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The effect of combined miR-200c replacement & cisplatin on apoptosis induction and inhibition of gastric Cancer cell line migration

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Author contribution statement

Mehri Ghasabi has provided constructing an idea or hypothesis for research.

Behzad Baradaran and Behzad Mansoori have provided planning methodology to reach the conclusion.

Behzad Baradaran and Behzad Mansoori have provided organizing and supervising the course of the project or the article and taking the responsibility.

Providing personnel, environmental and financial support, tools, and instruments that are vital for the project have been provided by Behzad Baradaran.

Mehri Ghasabi, Behzad Mansoori, and Ali Mohammadi have provided biological materials, reagents and referred patients.

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Taking responsibility in logical interpretation and presentation of the results have been provided by Mehri Ghasabi, Behzad Baradaran and Behzad Mansoori.

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Abstract

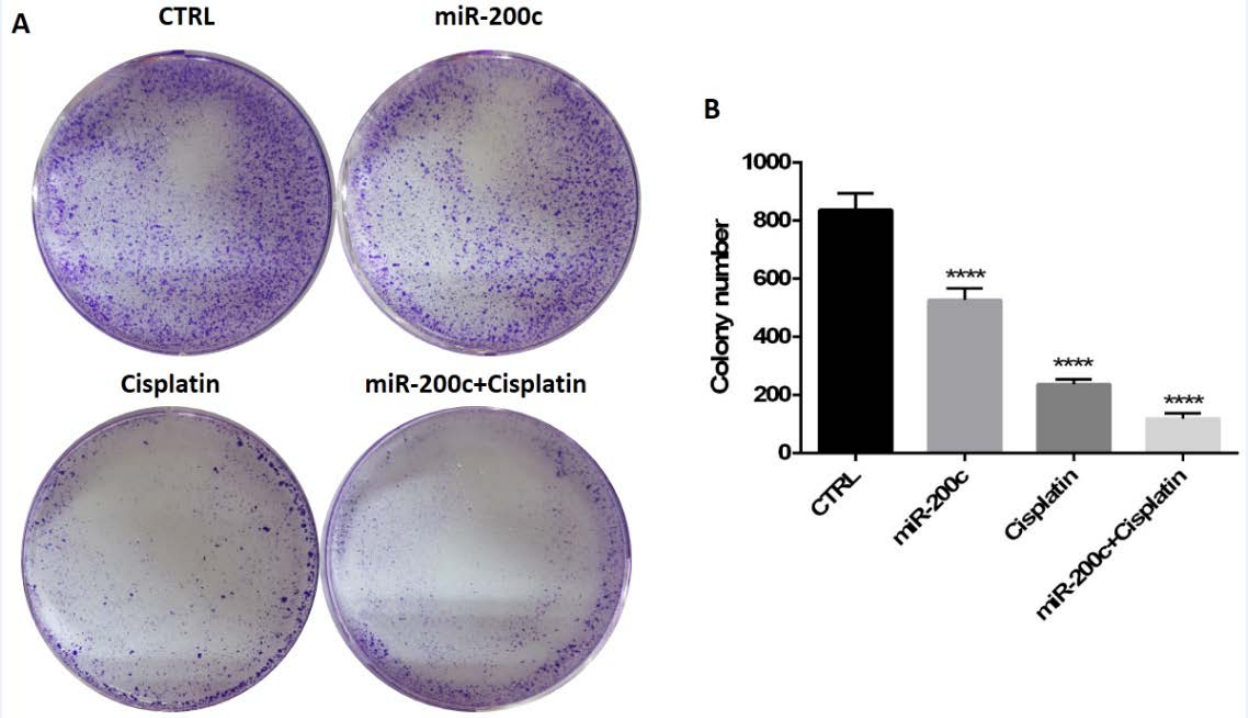
One of the major obstacles in the treatment of cancer is resistance to standard chemotherapeutic drugs. According to the numerous reports, miR-200c is involved in many cancers, especially gastric cancer, also miR-200c has been known as an effective factor in the elimination of chemotherapy resistance. The purpose of this study was to explore the potential function and mechanism of miR-200c and cisplatin in the inhibition of migration and induction of apoptosis in gastric cancer cells.

In this study, first, miR-200c mimics and LNA-anti-miR-200c were transfected into KATOIII cells**. Moreover**, MTT assay results revealed that increased miR-200c expression and cisplatin can more inhibited the proliferation of KATOIII cells. miR-200c overexpression inhibited the movement of KATOIII cells in wound healing assay. Subsequently, miR-200c /cisplatin could suppressed colony formation in KATO III III

cells. To identify a potential miR-200c target, we investigated the effect of miR-200c modulation on RhoE, PTEN, VEGFR and MMP9 expression levels. Increased miR-200c expression caused a reduction in VEGFR and MMP9 mRNA and protein, suggesting that VEGFR and MMP9 are targets of miR-200c. In addition, RT-PCR assays showed that RhoE, is target gene of miR-200c and LNA-anti-miR-200c suppressed the expression of PTEN. Flow cytometry and DAPI staining analysis indicated that, miR-200c increased the cisplatin-induced apoptosis which may be associated with suppression of RhoE expression in KATOIII cells, also cell cycle analysis showed the arrest of cell cycle progression at the G2 phase. These data demonstrated that miR-200c functioned as a suppressor gene in KATOIII cells. Also, miR-200c can be a potential therapeutic approach to overcome chemoresistance during cisplatin chemotherapy.

Graphical Abstract

Gastric cancer is one of the most common malignancy of the digestive system. There are, however, different problems in the common treatment methods, such as resistance to drugs, recurrence and metastasis in cancerous cells. Therefore, investigation to the use of new treatments like microRNA is more exposure to attention and miRNAs are progressively regarded as potential therapeutic targets. microRNAs are a small non-coding RNA molecule.(Heydari et al., 2018; Jiang et al., 2017; Liu et al., 2017b). In our study further reavled that miR-200c could enhance the sensitivity of gastric cancer cells to cisplatin and also, demonstrated miR-200c involvement in the regulation of cell proliferation, migration and apoptosis by targeting relative genes such as VEGFR, MMP9 and RhoE.



