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Research Article

Mir-497 Regulates Cisplatin Resistance of Human Gastric Cancer Cell Line by Targeting IGF1R, IRS1 and BCL2

Mingfeng He[1#], Jiaqi Qian[2#], Qin Shi[3], Jun Zhu[4], Yin Ding[5], Xin Zhou[2], Wei Zhu[2*], Wenfang Cheng[3] and Ping Liu[2*]

[1]Department of Anesthesiology, First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, PR China

[2]Department of Oncology, First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, PR China

[3]Department of Gastroenterology, First Affiliated Hospital of Nanjing Medical University, 300 Guangzhou Road, Nanjing 210029, PR China

[4]Department of Radiation Oncology, Jiangsu Cancer Hospital, No. 42 Bai Zi Ting, Nanjing 210009, PR China

[5]State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, No. 22 Hankou road, Nanjing 210093, PR China

[#]Contributed Equally

***Corresponding author:** Dr. Wei Zhu or Prof. Ping Liu, PR China

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Abstract

Purpose: Studies showed that drug resistance of gastric cancer cells could be modulated by the abnormal expression of miRNAs which targeted multiple cell signal pathways. Here we aimed to investigate the possible role of miR-497 in the development of cisplatin resistance in human gastric cancer cell line.

Methods: miRNA Quantitative real-time PCR was used to detect the different miRNAs expression level between drug resistant and parental cancer cells. MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to test the drug resistance phenotype changes of cancer cells via over or down regulation of miRNAs. Dual-luciferase activity assay was used to verify the target genes of miRNAs. Western blot analysis, Immunohistochemistry, Immunofluorescence staining, Cell proliferation assay, clonogenic assay and apoptosis assay were used to elucidate the mechanism of miRNAs on modulating drug resistance of cancer cells.

Results: miR-497 was significantly down-regulated in both gastric cancer tissues and various gastric cancer cell lines. Moreover, it was down-regulated in cisplatin-resistant gastric cancer cell line SGC7901/cisplatin (DDP) and the down-regulation of miR-497 was concurrent with the up-regulation of IGF1R/IRS1 pathway related proteins, such as IGF1R, IRS1 and BCL2, compared with the parental SGC7901 cell line, respectively. In vitro drug sensitivity assay demonstrated that over-expression of miR-497 sensitized SGC7901/DDP cells to cisplatin. The luciferase activity of the above proteins 3'-untranslated region-based reporters constructed respectively in SGC7901/DDP cells suggested that IGF1R, IRS1 and BCL2 were all the direct target genes of miR-497. Enforced miR-497 expression reduced its target proteins level, inhibited SGC7901/DDP cells proliferation and sensitized SGC7901/DDP cells to DDP-induced apoptosis.

Conclusions: Our findings suggested that hsa-miR-497 could modulate cisplatin resistance of human gastric cancer cell line at least in part by targeting IGF1R/IRS1 pathway.

Keywords: miR-497; Cisplatin resistance; IGF1R/IRS1 pathway; Gastric cancer

Abbreviations

miRNAs: microRNAs; DDP: Cisplatin; MTT: (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)

Introduction

Drug resistance is one of the leading cause of chemotherapy failure. Earlier studies have clarified several cytological mechanisms of drug resistance of cancer cells, such as increased efflux of hydrophobic drugs, decreased uptake of water-soluble drugs and various changes influencing the capacity of cytotoxic drugs to kill cells, including alterations in cell cycle and proliferation, enhanced DNA repair activity, defective apoptosis, altered metabolism of drugs, etc. [1-4]. Recently, people have realized that multiple paths could cause drug resistance phenotype of cancer cells, such as genetic changes including mutations, translocations, deletions and amplification of genes or promoter regions and epigenetic changes including aberrant DNA methylation, histone modifications and non-coding RNA expression, etc. [5,6]. Recent studies have also implicated that epigenetic mechanisms do not necessarily require a stable heritable genetic alteration, might play a more important role in acquired drug resistance of cancer cells, which is closely relevant to clinical practice [7].

