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Effect of miR-101 on proliferation and oxidative stress-induced apoptosis of breast cancer cells *via* Nrf2 signaling pathway

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Abstract. – OBJECTIVE: To explore the influence of micro ribonucleic acid (miR)-101 on breast cancer cell proliferation and apoptosis via nuclear factor (erythroid-derived 2)-like 2 (Nrf2) signaling pathway.

MATERIALS AND METHODS: All MCF-7 cells were divided into 3 groups, namely control group, miR-101 mimic group (the cells were treated with 50 nmol/L miR-101 mimic), and miR-101 inhibitor group (the cells were treated with 50 nmol/L miR-101 inhibitor). The impact of miR-101 expression level on MCF-7 cell proliferation was evaluated via cell counting kit-8 (CCK-8) and colony formation assays. After the MCF-7 cells in the three groups were treated with 100 nM H2O2 for 12 h, the change in the apoptosis rate was detected via flow cytometry. Moreover, the influence of miR-101 expression level on the Nrf2 signaling pathway was detected via reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting.

RESULTS: According to the CCK-8 assay results, compared with that in control group, the proliferation rate of cells notably declined at 48, 72, and 96 h in miR-101 mimic group, and the difference was statistically significant (p<0.01), while it was substantially raised in miR-101 inhibitor group, showing a statistically significant difference (p<0.01). Compared that in control group, the cell colony formation rate was remarkably lowered in miR-101 mimic group, and the difference was statistically significant (p<0.01), while it was substantially raised in miR-101 inhibitor group (p<0.01). According to the flow cytometry assay results, compared with that in control group, the apoptosis of MCF-7 cells was markedly enhanced in miR-101 mimic group, showing a statistically significant difference (p<0.01), while it was weakened in miR-101 inhibitor group, with a statistically significant difference (p<0.01). The influence of miR-101 on the expression level of Nrf2 was detected via RT-PCR, and it was found that the messenger RNA (mRNA) expression level of Nrf2 was notably lower in miR-101 mimic group than that in control group (p<0.01), while it was raised in miR-101 inhibitor group. Western blotting results showed that compared with control group, miR-101 mimic group had a substantially lowered protein expression level of Nrf2 in the cell nucleus, with a statistically significant difference (p<0.01), while it was notably raised in miR-101 inhibitor group and the difference was statistically significant (p<0.01), indicating that miR-101 can remarkably lower the nucleoprotein expression level of Nrf2.

CONCLUSIONS: The results of this study imply that miR-101 can inhibit the expression of Nrf2 to suppress the proliferation of breast cancer cells and enhance their sensitivity to oxidative stress, which provides a theoretical basis for reversal of tumor resistance.

Key Words:
Breast cancer, MiR-101, Nrf2, MCF-7 cells.

Introduction

Breast cancer is not only one of the most common malignancies, but also one of the leading causes of cancer-related deaths in women[1,2]. With significant progress made in the treatment of breast cancer in recent years, surgery, chemotherapy, and endocrine and targeted therapies have relieved the pain of patients and raised the 5- and 10-year survival rates in them, but breast cancer resistance remains the difficulty in treating breast cancer[3,4].

Prior to tumorigenesis, the decrease in the expression level of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) can enhance the sensitivity to tumorigenesis to accelerate tumor formation[5,6]. However, after tumorigenesis, Nrf2 protects tumor cells, namely it can raise the tolerance of such cells to chemotherapeutics, promoting their growth, because Nrf2 can bind to the antioxidant responsive element (ARE) in the nucleus to reg-

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ulate the expressions of several antioxidants, including glutathione S-transferase (GST), quinone oxidoreductase 1 (NQO1), γ glutamylcysteine synthetase (GCL), and uridine 5'-diphospho-glucuronosyltransferase (UGT), ultimately protecting cells from carcinogen-induced deoxyribonucleic acid (DNA) injury and cell death.