Keyword: gastric cancer, miR-200c, metastasis, apoptosis

Introduction

Gastric cancer is one of the most common malignancy of the digestive system. Despite of the many advances in cancer treatment, gastric cancer has a high rate of death among of the patients across the world. It is reported that, the second death from cancer diseases, belongs to gastric cancer. Gastric cancer is a multifactorial disease that various hereditary and environmental factors have contribution role on it (Jiang et al., 2017; Ni et al., 2017). Over the years, various treatments ,such as chemotherapy, targeted therapy surgery and radiation therapy are used for treatment of this type of cancer (Davudian et al., 2016; Ni et al., 2017). There are, however, different problems in the common treatment methods, such as resistance to drugs, recurrence and metastasis in cancerous cells(Mansoori et al., 2017a). Therefore, investigation to the use of new treatments like microRNA is more exposure to attention and miRNAs are progressively regarded as potential therapeutic targets. microRNAs are a small non-coding RNA molecule (17-25 nucleotide) that are involved in post-transcriptional regulation of gene expression (Zhang et al., 2017). Studies show that the function and expression of miRNAs can be changed in most tumor cells. Consequently, dysregulation of miRNAs expressions could interrupt different cellular processes such as apoptosis or proliferation(Hosseinahli et al., 2018; Mirzaei et al., 2018). Therefore, miRNA plays an important role in cancer treatment. On the other hand, studies show that miRNAs can be considered as an effective chemotherapy. Also, miRNAs can impact on the drug resistance-related proteins or drug target genes

expressions (Li and Yang, 2013; Mansoori et al., 2015; Tang et al., 2013b). To date, many studies have been done to clarify the role of miRNAs on gastric cancer. Such other cancers, miRNAs have a bilateral role in gastric cancer that means depending on their targeted genes (tumor suppressive or oncogene). They can help suppress or progress of the disease (Shomali et al., 2017; Tang et al., 2013a). Some of the studies showed that miRNAs play a crucial role in the prevention of proliferation and metastasis of cancer cells. Also, they are responsible for increasing of the sensitivity of cancer cells to chemotherapy(Ni et al., 2017; Tang et al., 2013b). In this regard, miR-200c is one of the mir-200 family that is mainly down-regulated in gastric cancer (Feng et al., 2014). Also, studies show that miR-200c can be effective in the reduction of chemoresistance (Chang et al., 2014; Jiang et al., 2017; Liu et al., 2017b). Many reports revealed that miR-200c plays a vital role in the metastasis and apoptosis processes of cancer cells by impacting of different genes, such as FAP1, FN1, ZEB1, ZEB2 and E-cadherin. In other words, down-regulation of miR-200c is strongly related to the proliferation and metastasis of cancer cells [3,9,12]. Another category of the studies indicated that miR-200c has an essential role in chemotherapy resistance. It is suggested that by increment of miR-200c expression in the cancer cells, the response of cancer cells to the chemotherapy would be improved (Heydari et al., 2018; Jiang et al., 2017; Liu et al., 2017b). In our study further reavled that miR-200c could enhance the sensitivity of gastric cancer cells to cisplatin and also, demonstrated miR-200c involvement in the regulation of cell proliferation, migration and apoptosis by targeting relative genes such as VEGFR, MMP9 and RhoE.

Materials and methods

Cell culture and transfection

Human gastric cancer cell line (KATO III) was purchased from Pasteur Institute (Tehran-Iran). The cell line was maintained in the supplemented RPMI-1640 with 10% fetal bovine serum (FBS), penicillin and streptomycin. RPMI-1640, penicillin and streptomycin, were obtained from Gibco (Gaithersburg, Maryland, Md.) The cells were incubated at 37 °C in a humidified atmosphere of CO2 (5%) in the air. The nucleotide sequence of miRNA-200c (CGUCUUACCCAGCAGUGUUUGG) was achieved from (http://www.nchi.nlm.nih.gov).Also, hsa-miR-200c and anti-miR-200c oligonucleotides were obtained from (Microsynth AG, Switzerland). In this manner, KATO III cells were seeded in a 6-well culture plates at a density of 10^5 cells/ml per well and then incubated for 24 h. After 24h of incubation ,wells washed with sterile PBS. Also, added 1000 µl of Antibiotic-free medium and no FBS (Optimum) to each well, then KATO III cells were transfected with miR-200c mimics using jetPEI1 protocol (Polyplus, Germany).

Quantitative RT-PCR

RNA was isolated from the harvested cells with Trizol reagent according to the manufacturer's instruction (Riboex, Gene All Biotechnology,Seoul,Korea) Then, the total concentration of RNA was measured by using a NanoDrop (Thermo Scientific,USA). The samples with optical density (OD) ratios of 1.5-1.9 were used for further experiments. To the synthesis of cDNA, cDNA synthesis kit(Biofact, South Korea) was used. To this purpose, 1- 0.5µg of total RNA of each sample,0.5 µl of random hexamer primer, 0.5µl of Oligo(dt) Primer,10µl of RT Pre-MIX to microtube and added RNase free water to the final volume reach 20µl. Subsequently, microtubes were placed into a thermocycler for 30 minutes at 42 ° C and for 5 minutes at 85 ° C. Real time-PCR was conducted with SYBR green master mix and specific primers of miR-200c, MMP9, VEGFR, PTEN, and RhoE (Table 1). The expression level of mRNA was measured by light cycler 96 (Roche, Germany) and reported by the $2^{-\Delta\Delta CT}$ method.