The microRNAs (miRNAs) are a group of small non-coding RNAs, which are single-stranded and consist of 19–25 nucleotides (~22 nt). The basic mechanism of miRNA action is that miRNA can imperfectly bind to the 3'UTR of target mRNAs, resulting in translational repression or target mRNA cleavage [8]. Recent studies suggested that the acquisition of drug resistance by cancer cells might be regulated via the change in miRNAs levels [9-12]. Emerging evidence have shown that knock-down or re-expression

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of specific miRNAs by synthetic anti-sense oligonucleotides or miRNAs precusors or mimics could modulate drug resistance [12]. For instance, plently of miRNAs, such as miR-15b, miR-16 and miR-181b, could sensitize drug-resistant gastric cancer cell lines to drug induced apoptosis at least in part by targeting BCL2 [13,14]. miR-21 was up-regulated in various chemoresistant cancer cells, including breast cancer, glioblastoma and gastric cancer, while transfection with a specific anti-miR-21 could sensitize cancer cells to anti-cancer drugs via regulating PDCD4, LRRFIP1 or PTEN [15-17].

In this study, we reported that miR-497 was significantly down-regulated in both gastric cancer tissues and various gastric cancer cell lines. Moreover, it was down-regulated in cisplatin-resistant gastric cancer cell line SGC7901/cisplatin (DDP). We demonstrated that miR-497 might modulate cisplatin resistance of human gastric cancer cell line at least in part by targeting IGF1R/IRS1 pathway.

Material and Methods

Clinical samples

Endoscopic biopsy specimens including 24 cases of gastric cancer and 30 cases of non-tumor gastric mucosa collected from the First Affiliated Hospital of Nanjing Medical University between March 2012 and December 2012 were included. All the samples were confirmed by pathological examination and stored in liquid nitrogen for miRNA analysis. While another 20 tissue samples obtained from surgical specimens diagnosed with gastric cancer from May 2011 to December 2012 were used for Immunohistochemistry detection. Approval for this study was obtained from the medical ethics committee of the First Affiliated Hospital of Nanjing Medical University (reference number: 2011-SRFA-058).

Cell culture

Human gastric epithelium cell line GES-1 and gastric adenocarcinoma cell lines MKN45, MGC803, BGC823 and SGC7901 were purchased from the National Institute of Cells (Shanghai, China). Cisplatin-resistant variant SGC7901/DDP was obtained from KeyGEN Biotechnology Company (Nanjing, China). All the cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum (Gibco BRL, Grand Island, NY) in a humidified atmosphere containing 5% CO2 at 37°C. To maintain the cisplatin-resistant phenotype, cisplatin (DDP, with final concentration of 1 μg/ml) was added to the culture media for SGC7901/DDP cells.

Quantitative real-time PCR analysis for *miRNA*

Both biopsy specimens and cells were isolated with Trizol reagent (Invitrogen, Carlsbad, CA) and miRNA fraction was further purified using a mirVana ™ miRNA isolation kit (Ambion, Austin, TX). The concentration and purity of the RNA samples were determined spectroscopically. Expression of mature miRNA was assayed using stem-loop RT followed by real-time PCR analysis [14]. The primers of reverse transcription and polymerase chain reaction were purchased from RiboBio Co., Ltd (Guangzhou, China) named Bulge-Loop ™ miRNA qRT-PCR Primer Set as previously described [18]. qRT-PCR was performed according to the protocol of the primer set. PCR product amplification was detected by the level of fluorescence emitted by SYBR Green (SYBR® *Premix Ex Taq*™ II, TaKaRa) which intercalated into double stranded DNA [18]. U6 gene was used for normalizing the each sample. The ΔCt method was used for miRNA expression analysis of biopsy specimens. First, the cycle number at the threshold level of fluorescence (Ct) for each sample was determined. Next, the ΔCt value was calculated. The ΔCt value was the difference between the Ct value of miR-497 and the Ct value of U6: ΔCt = Ct (miR-497) - Ct (U6). The fold-change for miRNA from cells relative to each control cells was calculated using the $2^{-\Delta\Delta Ct}$ method [14], where ΔΔCt =ΔCt MKN45, MGC803, BGC823, SGC7901 -ΔCt GES-1 or ΔΔCt =ΔCt SGC7901/DDP –ΔCt SGC7901. PCR was performed in triplicate.