Studies[7,8] have suggested that the increase in the expression level of Nrf2 can inhibit the occurrence of lung cancer and prostate cancer. Additionally, the *in vitro* and *in vivo* researches have reported that Nrf2 serves as a major player in the development of breast cancer. Nrf2 can activate the antigens on which Nrf2 depends on breast cancer cells to repress H_2O_2-induced death of them[9]. According to the results of an *in vivo* experiment, the carcinogenic factor estrogen is able to inhibit the expression of antioxidants *via* the pathway on which Nrf2 depends, thereby stimulating the formation of estrogen-dependent breast cancer in mice[10]. Moreover, the level of Nrf2 is elevated in breast cancer tissues, and its overexpression can enhance the drug resistance of breast cancer cells[11,12].

Micro-ribonucleic acids (miRNAs) are a class of non-coding RNAs consisting of 21-23 nucleotides, and a current study manifested that miRNAs can modulate about 30% of genes in organisms[13]. MiRNAs can directly bind to the 3′-untranslated region (3′-UTR) of messenger RNAs (mRNAs), thereby repressing the translation or degrading mRNAs. A single miRNA can directly regulate various mRNAs, thus playing an important role in the regulatory mechanism in organisms. According to the results of current studies, the abnormality of the expression of miRNAs is often accompanied by the occurrence of several human diseases, such as breast cancer. The current study findings have revealed that compared with that in normal breast tissues, the expression level of miR-101 is aberrantly lowered in breast cancer tissues, implying that miR-101 may be important in the pathological process of breast cancer.

According to the results of this investigation, miR-101 can reduce the expression level of Nrf2 to suppress the proliferation of breast cancer cells and enhance their sensitivity to oxidative stress, which serves as a theoretical basis for reversal of tumor resistance.

Materials and Methods

Cell Treatment and Culture

Human breast cancer MCF-7 cells (Shanghai Supan Biotech Co., Ltd., Shanghai, China) were cultured for passage in the Roswell Park Memorial Institute-1640 (RPMI-1640; Hyclone, South Logan, UT, USA) medium containing 10% fetal bovine serum (FBS; Hyclone, South Logan, UT, USA) under the conditions of 37°C and 5% CO_2, and the cells were centrifuged and sub-cultured when 80-90% of them were fused.

Main Reagents and Instruments

MiR-101-mimic and miR-101-inhibitor (Shanghai GenePharma Co, Ltd., Shanghai, China), RPMI-1640 medium, flow cytometer and bicinchoninic acid (BCA) protein concentration assay kit (Hangzhou ACEA Biosciences, Inc., Hangzhou, China), Real Time Polymerase Chain Reaction (PCR) instrument (Beijing Zhongke Keer Instrument Company Limited, Beijing, China) and automatic gel imaging analyzer (Shanghai Clinx Science Instruments Co., Ltd., Shanghai, China).

Cell Transfection and Grouping

The cells were evenly inoculated into 6-well plates at 1.2×10^6 cells/well and cultured in 2 mL of Dulbecco's Modified Eagle's Medium (DMEM; Hyclone, South Logan, UT, USA) containing 10% fetal bovine serum. All the cells were divided into control group, miR-101 mimic group (the cells were treated with 50 nmol/L miR-101 mimic) and miR-101 inhibitor group (the cells were treated with 50 nmol/L miR-101 inhibitor). After the three groups of cells were cultured for 24 h, the cell plates were added with miR-101 mimic or inhibitor diluted in 250 μL of serum-free opti-MEM, let stand at room temperature for 5 min, and before and after adding 10 μL of Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), they were slightly shaken to evenly mix the mixture with the culture solution in the wells. After 6 h, with the medium replaced with a normal one, all the cells were cultured in an incubator at 37°C with 5% CO_2 for 48 h.

Detection of Breast Cancer Cell Proliferation Using Cell Counting Kit-8 (CCK-8) Assay

Three groups of cells in the logarithmic growth phase were seeded into 96-well plates at 1×10^4 cells/well and cultured in the incubator for another 72 h, and with the original medium discarded, the cells were incubated with 20 μL of CCK-8 reaction solution (Dojindo Molecular Technologies, Kumamoto, Japan) and 180 μL of cell culture solution at 37°C in the dark for 2 h. Then the resulting cells were shaken on a micro-vibration

device for 3 min. Finally, the absorbance at the wavelength of 450 nm was measured using a microplate reader.

