# MiR-200c Sensitizes Breast Cancer Cells to Carboplatin Treatment by Decreasing MDR1 Expression

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**Short Report**

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# Abstract

**Background:** Breast cancer (BC) is one of the most common cancers worldwide and is associated with a high rate of cancer mortality in women. Resistance to chemotherapy is considered a significant problem and a major challenge for the treatment of patients with BC. miR-200c belongs to a family of miRNAs that act as tumor inhibitors. The expression level of miR-200c has been reported to be decreased in cancers, especially in BC. The increased miR-200c expression can be considered as a potent inhibitor of drug resistance and tumor progression.

**Methods and Results:** The current study examined the effect of miR-200c on enhancing the BC cells' sensitivity to Carboplatin through targeting MDR1 expression. To perform functional analyses, mimic miR-200c transfected to MCF7 cells. Then, the viability of the cells was investigated via MTT assay. Finally, the expression of associated genes assessed using qRT-PCR. The results indicated that downregulation of miR-200c was occurred in MCF7 cells in comparison to control. Besides, restoring miR-200c expression by regulating the expression level of the apoptotic gene reduces the viability of cancer cells. Moreover, miR-200c increased the sensitivity of MCF7 cells to Carboplatin via reducing the MDR1 gene expression.

**Conclusions:** This study provided valuable data showing that miR-200c enhances the effect of carboplatin as a clinically approved chemotherapeutic agent, and restoring its expression could be considered as a promising targeted adjuvant therapy for BC management.


# Introduction

Breast cancer (BC) is a widespread malignancy in females globally, causing the highest mortality and morbidity in this gender [1]. The development of new treatments has led to an increase in patients' survival rate with BC [2]. The first treatment of BC to prevent this cancer's relapse is often removing the tumor by surgery. When the disease is adequately advanced, there are several adjuvant treatments for BC patients, including a diversity of regimens related to chemotherapy and radiotherapy [3, 4]. Despite the significant advances in BC treatment and its early diagnostic, half of the patients' resistance to chemotherapy agents or failure to respond to them is considered a challenge in this cancer. Chemotherapy, as a pivotal approach, is efficient in the treatment of metastasis BC [5]. Carboplatin is a cisplatin derivative that is similar in function but differs in structure. It is widely used as monotherapy or in combination with other chemotherapy drugs to treat various cancers, comprising the neck, head, breast, ovary, and small cell lung cancer (SCLC) [6]. This drug targets DNA and, after binding, inhibits replication and transcription as well as induces apoptosis in tumor cells [6]. MicroRNAs (miRNAs) regard as short non-coding RNAs that form a new gene regulators group. They have a crucial function in regulating various processes like cell cycle, tumor invasion, and cell proliferation, and cell death [7, 8]. According to multiple studies, the miRNAs' role in almost all types of cancer is determined [9]. It is well-known that the initiation and development of many human cancers are related to miRNAs. For instance, the changes in the expression and regulation of miRNAs resulted in BC tumor development [10, 11].

miRNAs are classified into two categories of tumor-suppressors with decreased expression and oncomiRs with increased expression levels in cancers [12]. There are 5 members related to the family of miR-200, which are: miR-429, miR-200a, -b, -c, and miR-141, of these; miR-200c has been extensively examined in cellular sensitivity to chemotherapeutic drugs and also a tumor suppressor [13, 14]. In various cancers, the dysregulation of miR-200 members has been illustrated like gastric, lung, bladder, and breast cancer. It has been indicated that the increased miR-200c expression is a powerful inhibitor of drug resistance and advancement of the tumor [15–17]. In a study by Kopp and colleagues concerning the effect of the increased expression of miR-200c on the sensitivity of BC cells to Anthracyclines, they showed that increasing microRNA expression not only decreased the TRKB and BMI1 genes expression but also increased the treatment efficacy of Doxorubicin [13]. Another study of the effect of miR-200c on the triple-negative MDA-MB-231 BC demonstrated that increased miR-200c expression reduced the KRAS oncogene expression and inhibited the proliferation of cancerous cells. Besides, another study showed that the KRAS gene is suppressed by overexpression of miR-200. Accordingly, colony-forming capability also the proliferation of BC cells are inhibited in vitro and in vivo [18]. In this study, we indicated that miR-200c could increase BC cell's sensitivity to Carboplatin by targeting the relevant genes, including MDR1, BAX, Bcl2, and caspase-3.

# Materials And Methods

### *2.1. Cell culture*

Initially, the MCF7 cell line was obtained from the cell bank at the Pasture Institute (Tehran, Iran) and was grown in RPMI-1640 medium comprising 10% fetal bovine serum (FBS). The cells were then incubated in a humidified atmosphere of 95% at 37°C and containing 5% $CO_2$.