In vitro drug sensitivity assay

SGC7901/DDP and SGC7901 cells were plated in 6-well plates (6×10^5 cells /well), 100 nM of the miR-497 mimic or 100 nM miRNA mimic control were transfected in SGC7901/DDP cells, while 100 nM of the miR-497 inhibitor or 100 nM miRNA inhibitor control were transfected in SGC7901 cells, using lipofectamine 2000 (Invitrogen, Long Island, NY, USA) according to the manufacturer's protocol, respectively. The miR-497 mimic, miRNA mimic control, 2'-O-methyl (2'-O-Me) modified miR-497 inhibitor and miRNA inhibitor control were chemically synthesized by Shanghai GenePharma Company (Shanghai, China). The sequence of each was shown in Supplementary data. 1. Twenty-four hours after transfection cells were seeded into 96-well plates (5×10^3cells/well) for next step experiment. After cellular adhesion, freshly prepared cisplatin (DDP) was added with the final concentration being 0.01, 0.1, 1 and 10 times of the human peak plasma concentration for cisplatin as previously described [14]. The peak serum concentrations of cisplatin was 2.0 μg/ml [14]. 48 hr after the addition of drugs, cell viability was assessed by MTT assay. The absorbance at 490 nm (A490) of each well was read on a spectrophotometer. The concentration at which cisplatin produced 50% inhibition of growth (IC50) was estimated by the relative survival curve. Three independent experiments were performed in quadruplicate.

Dual-luciferase activity assay

The 3'UTR of human IGF1R, IRS1 and BCL2 cDNA containing the putative target site for the miR-497 (sequence shown in Supplementary data. 2) was chemically synthesized and inserted at the XbaI site, immediately downstream of the luciferase gene in the pGL3-control vector (Promega, Madison, WI) by Integrated Biotech Solutions Co., Ltd (Shanghai, China), respectively. Twenty-four hours before transfection, cells were plated at 1.5×10^5 cells/well in 24-well plates. 200 ng of pGL3-IGF1R-3'-UTR, pGL3-IRS1-3'-UTR or pGL3-BCL2-3'-UTR plus 80 ng pRL-TK (Promega) were transfected in combination with 60 pmol of the miR-497 mimic or miRNA mimic control using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol as described, respectively [14]. Luciferase activity was measured 24hr after transfection using the Dual Luciferase Reporter Assay System (Promega). Firefly luciferase activity was normalized to renilla luciferase activity for each transfected well. Three independent experiments were performed in triplicate.

Immunohistochemistry

Twenty tissue samples were obtained from surgical specimens diagnosed with gastric cancer, from May 2011 to December 2012 at the First Affiliated Hospital of Nanjing Medical University. Tissue samples were formalin-fixed and paraffin-embedded; 4-μm-thick

sections were cut and stained by using the avidinbiotin complex method. After that, the slides were pretreated with microwaves for antigen retrieval in 10 mM citrate buffer (pH 6.0) and incubated in the primary antibody at 4°C overnight. The antibody of IGF1Rα (Catalog: sc-271606), IRS1 (Catalog: sc-559) and BCL2 (Catalog: BS1511) were purchased from Santa Cruz Biotechnology and Bioworld Technology, respectively. If the staining was uncertain, we repeated to confirm it. For the scoring of the above proteins, the slides were scored by two separate observers blinded to the clinical data. They evaluated the immunostainings of the slides under an optical microscope of a magnification of 400×. If there were any intra-observer differences, the slides were reevaluated to reach consensus. The staining intensity of the above proteins expression was scored on a scale of 1–3 as follows: 0 score for no staining; 1 for weak staining; 2 for moderate staining; and 3 for strong staining. The percentage of positive cancer cells was scored as follows: 0 score for 0 %; 0.1 for 1–9 %; 0.5 for 10–49 %; and 1.0 for 50 % or more. We multiplied the staining intensity by the proportion score of the percentage of positive cancer cells. Thus, we separated the patients into positive ones (the product >1) and negative ones (the product ≤1).

Immunofluorescence staining

GES-1, MKN45, MGC803, BGC823, SGC7901 and SGC7901/DDP cells were grown on glass coverslips and fixed with 4% paraformaldehyde at 4°C for 15 min and were further permeabilised and blocked with 0.5% Triton X-100 and 5% bovine serum albumin in phosphate buffered saline (PBS) for 30 min. The coverslips were then exposed to primary antibodies as mentioned above at 4^0C overnight, followed by the appropriate secondary antibodies. The preparations were visualised using an Olympus IX70 fluorescence microscope.