Table 1.The primers sequences

primer	**Target sequence**
has-miR-200c	CGUCUUACCCAGCAGUGUUUGG
RhoE	Forward: GTGGGAGACAGTCAGTGTGG Reverse: CACAGCATCCGAATCAGGGT
MMp9	Forward: TTGACAGCGACAAGAAGTGG Reverse: GCCATTCACGTCGTCCTTAT
PTEN	Forward:CAGCTTGGGGATAAGTGTCACG Reverse: ACAGCTAGGACGCGATCAC
VEGFR	Forward:CAAGACAAGAAAATCCCTGTGG Reverse: CCTCGGCTTGTCACATCTG

Western blot analysis

After 48h of the transfection with miR-200c mimic, KATO III cells were lyzed in RIPA buffer (25 mM Tris HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS). Then, pellets were washed twice with cold PBS,and following that the cell lysates were mixed with 25 macrogeram of the protein sample buffer (10% Glycerol, 50 Mm Tris pH 8.6, 2% SDS, 1% Bromophenol blue, and 100 mM DTT) . Next, the samples were incubated for 5 min in a boiling water bath, and the samples were resolved by 12% SDS-PAGE. After transferring of detached proteins to an activated polyvinylidene difluoride membranes (Roche Diagnostics GmbH), the blots were incubated overnight at room temperature in blocking buffer (0.5% Tween 20). After that, goat polyclonal antibodies was used specifically for MMP9, VEGFR primary antibody (1:1000, sc-14700, Santa Cruz Biotechnology) and B-actin (1:1000, monoclonal antibody, Abcam). After washing, the blots were incubated with (HRP) the conjugated rabbit anti-goat secondary polyclonal antibody (1:1000, Santa Cruz Biotechnology). ECL kit (Roche Diagnostics GmbH) was used for signal detection. Analyses of the band intensity were performed by using of the ImageJ 1.63 Software.

Cell viability assay

The cell viability was detected by MTT assay. Firstly, MTT assay was used to assess the rate of IC50 (50% of inhibition) value for cisplatin and miR-200c. After obtaining of the IC50 values for cisplatin and miR-200c , individually. Subsequently, cells transfected with miR-200c and treated with different doses of cisplatin. In this way, after 24h of the transfection, different doses of cisplatin with ranging from 0.25 to 100 μM (Mylan united states) were added in to the each transfected wells. The cell viability was analyzed by MTT assay kit (Sigma). All experiments were carried out in triplicate.

Migration assay

Migration assay was determined by using 24 well plates (pore size, 8 mm; SPL life science, South Korea) in accordance with the manufacturer's instructions. Briefly, 10×10^4 of the cells were seeded in the plates. After 24h of incubation at 37˚C, the cells were divided into the four groups which includes control, miR-200c, cisplatin and combined miR-200c / cisplatin groups. The cells were seeded in 24-well plates at a 300,000cells/well density and yellow pipette tips (10–100 ml) was used to build an artificial gap. Then, the plates were incubated in 37˚C for 72h. This time frame would allow the cells to migrate into the scratch area. Later on, the images were captured from the wells for each group. The images were captured from time 0 to 72h by the inverted microscope (Optika, Italy).

Apoptosis analysis

Apoptosis was measured by AnnexinV/PI assay. In this way, the cells were seeded in to 6-well plates (SPL life science, Korea) at a density of (2×10^5) cells per well. The cells were divided into four groups, including: control, miR-200c, cisplatin and combined miR-200c / cisplatin groups. F Briefly, the treated cells were trypsinized and centrifuged at 1500 g for 5 min. Then, the cells were stained with annexin V and PI according to the manufacturer's instructions (Roche, Germany). For AnnexinV staining, 5μL of Annexin V, 5μL of propidium iodide and 200 μL of binding buffer were added to the samples. Next, they were incubated for 15 min at room temperature under the dark condition. Then the samples were analyzed by flow cytometer instrument (milteny Biotec ™ FACS Quant 10, Germany). The rate of apoptosis was assessed by Flowjo software (Tree Star, San Carlos, Calif.)

DAPI staining

The cells were seeded in to 96-well plates (SPL life science, Korea) at a density of (15×10^3) cells per well. Four groups were established (control, miR-200c, cisplatin and miRNA-200c + cisplatin). In the following two cluster of cells transfected with miR-200c (Sigma-Aldrich.USA) 24 h after transfection, cells were treated with cisplatin in two groups (cisplatin and miRNA-200c + cisplatin). Cells were incubated for 24h at 37°C then washed with PBS three times. cells were first fixed with 4% paraformaldehyde for 20 min after that cells washed with PBS and permeabilized with 0.3% Triton X-100 for 10 min, after that cells were incubated with 10 μg/mL DAPI for 10 min at darkness, Then wells washed with PBS and were analyzed with a Cytation 5 Cell Imaging Multi-Mode Reader(BioTek,USA)

Cell cycle

Kato III cells were seeded in to 6-well plates (SPL life science, Korea) at a density of (2×10^5) cells per well. In following four groups were determined: control, miRNA-200c, cisplatin and miRNA-200c + cisplatin .Cells were transfected with miR-200c (Sigma-Aldrich.USA) 24 h after transfection, cells were treated with cisplatin in two groups(cisplatin and miRNA-200c + cisplatin) in flowing cells were further incubated for 24 h. Floating and adherent cells were harvested and washed with phosphate buffer saline (PBS), and fixed with 70% ethanol overnight .After that cells stained with 50 μg/ml of DAPI for DNA content analysis by flow cytometry analysis on a FACS Calibur system (milteny biotec ™ FACS Quant 10, Germany). The data were processed using the FlowJo FACS analysis software (Tree Star, San Carlos, Calif.).

Colony formation

About 1000 cells per well were seed to a 6-well culture plate. In this way, KATO III cells transfected and treated with miR-200c and cisplatin Kato III cells were kept in 37 °C incubator with 5 % CO_2 for 10 days and media exchange every 3 days. After ten days incubation, the cells were washed with PBS and stained with 0.5 % crystal violet solution, After 20 min ,number of colonies was counted using an inverted microscope (Optika, Italy).