### *2.2. MiR-200c transfection*

To the transfection of microRNA, MCF7 cells were at first seeded in a 6-well plate (the seeding density: $5\times10^5$ cells per well). miR-200C was then transfected by electroporation by the BioRad guideline. miRNA was transfected to the final concentration of 10 nm, selected according to the previously reported research [19]. After transfection, the cells were incubated for 24 h at 37°C.

### *2.3. MTT assay*

To assess viability, transfected and control (not transfected) cells were seeded in a 96-well plate (the seeding density: $15\times10^3$ cells per well) and incubated at 37°C for 24 hours. The cells were then treated with Carboplatin for 24 hours. MTT (Sigma-Aldrich, USA) assay was performed to evaluate the viability of the cells. In short, 5 mg/ml of the MTT was dissolved in PBS, and cells were treated with 20 µl of the solution. After removal of the medium, 200µl dimethyl sulfoxide (DMSO) was added into wells. The absorption of wells at 570 nm was evaluated using a spectrophotometer. The following formula was used to calculate the ratio of viable cells.

### *2.4. Quantitative real-time PCR*

According to the constructor's protocol, the TRIZOL reagent (geneAll biotechnology Seoul, Korea) was used to isolate the cells' total RNA. Then, the measurement of total RNA concentration was conducted by the Thermo Scientific NanoDrop instrument. The RNA sequence was then reverse-transcribed to cDNA using the miScript II RT Kit (obtained from QIAGEN, Hilden Germany) for miRNA and other genes (BIOFACT, Korea). Quantitative reverse transcription PCR was conducted using miScript SYBR Green Kit (QIAGEN, Hilden, Germany). GAPDH was used as a housekeeping gene for Caspase-3, Bcl-2, MDR1, and BAX genes. The expression level of mRNA was evaluated by the $2^{-\Delta\Delta ct}$ method. The primer sequences are reported in table 1.

### *2.5. Statistical analysis*

analysis of statistical was accomplished on GraphPad Prism 6 software. All values were expressed as mean ± standard deviation (SD). Statistical differences between treated and control cells were performed using one-way, and two-way ANOVA followed by Sidak and Tukey tests. P-values < 0.05 were depicted to display a statistically significant difference. Each experiment was conducted triple times independently.

## Results

### *3.1. Combination of Carboplatin and miR-200c reduced cell viability in MCF7 cells in vitro*

To determine the effect of Carboplatin and miR-200c on the proliferation and cell death of MCF7 cells, an MTT assay was carried out to detect the Carboplatin IC50 value (IC50 = 222.6 ug/ml). The finding revealed that the Carboplatin IC50 value (IC50 = 139.4 ug/ml) was reduced through the transfection of miR-200c. Therefore, it was shown that the combined use of miR-200c and Carboplatin decreases the proliferation rate of MCF7 cells (Figure 1 A and B). These findings represented that miR-200c overexpression, in combination with Carboplatin simultaneously, could inhibit MCF7 cell proliferation.

### *3.2. The combination effect of Carboplatin and miR-200c on the expression levelof BAX, Bcl2, Caspase-3, and MDR1 genes in MCF7 cells*

QRT-PCR was used to evaluate the levels of BAX, Bcl2, Caspase-3, and MDR1 gene expression in MCF7 cells. In this regard, after the transfection of MCF7 cells with miR-200c, cells were treated with Carboplatin. The results demonstrated that the expression of genes like BAX and Caspase-3 was significantly increased in the cells which are treated with the combination of miR-200c and Carboplatin. Also, Bcl-2 and MDR1 gene expression was decreased in comparison to the control group. GAPDH was utilized as an appropriate housekeeping gene for the MDR1, Bax, Bcl2, and Caspase-3 (Figure 2).