Western blot analysis

SGC7901/DDP cells were plated in 6-well plates (6×10^5 cells / well), 72 hr after the transfection of miR-497 mimic or miRNA mimic control, cells were harvested and homogenized with lysis buffer. Total protein was separated by denaturing 10% SDS–polyacrylamide gel electrophoresis. Total protein of GES-1, MKN45, MGC803, BGC823, SGC7901 and SGC7901/DDP was also extracted and separated as described above. Western blot analysis was performed as described [14]. The primary antibodies for IGF1Rα (Catalog: sc-271606), IRS1 (Catalog: sc-559), IGF1R (Catalog: BS1183), BCL2 (Catalog: BS1511) and GAPDH (Catalog: BS6945) were purchased from Santa Cruz Biotechnology and Bioworld Technology, respectively. Protein levels were normalized to GAPDH. Fold changes were determined.

Cell proliferation assay

Six hours after the transfection of miR-497 mimic or miRNA mimic control, SGC7901/DDP cells were trypsinized and seeded into 96-well culture plates at a density of 5×10^3cells/well in growth medium supplemented with 10% serum. The MTT assay was performed 24, 48 and 72 hr post-transfection and absorbance was measured using a spectrophotometer at 490 nm. Each assay was performed in triplicate with three independent replicates.

Clonogenic assay

SGC7901/DDP cells were transfected with miR-497 mimic or miRNA mimic control as previously described and plated into 6-well plates at a density of 200 cells per well, incubated at 37°C for 2 weeks, fixed and stained with crystal violet. The mean ±SEM number of colonies containing > 50 cells were counted under a microscope from three independent replicates.

Apoptosis assay

SGC7901/DDP cells were plated in 6-well plates (6×10^5 cells / well). Twenty-four hours after the transfection of miR-497 mimic or miRNA mimic control as described above, cells were treated by DDP, with final concentration of 10 μg/ml, respectively. 48 hr after the treatment of DDP, flow cytometry was used to detect apoptosis of the transfected SGC7901/DDP cells by determining the relative amount of AnnexinV-FITC-positive- PI-negative cells as previously described [14], respectively.

Statistical Analysis

Each experiment was repeated at least 3 times. Numerical data were presented as mean±SD. The difference between means was analyzed with Student's t test. All statistical analyses were performed using SPSS11.0 software (Chicago, IL).

Differences were considered significant when $p < 0.01$.

Results

miR-497 was significantly down-regulated in gastric cancer tissues and gastric cancer cell lines

We examined miR-497 expression in endoscopic biopsy specimens including 24 cases of gastric cancer and 30 cases of non-tumor gastric mucosa using quantitative real-time PCR, to determine whether miR-497 expression was associated with gastric cancer. We found that miR-497 expression was significantly down-regulated in gastric cancer tissues, with an average 5.21-fold decrease, compared with the non-tumor gastric mucosa (Supplementary data. 3A). Meanwhile, we also found that miR-497 was significantly down-regulated in human gastric adenocarcinoma cell lines MKN45, MGC803, BGC823 and SGC7901, compared with human gastric epithelium cell line GES-1, with the average 3.57-fold, 2.77-fold, 3.22-fold and 4.75-fold decrease, respectively (Supplementary data. 3B).

miR-497 was significantly down-regulated in cisplatin-resistant gastric cancer cell line SGC7901/ DDP

Quantitative real-time PCR for miR-497 verified that miR-497 was also significantly down-regulated in cisplatin-resistant gastric cancer cell line SGC7901/DDP. The average decreased fold change