Detection of Nrf2 Expression in MCF-7 Cells Via Reverse Transcription (RT)-PCR

The expression of Nrf2 in the three groups of MCF-7 cells was detected *via* RT and quantitative PCR (qPCR). The chloroform/TRIzol (Invitrogen, Carlsbad, CA, USA) reagent was prepared at the volume ratio of 1:5, shaken and mixed evenly in 6-well plates, let stand at room temperature for 15 min, transferred into Eppendorf (EP) tubes and centrifuged at 4°C and 12,000 rpm for 15 min. Then, the supernatant water-phase was sucked into another centrifugal tube, added with isopropanol by 0.7-1-fold volume of the supernatant, placed at room temperature for 10-30 min and centrifuged at 12,000 rpm for 10 min. The supernatant was discarded, while RNAs were precipitated at the bottom of tubes. Subsequently, the centrifugal tubes were added with 75% ethanol (75% ethanol/TRIzol: 1:1), moderately shaken to suspend the precipitates, centrifuged at 4°C and 12,000 rpm for 5 min. The products were blown dry on a super clean bench for 10-20 min to try discarding the supernatant, added with 10-50 μL of diethyl pyrocarbonate-treated ddH_2O to dissolve the precipitates. Finally, the concentration of RNAs was determined using OneDrop micro-spectrophotometer. reverse transcription (RT) reaction was performed using 4.5 μL of RNase-free ddH_2O, 2 μL of 5× RT reaction buffer, 0.5 μL of random primers, 0.5 μL of oligo (dT), 0.5 μL of reverse transcriptase and 2 μL of RNAs. The samples of complementary DNAs (cDNAs) were divided into three groups and each of them was diluted by 20 folds, from which 3 μL of cDNA was selected for PCR amplification. The amplification level of the target gene was determined *via* 5% agarose gel electrophoresis. Then, the LabWorks 4.0 image acquisition and analysis software was adopted for quantification and data processing. To obtain reliable data, the above operations were performed for three times in each group. In the present study, the change in the relative expression level of the target gene was analyzed using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used in this study are shown in Table I.

Detection of MCF-7 Cell Apoptosis Via Flow Cytometry

The changes in the apoptosis of MCF-7 cells treated were detected using the annexin V/propidium iodide (PI) double-staining kit. A total of 5×10^5 cells were digested using trypsin and then rinsed using phosphate-buffered saline (PBS) at 4°C twice. Subsequently, the cells centrifuged were re-suspended in 500 μL of staining buffer and stained with 5 μL of annexin V-FITC (fluorescein isothiocyanate) and 5 μL of Propidium Iodide (PI) staining solution at 37°C in the dark for 15 min. Finally, the cells were loaded for flow cytometry assay using the Guava flow cytometer.

Detection of Influence of MiR-101 on the Nrf2 Proteins in MCF-7 Cell Nucleus Via Western Blotting

The Nrf2 nucleoproteins were extracted as follows: after adding a small amount of cooled PBS, the cells were scraped down using cell scrapers. Then, they were centrifuged in a pre-cooled centrifugal machine at 4°C and 1,000 RCF for 3 min and precipitated. The resulting cells were re-suspended in 1 mL of cell lysis buffer, added with protease inhibitor and placed on ice for 1 h. After centrifugation at 4°C and 1,000 RCF for 20 min, the precipitates were re-suspended in 100 μL of nuclei lysis buffer, added with protease inhibitor, let stand on ice for 1 h, shaken once every 5 min and fully lysed. The resulting precipitate suspension was centrifuged at 4°C and the maximum rotational speed for 10 min, and the supernatant was nucleoprotein. The protein samples prepared were stored in the refrigerator at –80°C for later use. The proteins were quantified using bicinchoninic acid (BCA) kit. Subsequently, with dodecyl

Table I. Primer sequences.