## Discussion

Discovering a powerful method to treat cancer has been a major concern and challenge in the world. Many studies have revealed that miRNA expression changes have an essential function in cancers and cancerous tissues. Therefore, regulating miRNA expression can be considered a new treatment [20, 21]. Chemotherapy as a standard treatment, is used to treat cancer. However, one of the main challenges is resistance to chemotherapeutic agents. Recent studies have shown that miR-200c is an efficient factor in chemotherapy resistance in different cancers [22, 23]. In this investigation, we analyzed the simultaneous effect of Carboplatin and miR-200c in the MCF7 cell line of BC. Primary results showed that miR-200c functions as a tumor-inhibiting miRNA in BC via targeting specific genes. MTT assay results demonstrated that the survival rate of MCF7 cells after treatment with the combination of miR-200c and Carboplatin was significantly reduced compared to the carboplatin treatment and miR-200c transfection alone and in the control group. These findings indicated that miR-200c increases Carboplatin's function in inducing apoptosis via inhibition of the Bcl2 expression. Bcl-2 belongs to the family of Bcl-2 proteins which has a regulatory role and controls the death of cells via induction or inhibition of apoptosis [24]. Besides, Bcl-2 is the cause of cancer therapeutic resistance, and its negative expression as a marker represented that BC patients have an acceptable clinical response to chemotherapy [25]. Our results showed that miR-200c restoration downregulated the Bcl-2 expression in the transfected cells and inhibited the Bcl-2 expression as a possible target for miR-200c. By this causes resensitization of BC cells to Carboplatin. In a study, DM et al. represented that restoring the expression of miR-200c increased the ovarian cancer cells' response to Paclitaxel [26].

Moreover, in another research, Qian and colleagues reported that miR-200c mediates increased colorectal cancer cell's sensitivity to 5-FU by suppressing Bcl-2 expression [27]. Many studies have illustrated the effective and positive role of combination therapies with miR-200c and chemotherapeutic agents in inhibiting proliferation and increasing sensitivity to chemotherapy drugs by inhibiting target genes [7, 28]. Kopp et al. illustrated that miR-200c by targeting genes like Bmi1 and TrkB causes BC cells to become sensitive to Doxorubicin [13]. Besides, studies have shown that miR-200c counteracts Trastuzumab resistance of BC cells via inhibiting the pathway of TGF-β as well as targeting the ZNF217 and ZEB1 genes [29]. The results of QRT-PCR demonstrated that the levels of Bcl-2 and MDR1 gene expression are reduced by transfection of miR-200c.

Further, suppression of miR-200c in MCF7 (BC) cells was displayed to be associated with loss or reduced expression of BAX and Caspase-3. The BAX gene is part of the Bcl-2 family and acts as an apoptotic activator [30]. The Caspase-3 gene is also active in apoptotic cells via extrinsic and intrinsic pathways. Aberrant expression of caspases has been identified in various cancers. The presence of caspase-3 at a high amount has been reported in acute myelogenous leukemia (AML) compared with normal cells [31]. Also, low levels of caspase-3 have been shown in pancreatic cancer cells compared to normal tissues [32]. In BC patients, MDR1 expression widely has a significant role in chemotherapy resistance in the therapeutic approaches. Extensively, the function of MDR1 has been investigated in BC because chemotherapy drugs like Paclitaxel and Doxorubicin, which are universally used to treatment of BC, develop drug resistance [33]. In this study, miR-200c appears to increase Carboplatin's efficacy by

inhibiting proliferation and reducing drug resistance via inhibiting Bcl-2 and MDR1 gene expression also increasing BAX and Caspase-3 gene expression.

# Conclusions

In conclusion, the results of our study indicate that miR-200c could inhibit BC cell proliferation by reducing Bcl-2 expression. Besides, miR-200c can be efficiently enhanced breast cancer cell's sensitivity to Carboplatin by targeting MDR1. Therefore, further studies on combination therapy with miR-200c and Carboplatin are required to evaluate its efficacy as a new treatment.

# Declarations

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*Statement of Ethics*

This study was conducted in compliance with the ethical principles of Tabriz University of Medical Science, Tabriz, Iran and approved by the regional ethical committee for medical research.

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*Authors' contributions*

S.N. and B.B. devised the main conceptual ideas and participated in the design of the work. B.B. provided biological materials and reagents. S.N., S.S., M.A., and N.A. performed the experiments. S.N. and B.B. wrote the initial draft of the manuscript. A.M., N.B., and K.H. participated in the analysis of the work and reviewed and edited the manuscript. B.B. supervised the study.

*Availability of data and material*

The data that support the findings of this study are available from the corresponding author upon

reasonable request.

*Conflict of Interest statement*

The authors declare that there are no conflicts of interest.