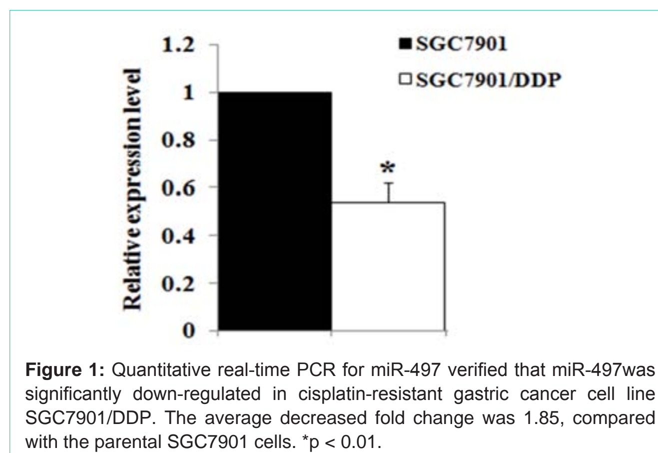

Figure 1: Quantitative real-time PCR for miR-497 verified that miR-497was significantly down-regulated in cisplatin-resistant gastric cancer cell line SGC7901/DDP. The average decreased fold change was 1.85, compared with the parental SGC7901 cells. *p < 0.01.

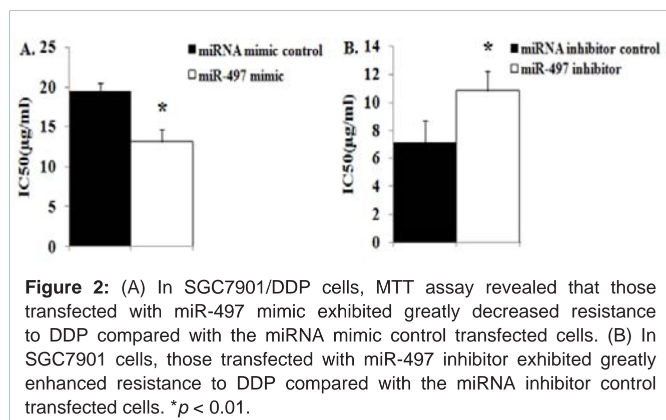

Figure 2: (A) In SGC7901/DDP cells, MTT assay revealed that those transfected with miR-497 mimic exhibited greatly decreased resistance to DDP compared with the miRNA mimic control transfected cells. (B) In SGC7901 cells, those transfected with miR-497 inhibitor exhibited greatly enhanced resistance to DDP compared with the miRNA inhibitor control transfected cells. *$p < 0.01$.

was 1.85, compared with the parental SGC7901 cells (Figure 1).

miR-497 modulated cisplatin resistance of SGC7901/DDP cell line

In SGC7901/DDP cells, MTT assay revealed that those transfected with miR-497 mimic exhibited greatly decreased resistance to DDP compared with the miRNA mimic control transfected cells (Figure 2A), while in SGC7901 cells, those transfected with miR-497 inhibitor exhibited greatly enhanced resistance to DDP compared with the miRNA inhibitor control transfected cells (Figure 2B). These results suggested that miR-497 might modulate cisplatin resistance of SGC7901/DDP cell line.

IGF1R, IRS1 and BCL2 were the target genes of miR-497

TargetScanHuman (http://www.targetscan.org) predicted that IGF1R, IRS1 and BCL2 were all the target genes of the miR-497 conservely between different species (Supplementary data. 4). To explore whether the above 3 genes were the target genes of the miR-497, we constructed the luciferase reporter vectors with the putative IGF1R, IRS1 and BCL2 3' UTR target sites for the miR-497 downstream of the luciferase gene (pGL3-IGF1R-3'-UTR, pGL3-IRS1-3'-UTR and pGL3-BCL2-3'-UTR), respectively. Luciferase reporter vectors together with the miR-497 mimic or the miRNA mimic control were transfected into SGC7901/DDP cells, respectively. In SGC7901/DDP cells, significant decrease in relative luciferase activity was noted when

