present study,<sup>24</sup> we demonstrated that miR-338-5p enhanced the sensitivity of gastric cancer to DDP by directly targeting ZEB2. However, there are still some limitations in this study. For example, only the SGC7901/DDP cell line was used in this study and the detailed molecular mechanism involved remains unclear. The molecular mechanism of miR-338-5p and ZEB2 with respect to DDP-induced apoptosis needs further analysis in the future. In conclusion, we assessed and reported the effect of miR-338-5p and its target gene ZEB2 on DDP resistance in gastric cancer. Our findings illustrated that miR-338-5p expression was significantly downregulated in gastric cancer, while ZEB2 expression exhibited the opposite trend. Moreover, ZEB2 was found to be a direct target of miR-338-5p.<sup>25</sup> Our data further demonstrated that upregulation of miR-338-5p and downregulation of ZEB2 could increase the chemotherapeutic sensitivity of gastric cancer and chemotherapy-induced apoptosis. Collectively, all of these data suggest that miR-338-5p enhanced the sensitivity of gastric cancer to chemotherapy by directly targeting and regulating the expression of ZEB2. Thus, both miR-338-5p and ZEB2 exhibit great potential to serve as effective therapeutic targets for increasing the sensitivity of gastric cancer to DDP.<sup>26</sup>

## CONCLUSION

1- The data further demonstrated that upregulation of miR-338-5p and downregulation of ZEB2 could increase the chemotherapeutic sensitivity of gastric cancer and chemotherapy-induced apoptosis. Collectively, all of these data suggest that miR-338-5p enhanced the sensitivity of gastric cancer to chemotherapy by directly targeting and regulating the expression of ZEB2. Thus, both miR-338-5p and ZEB2 exhibit great potential to serve as effective therapeutic targets for increasing the sensitivity of gastric cancer to DDP.

2- The findings illustrated that miR-338-5p expression was significantly downregulated in gastric cancer, while ZEB2 expression exhibited the opposite trend. Moreover, ZEB2 was found to be a direct target of miR-338-5p.

The upregulation of miR-338-5p or downregulation of ZEB2 could enhance the sensitivity of SGC7901/DDP cells to DDP-induced apoptosis and therefore miR-338-5p and ZEB2 can potentially regulate chemotherapy sensitivity through the apoptotic signaling pathway in gastric cancer patients

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