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# Tables

**Table 1.** The primers sequences and miRNA used in the current study.

| Name | Strand | Sequences |
|---|---|---|
| *miR-200c* | Target sequence | 5′- UGGCAGUGUCUUAGCUGGUUGU-3′ |
| *U6* | F | CTTCGGCAGCACATATACTAAAATTGG |
| | R | TCATCCTTGCGCAGGGG |
| *GAPDH* | F | AAGGTGAAGGTCGGAGTCAAC |
| | R | GGGGTCATTGATGGCAACAA |
| *Caspase-3* | F | CAAACCTCAGGGAAACATTCAG |
| | R | CACACAAACAAAACTGCTCC |
| *Bcl-2* | F | GAGTTCGGTGGGGTCATGTG |
| | R | CACCTACCCAGCCTCCGTTA |
| *BAX* | F | TTTGCTTCAGGGTTTCATCCA |
| | R | TCTGCAGCTCCATGTTACTGTC |
| *MDR1* | F | TTCCGCTTCTTCGTCTGCTT |
| | R | TCTTGCCATCTTCCGACCAC |

# Figures

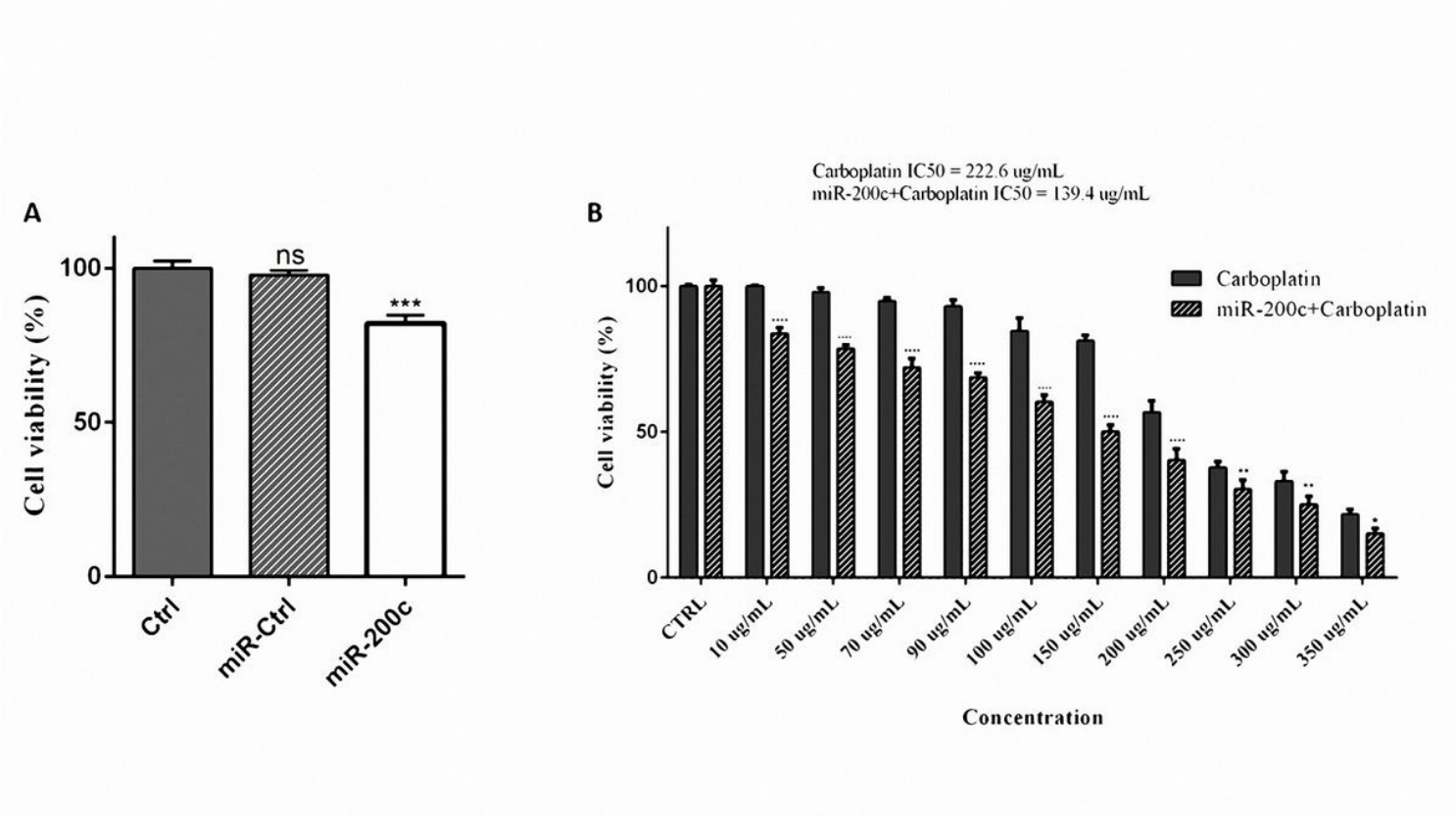
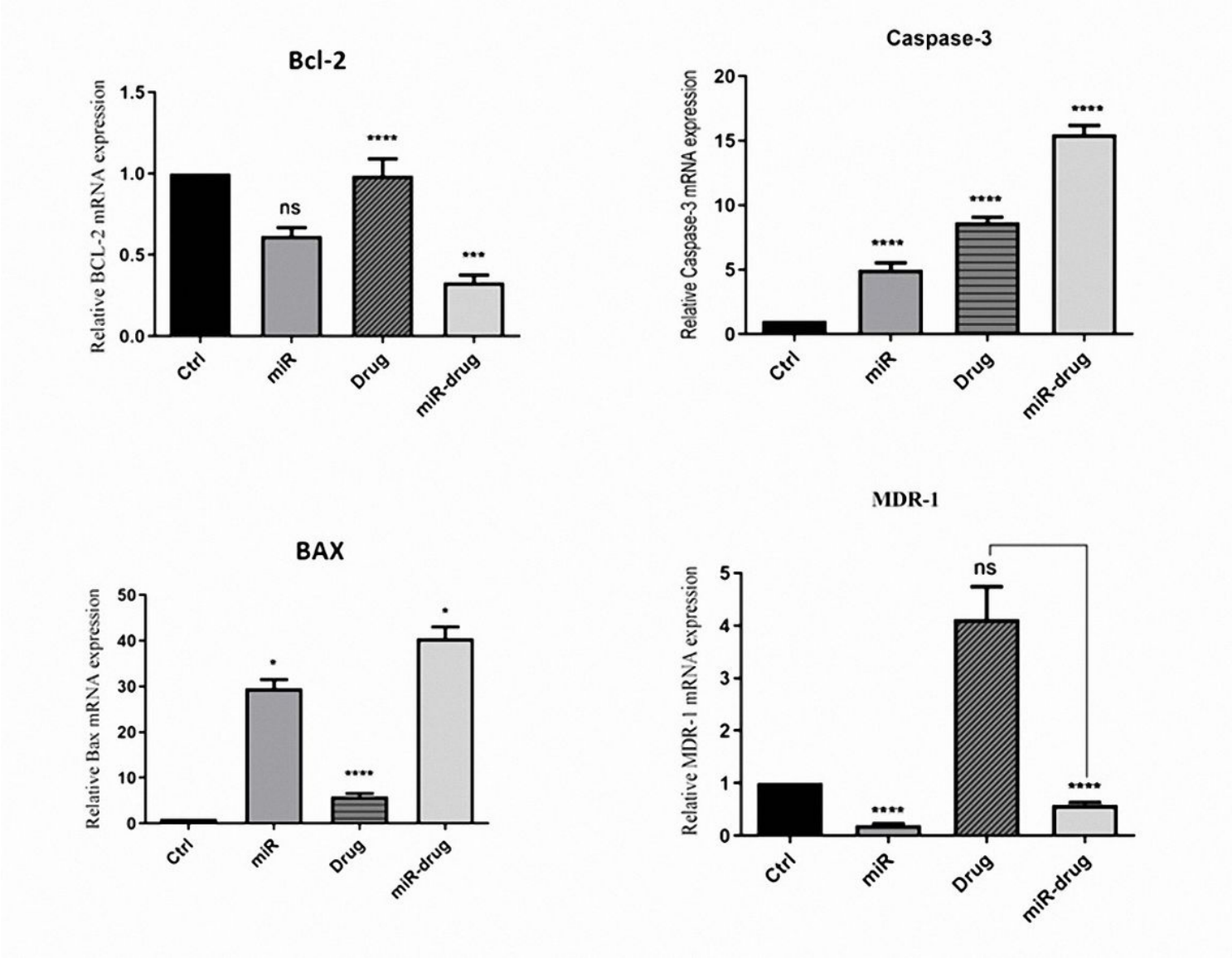



**Figure 1**

(A) The effects of miR-200c incubation on the viability of MCF7 cells. (B) The effects of miR-200c/ Carboplatin combination on the viability of MCF7 cells. MCF7 cells were treated with miR-200c alone or in combination with carboplatin at different concentrations for 48 hours, and then cell viability was evaluated using MTT assay. *P <0.05, **P <0.01, ***P <0.001, ****P <0.00001. Results are expressed as the mean ± SD of three experiments (triplicate). ns refer to non-significant.




## Figure 2

miR-200c regulated the BAX, Bcl2, Caspase-3, and MDR1 mRNAs in the MCF7 cells. The qRT-PCR analysis of BAX, Bcl2, Caspase-3, and MDR1 mRNA was evaluated in the control, miR, drug, and miR-drug groups. MiR-200c repressed the Bcl-2 gene expression. *P <0.05, ***P <0.001, ****P <0.00001. Results are indicated as the mean ± standard deviation of 3 experiments (triplicate). ns refer to non-significant.