Autophagy

Kato III cells were seeded in to 6-well plates (SPL life science, Korea) at a density of (2×10^5) cells per well. In following four groups were determined: control, miRNA-200c, cisplatin and miRNA-200c + cisplatin. Cells were transfected with miR-200c (Sigma-Aldrich.USA), 24 h after transfection, cells were treated with cisplatin in two groups(cisplatin and miRNA-200c + cisplatin) then incubated for 24 h. In following cells washed 3 times with PBS to remove the media and added 1000 µl of monodansylcadaverine (MDC) 0.05% to each well at and incubated for 10 min at 37 °C. After incubation, the cells are washed with PBS and detached by trypsin/EDTA and centrifuged. Subsequently, samples immediately analyzed by flow cytometer instrument (milteny biotec ™ FACS Quant 10, Germany) and FlowJo FACS analysis software (Tree Star, San Carlos, Calif.)

Statistical analysis

All statistical analyses (except AnnexinV/PI assay) were performed by Graph Pad Prism version 6.00. All values were expressed as mean ± standard deviation (SD) and the experiments were performed in triplicate (n=3). Student's T-test and ANOVA were used to determine the statistical significance of inter-group differences. $P<0.05$ was considered to show a statistically significant difference. Flow Jo 7.6.1software was also used for analysis of the annexin V- PI data.

Results

miR-200c mimic sequence induced the level of miR-200c in gastric cancer cells

The expression of miR-200c was detected by qRT-PCR. The result described that the expression levels of miR-200c were significantly increase in dose-dependent manner in the transfected cells (Figure 1). Transfection 5, 7.5 and 10 nmol concentrations of miR-200c, increased expression level in quantities 1.8±0.3, 3.8±0.5 and 6.5±0.4 compare to negative control, respectively. In addition, anti-miR-200c could reduce the level of miR-200c in dose-dependent manner (Figure 1).

The replacement of miR-200c and LNA-anti-miR-200c altered expression level of MMP9, VEGFR, PTEN, and RhoE in KATOIII cell line

RT-PCR was used to detect the expression levels of MMP9, VEGFR, PTEN and RhoE, in KATOIII cells. After transfection of the miR-200c mimics into KATOIII cells, a marked reduction was revealed in the levels of VEGFR, MMP9and RhoE compared to the anti-miR-200c and control groups. While the transfection of miR-200c cloud up regulate the expression of PTEN as a tumor suppressor The mRNA expression levels of MMP9, VEGFR, and RhoE were decreased to 3.3±0.2, 1.2±0.15, 1.5±0.28 and 25±0.02, respectively. miR-200c also inhibited MMP9, VEGFR protein levels. β-actin was used as a loading control (Figure 2).

Combination of miR-200c and cisplatin decrease cell viability in KATO III cells in vitro

To clarify whether miR-200c and cisplatin could affect on the cell proliferation or apoptosis in KATO III cells, MTT assay was performed to be identify the IC50 value of cisplatin (IC50 = 0.6916). The results indicated that the transfection of miR-200c could reduce the IC50 value of cisplatin (IC50 = 0.1405). Therefore, it was shown that the transfection of miR-200c and cisplatin in combination caused a significant difference in the cell proliferation (Figure 3). These data demonstrated that over expression of miR-200c and cisplatin simultaneously could suppress the proliferation rate of KATO III cells.

miR-200c and cisplatin together inhibit gastric cancer cell migration strongly

Wound healing assays were used to detect the effects of miR-200c and cisplatin on the invasion and migration of KATOIII cells in the in vitro condition. These data suggest that miR-200c and cisplatin probably are able to inhibit invasion and migration of KATOIII cells, however the combination of them could remarkably improve their effects (Figure 4).

miR-200c replacement could sensitize the gastric cancer cell to apoptosis in combination to cisplatin

To understand the effects of miR-200c, cisplatin, and combination miR-200c/ cisplatin on apoptosis, annexinV/PI assay was performed. The percentage of apoptosis rate in the miR-200c, cisplatin and combination miR-200c/cisplatin was estimated to be 2.25±0.4, 6.44±0.3 and 43.44±0.6, respectively (Figure 5). Together, these results indicated that the overexpression of miR-200c could reduce the resistance of KATO III cells to cisplatin (Figure 5B).

Combination of miR-200c with cisplatin significantly induced apoptosis

To confirmed the effect of miR-200c on the apoptosis of KATO III cells, apoptosis rate was further accessed by DAPI staining. These data demonstrated that replacement of

miR-200c and cisplatin simultaneously could increase the apoptosis rate of KATO III cells in compared with other groups (Figure 6).

Combination of miR-200c/cisplatin regulates KATO III cell cycle progression

To further explore the role of miR-200c and cisplatin in promoting cell proliferation, we examined cell cycle distribution after transfection with miR-200c and treated with cisplatin in KATO III cells by flow cytometry. Our data indicated that miR-200c with cisplatin could enhance accumulation of KATO III cells in G2 phase, Actually the combination of miR-200c with cisplatin could induce cell cycle arrest in the G2 phase (Figure 7).

MiR-200c/ cisplatin inhibits colony formation of KATO III cells

To further characterize the function of miR-200c in the KATO III, we examined the effects of miR-200c, cisplatin and combination of miR-200c/cisplatin in the KATO III cells about colony formation. Results showed that, the cluster miR-200c/ cisplatin caused colony formation suppression in compered with other groups (Figure 8).

MiR-200c expression did not significantly change on autophagy in KATO III cells

In order to determine autophagy induction, we used detecting labeled cells with MDC by flow cytometry. The percentage of autophagy rate (MDC POS) in the miR-200c, cisplatin and combination miR-200c/cisplatin were estimated to be 1.01, 1.76 and 3.75, respectively. Together, these results indicated that combination of miR-200c/cisplatin could induced autophagy in KATO III cells in compared with control group but did not significantly alter the rate of autophagy (% 3.75) (Figure 9).