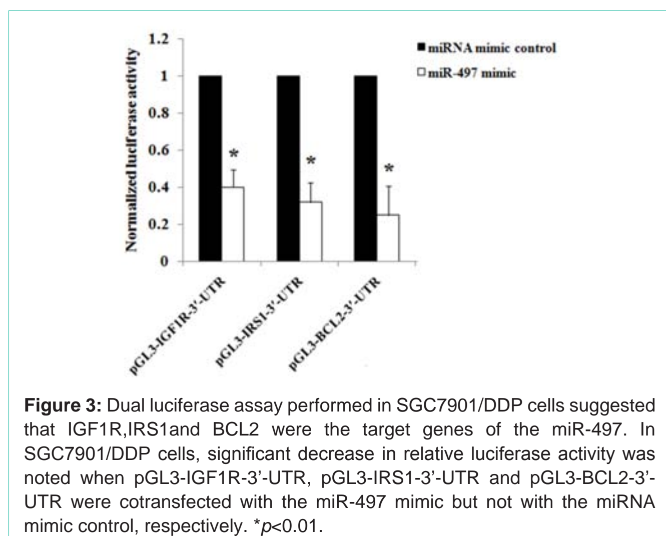

Figure 3: Dual luciferase assay performed in SGC7901/DDP cells suggested that IGF1R,IRS1and BCL2 were the target genes of the miR-497. In SGC7901/DDP cells, significant decrease in relative luciferase activity was noted when pGL3-IGF1R-3'-UTR, pGL3-IRS1-3'-UTR and pGL3-BCL2-3'-UTR were cotransfected with the miR-497 mimic but not with the miRNA mimic control, respectively. *$p<0.01$.

pGL3-IGF1R-3'-UTR, pGL3-IRS1-3'-UTR and pGL3-BCL2-3'-UTR were cotransfected with the miR-497 mimic but not with the miRNA mimic control, respectively. These results showed that IGF1R, IRS1 and BCL2 were all the target genes of the miR-497 (Figure 3).

IGF1R, IRS1 and BCL2 were differently expressed in gastric cancer tissues and gastric cancer cell lines

We checked IGF1Rα, IRS1 and BCL2 expression in 20 cases of gastric cancer tissue samples by immunohistochemistry. We found that the above proteins were differently expressed, with the positive rate of 20%, 60% and 65%, respectively (Supplementary data. 5A). Meanwhile, we also detected the expression levels of IGF1Rα or IGF1R, IRS1 and BCL2 in human gastric adenocarcinoma cell lines MKN45, MGC803, BGC823 and SGC7901 and human gastric epithelium cell line GES-1, using immunofluorescence staining and Western blot, respectively. We found that the above proteins were over-expressed in different degrees in gastric cancer cell lines, compared with the human gastric epithelium cell line GES-1, respectively (Supplementary data. 5B&5C). These results proved that IGF1R, IRS1 and BCL2 were closely related with gastric cancer.

miR-497 modulated cisplatin resistance by repressing IGF1R/IRS1 signal pathway related proteins

Worth of note, in our study the decreased expression of miR-497 in SGC7901/DDP cells was concurrent with the over-expression of the IGF1R, IGF1Rα, IRS1 and BCL2 proteins, compared with the parental SGC7901 cells, respectively (Figure 4A and 4B). Since the above IGF1R/IRS1 signal pathway related proteins were all the targets of the miR-497, we hypothesized that the miR-497 might modulate cisplatin resistance of gastric cancer cells by repressing the expression levels of them. To ascertain our hypothesis, we transfected the miR-497 mimic and the control miRNA mimic into SGC7901/DDP cells to detect the above IGF1R/IRS1 signal pathway related proteins expression level changes, respectively. In SGC7901/DDP cells, 72hr after the transfection Western Blot demonstrated significantly decreased IGF1R, IGF1Rα, IRS1 and BCL2 proteins level in miR-497 mimic transfected cells compared with the miRNA mimic control transfected cells (Figure 4C). These results showed that miR-497 might modulate cisplatin resistance of gastric cancer cells at least in part by repressing the IGF1R/IRS1 signal pathway related proteins.

miR-497 significantly inhibited SGC7901/DDP cells proliferation

Recently, studies had shown that the abnormality of IGF1R/IRS1 signal pathway was closely related with the drug resistance and poor chemotherapy reactivity of gastric cancer [19-21]. Since the miR-497 might modulate cisplatin resistance of gastric cancer cells at least in part by repressing the IGF1R/IRS1 signal pathway related proteins, considering the well-characterized role of IGF1R/IRS1 signal pathway, we suggested the hypothesis that miR-497 might play a role in the development of cisplatin resistance at least in part by modulation of proliferation of gastric cancer cells. Exactly, in SGC7901/DDP cells, both MTT and clonogenic assay suggested that those transfected with miR-497 mimic exhibited greatly decreased proliferation, compared with the miRNA mimic control transfected cells, respectively (Figure 5A,5B).