Gene	Forward/reverse	Primer sequences
Nrf2	R	5'-TICCTCTGCTCCCA-TTACTCAGTC-3'
	F	5'-GCTCTTCCATTT-CCGAGTCACTG-3'
β-actin	R	5'-GAGAGGGAAATCGTGCGTGAC-3'
	F	5'-GACGTAGCACAGCTTCTCCTTAATG-3'

sulfate, sodium salt-polyacrylamide gel electrophoresis (SDS-PAGE) gel prepared, the protein samples were loaded into the SDS-PAGE gel wells for electrophoresis under the constant voltage of 80 V for 2.5 h. Then, the proteins were transferred onto polyvinylidene difluoride (PVDF) membranes (Roche, Basel, Switzerland) by the semi-dry transfer method, and the membranes were immersed in Tris-Buffered Saline with Tween-20 (TBST) buffer containing 5% skim milk powder, shaken slowly using a shaker and sealed. The resulting membranes were incubated with the primary antibody diluted with 5% skim milk powder and washed using TBST for 3 times (10 min/time), followed by incubation with the secondary antibody at room temperature for 2 h and rinsing using TBST and TBS twice and once, respectively (10 min/time). The resulting proteins were detected using enhanced chemiluminescence (ECL) reagent and exposed in a darkroom, and the relative expression level of proteins was analyzed using Image-Pro Plus v6 software (Media Cybernetics, Silver Spring, MD, USA). With the β-actin as the internal reference, the expression of proteins was detected.

Cell Colony Formation Assay

MCF-7 cells in the three groups were inoculated into the culture dishes containing 10 mL of culture solution and incubated at 37°C for 14 d. When the cell colony was visible to the naked eye, the cells were fixed using 4% paraformaldehyde for 15 min, and the cell colonies were stained with the crystal violet staining solution for 30 min, rinsed using deionized water for 3 times and dried in air. The number of colonies formed was counted under an inverted microscope, and the colony formation rate was calculated based on colony formation rate (%) = (the number of colonies formed/that of cells inoculated) ×100%.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 (SPSS, Chicago, IL, USA) was used for statistical analysis in the present study, and all the data were expressed mean ± standard deviation. The *t*-test was used for analyzing measurement data. Differences between two groups were analyzed by using the Student's *t*-test. Comparison between multiple groups was done using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ denoted that the difference was significant.

Results

Impact of MiR-101 Expression Level on the Proliferation of MCF-7 Cells

According to the CCK-8 assay results, compared with that in control group, the proliferation rate of cells notably declined at 48, 72, and 96 h in miR-101 mimic group, showing a statistically significant difference ($p<0.01$), while it was substantially raised in miR-101 inhibitor group, displaying a statistically significant difference ($p<0.01$) (Figure 1), implying that the increase in the expression level of miR-101 can lower the proliferation rate of MCF-7 breast cancer cells.

Influence of MiR-101 Expression Level on Colony Formation of MCF-7 Cells

To further study the influence of miR-101 on the proliferation rate of MCF-7 cells, the impact of miR-101 on cell proliferation was detected *via* cell colony formation assay. It was found that compared with control group, miR-101 mimic group exhibited a remarkably lowered colony formation rate, with a statistically significant difference ($p<0.01$), while it was substantially raised in miR-101 inhibitor group ($p<0.01$), indicating that miR-101 mimic inhibits cell proliferation, while miR-101 inhibitor promotes cell proliferation, which is consistent with those of the CCK-8 assay (Figure 2).

Impact of MiR-101 Expression Level on the H_2O_2-Induced Apoptosis of MCF-7 Cells

To explore the impact of miR-101 on H_2O_2-induced MCF-7 cell apoptosis, the MCF-7 cells

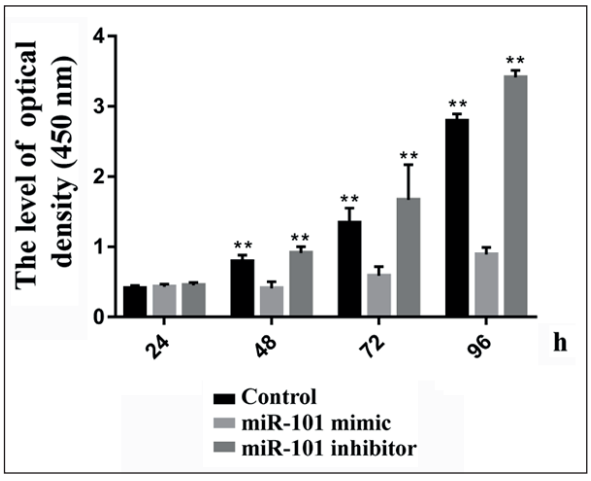

Figure 1. MiR-101 lowers the proliferation rate of MCF-7 breast cancer cells. **: $p<0.01$, *vs.* control group.