Discussion

Finding an effective treatment for cancer treatment has always been a concern in the world. Many studies indicated that the changes of miRNAs expressions have a crucial role in cancerous tissues. Therefore regulation of miRNAs expressions could be considered as a new therapeutic approach (Lei et al., 2019; Mansoori et al., 2017b; Shirafkan et al., 2018). Furthermore, chemotherapy is a common therapeutic strategy for cancer treatment, but resistance to the chemotherapy drugs is an obstacle to this therapeutic strategy. As previously reported, miR-200c plays an impressive role in the chemoresistance of different types of cancers (Gao et al., 2016; Jiang et al., 2017; Liu et al., 2017b) . In the present study the combined effect of miR-200c transfection and cisplatin was investigated. Our data indicated that miR-200c acts as an oncosuppressive microRNA in gastric cancer cell line by targeting specific genes. The result of MTT assay and DAPI staining indicated that simultaneous use of miR-200c and cisplatin had a positive effect on the increasing of apoptosis rate and also inhibit significantly the cell

growth. These data suggested that miR-200c is able to enhance the function of cisplatin in apoptosis induction through suppressing of expression of the related genes such as RhoE. RhoE as a member of GTPase family that is involved in different cellular processes, including migration and apoptosis. Also ,according to the previous studies, RhoE is a factor that contributes to the resistance of chemotherapy drugs and up-regulates the resistant of gastric cancer(Chang et al., 2014; Li et al., 2009) From the results, it is concluded that the overexpression of miR-200c can down-regulate the expression of RhoE in the transfected cells. Therefore, knocking down of RhoE as a potential target for miR-200c can restore the sensitivity of the cells to cisplatin. Wei Zhu and et al in 2011 investigated about the role of miR-200bc/429 cluster in the elimination of drug resistance and apoptosis induction in human gastric and lung cancer cell lines ,results showed, simultaneous use of miR-200bc/429 cluster with cisplatin,5-fluorouracil enhanced apoptosis rate (Zhu et al., 2012). In another study, Liu and et al. (2017) showed the inhibitory effect of the combined taxol with miR-200a on gastric cancer by targeting of β -catenin(Liu et al., 2017a). In contrast, some studies are in contradiction with these results, a study in 2011 indicated that inhibition of miR-200c expression could raise the chemosensitivity of esophageal cancer cells to cisplatin and enhanced apoptosis rate in cancer cells (Hamano et al., 2011). Many reports confirmed the positive effect of combination therapy of miR-200c with chemotherapy drugs in the inhibition of migration, proliferation and restoring of drug resistance by the target genes (Chang et al., 2015; Ma et al., 2015; Mutlu et al., 2016). Zhou and et al. showed that the overexpression of miR-200c could down-regulate ZEB1/ZEB2 and resensitize drugs resistance, in gastric cancer (Zhou et al., 2018). In Zhou and colleagues Study, miR-200c transfection intensify gefitinib sensitivity. Also, miR-200c significantly down-regulated ZEB1expression and inhibited invasion of non-small cell lung cancer by suppression of PI3K/Akt signaling pathway(Zhou et al., 2017). The obtained results of wound assay suggested that the migration ability of cancer cells was considerably reduced in the transfected cells with combined miR-200c /cisplatin groups, also combination therapy markedly reduced the cells ability to grow and colony formed. These findings demonstrated that there is a correlation between the expression level of miR-200c and effectiveness of cisplatin in the inhibition of colony formation and migration of cancer cells.

Data from qRT-PCR analysis indicated that miR-200c transfection decreases the expression level of oncogenes, such as VEGFR, MMP9 and RhoE. It is reported that the down-regulation of miR-200c in KATO III cells is correlated to the loss of PTEN expression. Western blot analysis confirmed that miR-200c can suppress VEGFR, MMP9 protein levels and also could inhibit the migration of KATO III cells. According to the studies, VEGFR, MMP9 genes are involved in the invasion process of different cancers. It is known that the MMPs and VEGF signaling networks such as PI3K–Akt are involved in the migration of cancer cells. VEGFRs and MMPs for can promote malignant

phenotypes of cancer cells because of angiogenesis stimulation ability, that leads to metastasis(Brown and Murray, 2015; Egeblad and Werb, 2002; Hicklin and Ellis, 2005). Also, reduced level of PTEN as a suppressor tumor gene is associated with the invasion and poor survival rate in various cancers. Losing of PTEN functionality could promote the migration rate through (PI3K)/Akt signaling. Studies have shown that the reduced level of PTEN also causes to the resistance of chemotherapy (Chen et al., 2013; Kang et al., 2002; Lee et al., 2003; Soubani et al., 2012).

Multiple studies suggests that microRNAs (miRNAs) play an important role in cancer cell cycle arrest(Bian et al., 2012; Christensen et al., 2013) The expression and function of miR-200c in cell cycle, however, have been investigated in different human cancer(Schubert and Brabletz, 2011; Zheng et al., 2017). To determine the function of miR-200c in promoting cell proliferation in gastric cancer, we investigate cell cycle diffusion after transfection with miR-200c in KATO III cells. Compared with control cells, combination of miR-200c transfected / cisplatin- treated cells showed a substantial increased in G2 phase. Our data suggest that inhibition of gastric cell growth by miR-200c may be interfere through induction of G2 arrest. Autophagy occurrence in cancer is different and depends on tumor type At present, the role of autophagy in cancer cells is not well determine Therefore, it is interesting to investigate whether miR-200c has any effect on autophagy, because, autophagy can also have effect chemotherapy agents(Sui et al., 2013). In this regard, we investigated autophagy an important factor for cancer treatment or apoptosis induction. Our result from autophagy assay didn’t show significant change after transfection and treated with miR-200c and cisplatin. In the present study miR-200c appeared to participate in developing efficacious effect of cisplatin and suppresses the proliferation and migration of the cells via the suppression of VEGFR, MMP9 and RhoE and increasing the expression of PTEN as tumor suppressor gene.