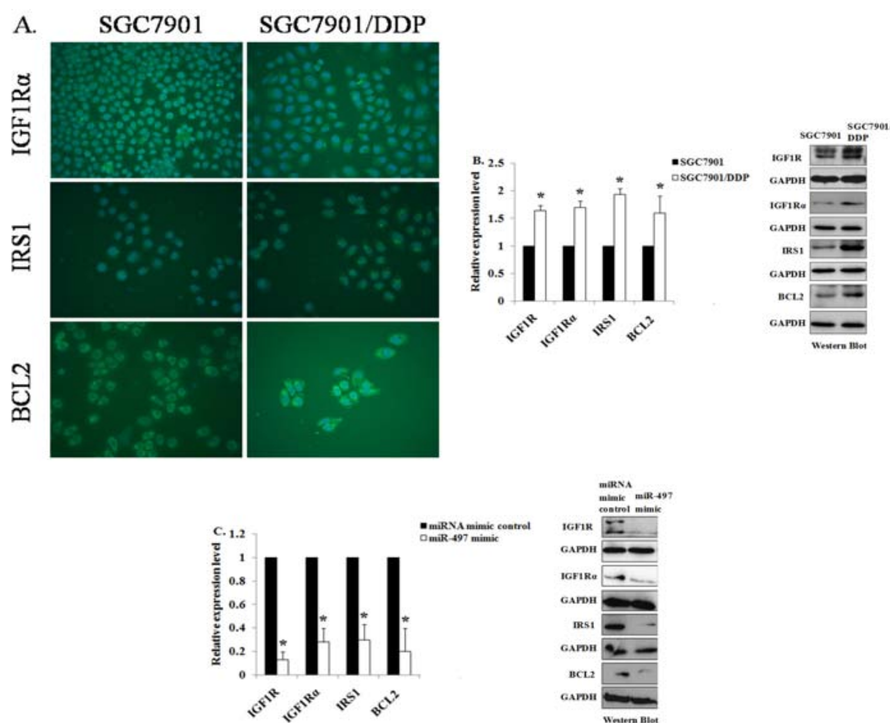

Figure 4: (A)&(B) Immunofluorescence staining and Western blot showed that IGF1R, IGF1Rα, IRS1, and BCL2 were significantly over-expressed in cisplatin-resistant gastric cancer cell line SGC7901/DDP, compared with the parental SGC7901 cells, respectively. Green color standed for IGF1Rα, IRS1, and BCL2 staining while blue color represent nuclear staining by DAPI.(C) In SGC7901/DDP cells, 72hr after the transfection Western Blot demonstrated significantly decreased IGF1R, IGF1Rα, IRS1 and BCL2 proteins level in miR-497 mimic transfected cells compared with the miRNA mimic control transfected cells, respectively. *p<0.01.

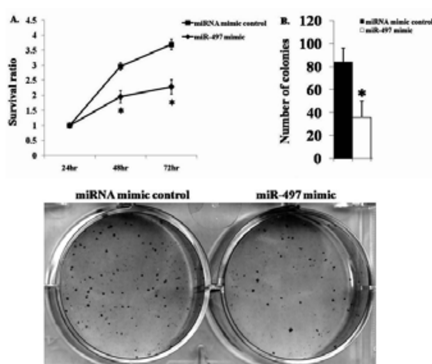

Figure 5: (A)&(B) In SGC7901/DDP cells, both MTT and clonogenic assay suggested that those transfected with miR-497mimic exhibited greatly decreased proliferation, compared with the miRNA mimic control transfected cells, respectively. Representative clonogenic assay result was attached under the graphs. *p<0.01.

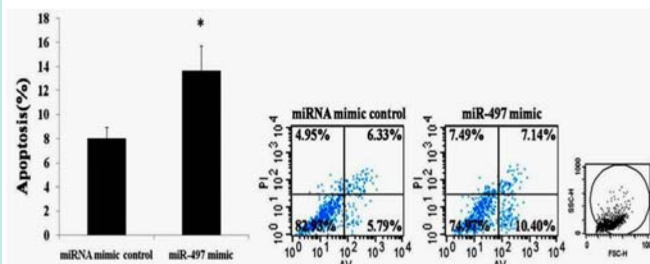

Figure 6: miR-497 mimic sensitized SGC7901/ DDP cells to DDP-induced apoptosis. In SGC7901/DDP cells, apoptosis evaluated by flow cytometry showed a marked increase of apoptosis in miR-497 mimic transfected cells after DDP treatment, compared with the miRNA mimic control transfected cells. Representative flow cytometry report was attached beside the graphs. The results shown represent the mean±SD from 3 independent experiments. *p <0.01.