Conclusions

In conclusion, our data showed miR-200c could inhibit gastric cancer cells growth, cell cycle and migration in vitro through down-regulating the expression of invasion-related factors such as VEGFR, MMP9. Also, miR-200c could improve the sensitivity of gastric cancer cells to cisplatin via targeting RhoE. Therefore, more researches are needed about combination therapy as a new therapeutic approach.

Conflict of Interest

All the authors declare no conflict of interest.

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Figure 1. miR-200c levels were induced after transfection of mimic sequence. The level of miR-200c evaluated by qRT-PCR. The data signify mean ± SD **P<0/001, ****P<0/0001 versus control cells.

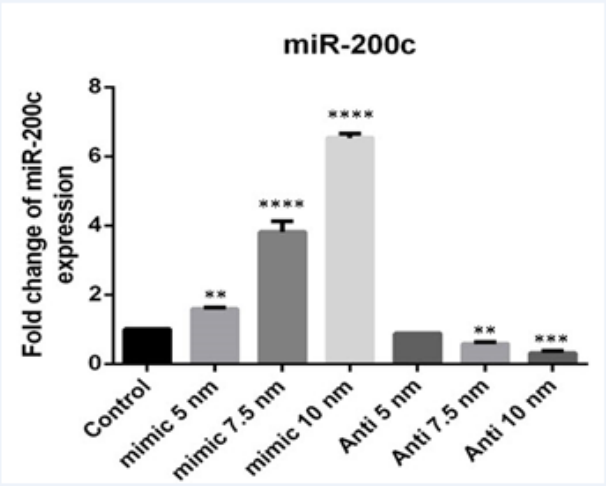

Figure 2. miR-200c regulated the MMP9, VEGFR, PTEN, and RhoE mRNAs in the gastric cancer cell line (Kato III). The qRT-PCR analysis of MMP9, VEGFR, PTEN, and RhoE mRNA were evaluated in the miR-200c, anti-miR-200c and, control groups.miR-200c suppressed the protein levels of MMP9, VEGFR, tumorgenesis potential and migration.The data represent mean ± SD (n=3); *p<0.05, **p<0.01, and ****p<0.01 versus control cells.

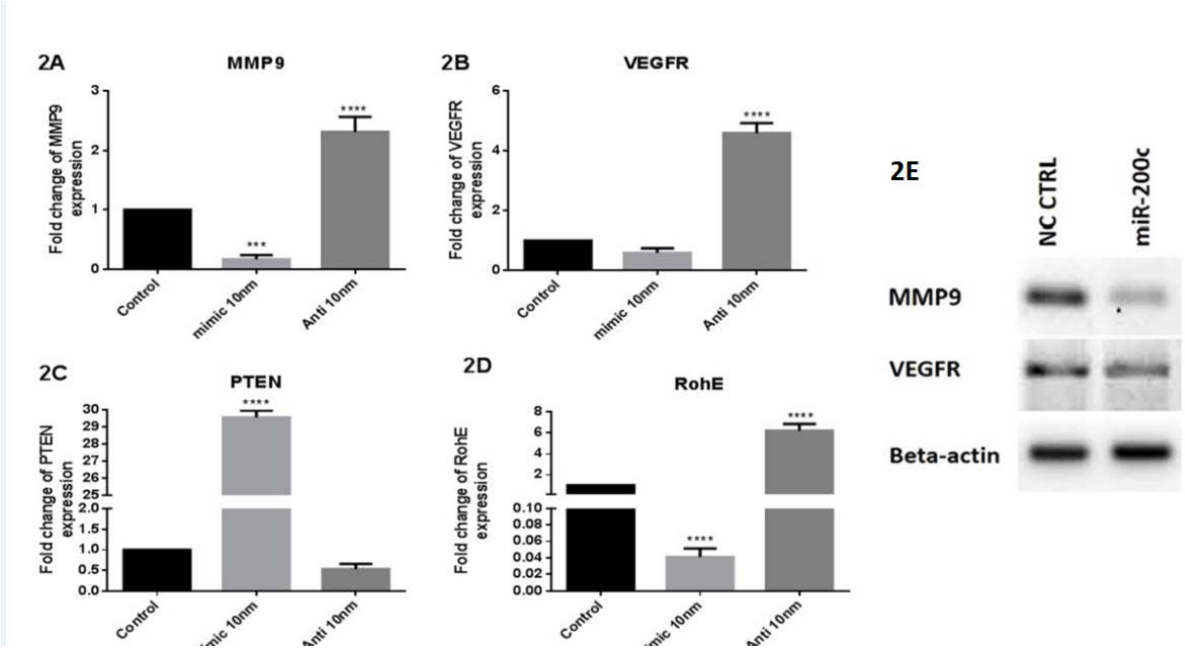

Figure 3. Effect of miR-200c /cisplatin on KATO III cells viability. KATO III cells were treated with a various doses of miR-200c / cisplatin for 24, 48h and then their viability was examined by MTT assay.

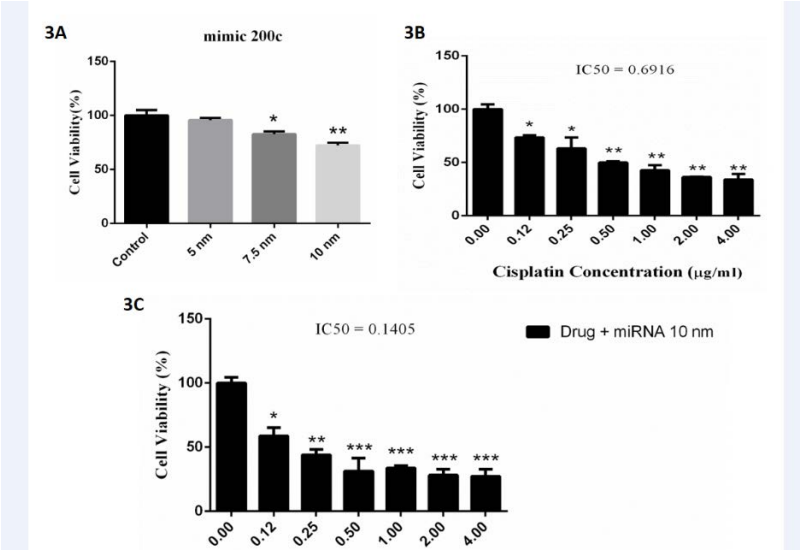

Figure 4. Effect of miR-200c / cisplatin on KATOIII cells migration. Representative image of the transfected and untransfected cells that were treated with cisplatin at time of zero, 24, 48, and 72h after treatment.

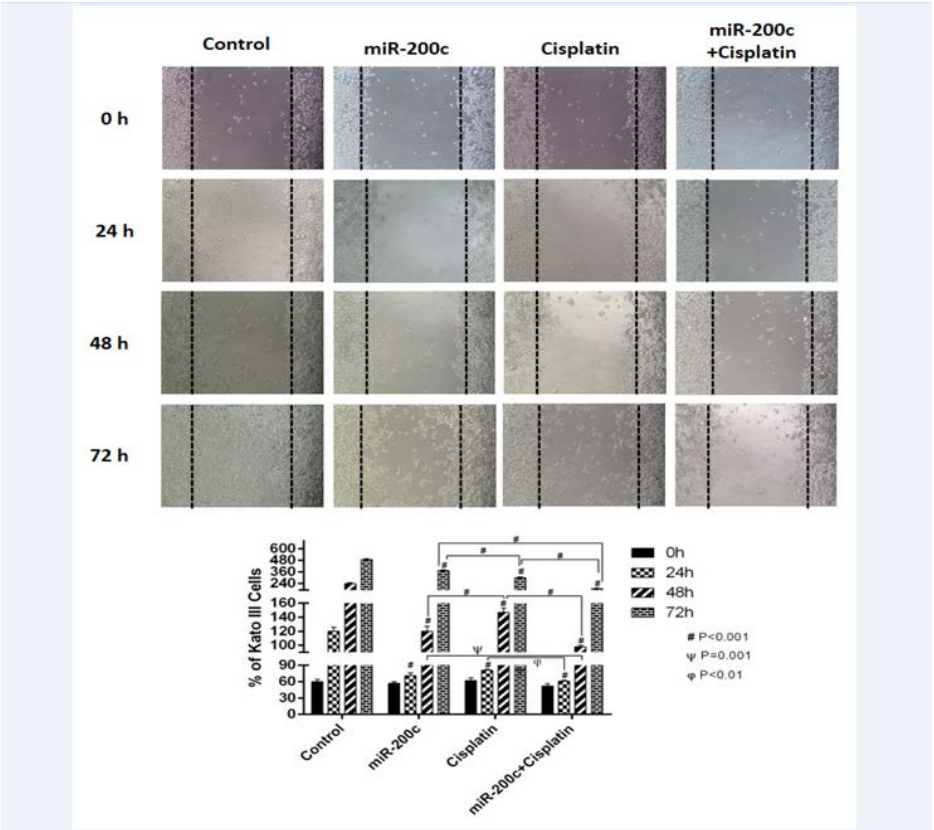

Figure 5. The combined effects of miR-200c/cisplatin on KATO III cells apoptosis. Transfected KATO III cells and untransfected cells were treated with cisplatin. Apoptosis was quantified using Annexin V-PI staining.

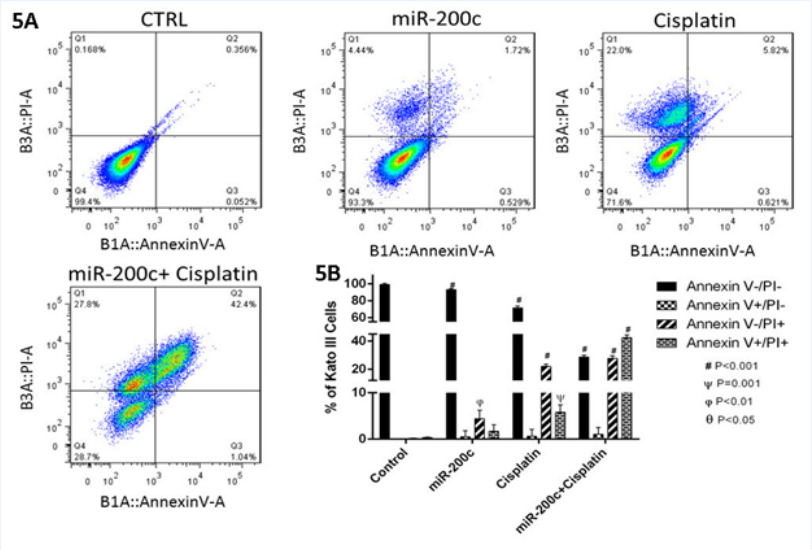

Figure 6. DAPI staining. miR-200c overexpression increased apoptosis rate in KATO III cells. The percentage of apoptotic cells were determined by citation 5. White arrows indicate apoptotic cells in different groups.