miR-497sensitized SGC7901/DDP cells to DDP-induced apoptosis

Moreover, the development of drug resistance in various cancer cells had been linked to a reduced susceptibility to drug-induced apoptosis, which was shown to be a consequence, at least in some cases, of over-expression of anti-apoptotic proteins, such as BCL2, IAPs, BCL-XL [13,14,22]. Since the miR-497 might modulate cisplatin resistance of gastric cancer cells at least in part by repressing the anti-apoptotic BCL2 protein expression, we suggested the hypothesis that miR-497 might also play a role in the development of cisplatin resistance at least in part by modulation of apoptosis of gastric cancer cells. To confirm this hypothesis, we evaluated DDP-induced apoptosis after transfection SGC7901/DDP cells with the miR-497mimic and the miRNA mimic control, respectively. In SGC7901/DDP cells, a marked increase in apoptosis, as assessed by flow cytometry, was observed in the miR-497 mimic transfected cells after DDP treatment, compared with the miRNA mimic control transfected cells (Figure 6).

Discussion

Researches had proved that IGF1R/IRS1 pathway was involved in cell proliferation and apoptosis control (http://www.uniprot.org/uniprot/P08069). Currently, the over-expression of IGF1R/IRS1

pathway proteins such as IGF1 and IGF1R were closely related to poor chemotherapy response and prognosis in gastric cancer [19,20]. Moreover, IGFIR expression level was also found increased during cisplatin treatment cycles and correlated with cisplatin resistance in ovarian cancer [21]. Those all suggested that IGF1R/IRS1 pathway might play a important role in drug resistance of cancer cells. Exactly, in our study, we found that miR-497 was down-regulated in cisplatin-resistant gastric cancer cell line SGC7901/DDP and its down-regulation was concurrent with the up-regulation of IGF1R/IRS1 pathway related proteins, such as IGF1R, IRS1 and BCL2. We verified that miR-497 could modulate cisplatin resistance of human gastric cancer cell line by inhibiting cell proliferation and promoting apoptosis at least in part via targeting IGF1R/IRS1 pathway.

miR-497 was located on chromosomal band 17p13.1. Recently, plenty of studies had showed that miR-497 was down-regulated in a range of human cancers, such as prostate carcinoma [23], adrenocortical carcinoma [24], malignant pleural mesothelioma [25], breast carcinoma [26], colorectal cancer [27], malignant astrocytomas [28], neuroblastoma [29], non-small cell lung cancer [30] and hepatocellular carcinoma [31], suggesting that it might have potential roles as tumour-suppressor gene in these cancers mentioned above. Moreover, the study by Bloomston M et al. [32] found that miR-497 was up-regulated in pancreatic cancer compared with chronic pancreatitis while down-regulated in chronic pancreatitis compared with the normal pancreas, which showed that the function of miR-497 on pancreatic tumourigenesis might be more conflicting. In our study, we also found that miR-497 was down-regulated in gastric cancer and could inhibit proliferation and increase apoptosis of drug-resistant gastric cancer cells at least in part via targeting IGF1R, IRS1 and BCL2, suggesting that miR-497 might also play a role of tumor suppressor gene in gastric cancer. This was in somewhat in concordance with recent study by Guo et al. They also found that IGF1R was the direct target gene of miR-497 [27]. However, the mechanism of the down-regulation of miR-497 was still unknown. The aberrant DNA methylation of the promoter region of miR-497 might be an important cause of the down-regulation of miR-497 in drug resistant cells [26], however, more research was needed to elucidate the underlying mechanism.

In summary, the findings we reported here presented the evidence that miR-497 might be involved in the development of cisplatin resistance in human gastric cancer cell line at least in part via targeting IGF1R/IRS1 pathway. However, it should be noted that our data were derived from cell line which had been removed from their in vivo context and could not be considered accurate surrogates for clinical tumors. Thus, future studies to assess the roles of miR-497 in vivo and in clinical context were warranted.

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