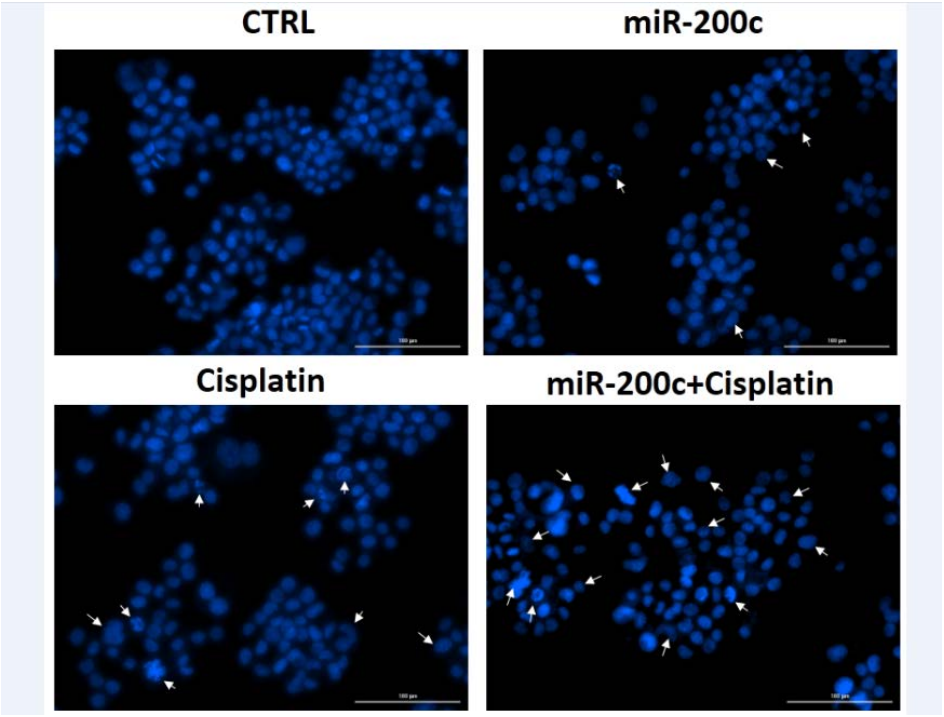

Figure 7. Analysis of cell cycle KATO III cells after transfection of miR-200c and treatment with cisplatin. 24 h after treatment with cisplatin (transfected and un transfected) KATO III cells were stained with DAPI and cell cycle was assessed by flow cytometry. The percentage of cells in different phases of cell cycle were displayed on bar diagram. miR-200c /cisplatin could result in increased accumulation of KATO III cells in G2/M phase(34.4%). The data represent mean ± SD (n=3); *p<0.05, **p<0.01, and ****p<0.01 versus control cells.

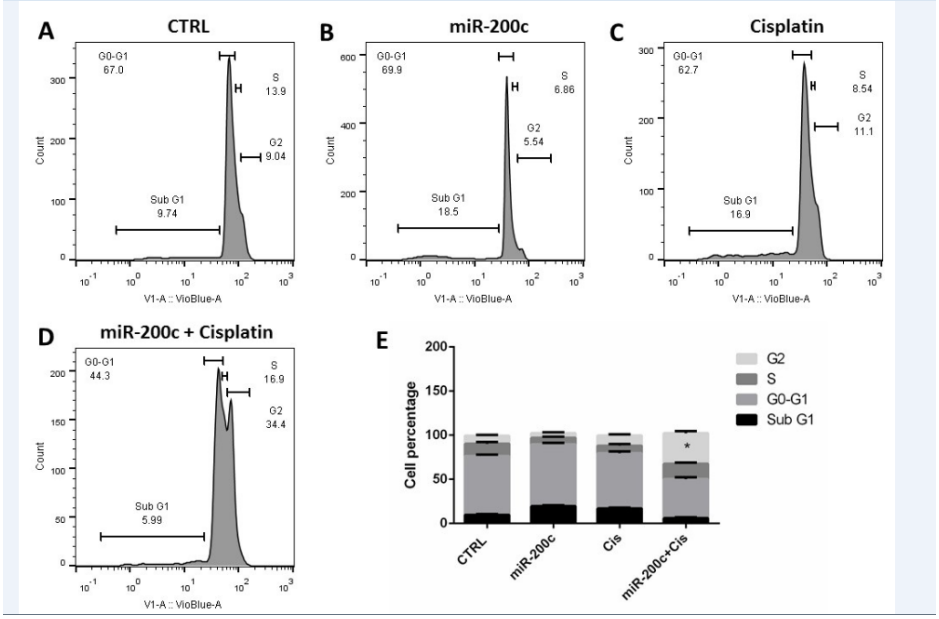

Figure 8. KATO III colony formation assays were performed in four cluster (control, transfected with miR-200c,cisplatin-treated cells, cisplatin/miR-200c).Number of colony decreased after miR-200c transfection or cisplatin-treated cells).Consistent with these data colony formation rates were significantly low in miR-200c/cisplatin cluster in compared with control group.

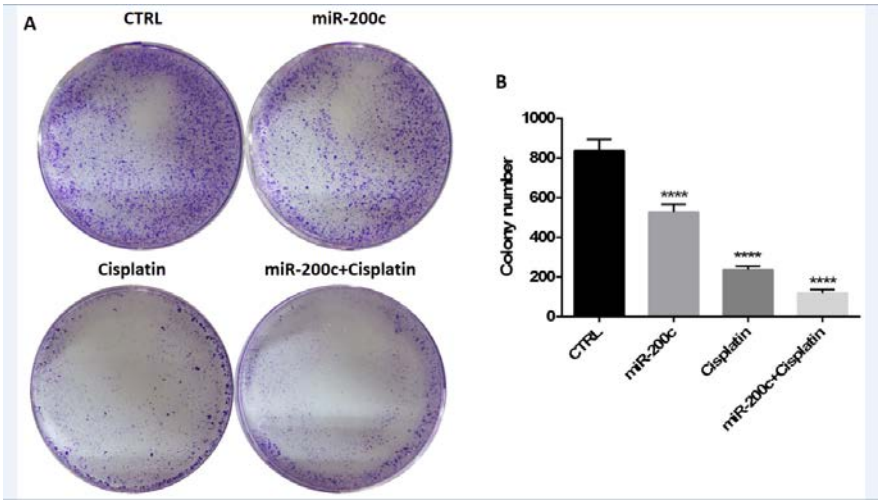

Figure 9. These cells labeled by autophagic marker monodansylcadaverin (MDC). Analysis of autophagy by flow cytometry after transfection and treatment didnt show significant change. The data represent mean ± SD (n=3); *p<0.05, **p<0.01, and ****p<0.01 versus control cells.