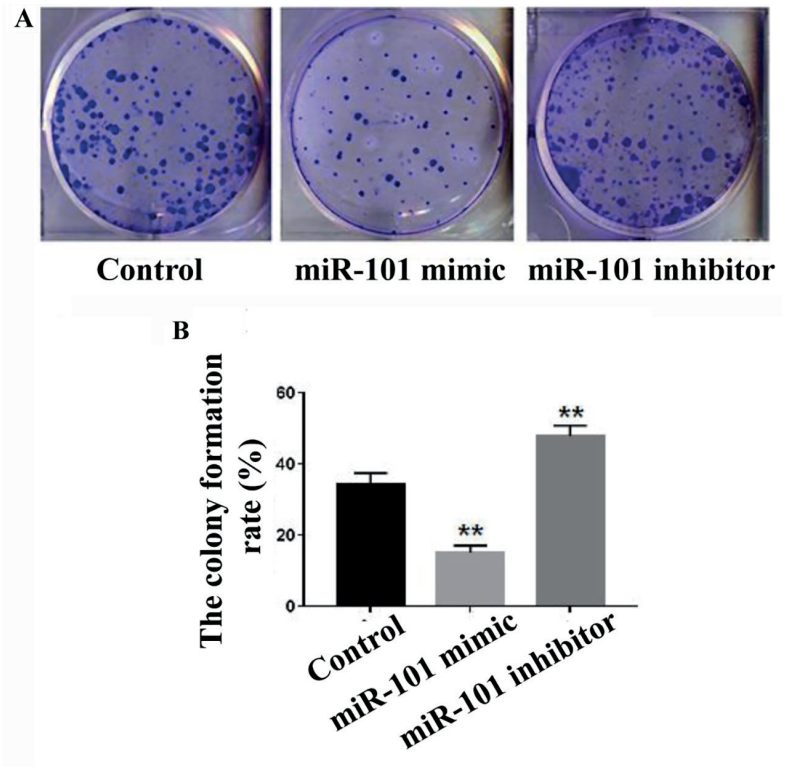

Figure 2. MiR-101 reduces the colony formation rate of MCF-7 breast cancer cells (magnification: 10×). **: $p<0.01$, *vs.* control group.

were transfected with miR-101 mimic or miR-101 inhibitor and then treated with 100 nM H_2O_2 for 12 h, and finally, the changes in the apoptosis rate were detected using the flow cytometer. According to the results, compared with that in control group, the apoptosis rate of MCF-7 cells was markedly raised by miR-101 mimic, showing a statistically significant difference ($p<0.01$), while it was lowered by miR-101 inhibitor, with a statistically significant difference ($p<0.01$) (Figure 3). The above findings indicate that miR-101 mimic promotes cell apoptosis, while miR-101 inhibitor represses it (Figure 3).

Influence of MiR-101 on the MRNA Expression Level of Nrf2

The influence of miR-101 on the expression level of Nrf2 was detected *via* qRT-PCR to reveal the related mechanism by which miR-101 accelerates the H_2O_2-induced apoptosis of MCF-7 cells and inhibits their proliferation. It was discovered that compared with that in control group, the mRNA expression of Nrf2 was notably lowered in miR-101 mimic group ($p<0.01$), while it was raised in miR inhibitor group, showing a statistically significant difference ($p<0.01$) (Figure 4).

Influence of MiR-101 on the Protein Expression Level of Nrf2

To further verify the results of RT-PCR experiment, the protein level of Nrf2 was measured *via* Western blotting, and according to the results, the protein expression level of Nrf2 in the cell nucleus was substantially lower in miR-101 mimic group than that in control group, showing a statistically significant differences ($p<0.01$), while it was notably raised in miR-101 inhibitor group, and the difference was statistically significant ($p<0.01$), indicating that miR-101 can remarkably lower the nucleoprotein expression level of Nrf2. The above results suggest that the increase in the expression level of miR-101 can reduce the protein expression level of Nrf2 in the cell nucleus (Figure 5).

Discussion

Over the past half century, early diagnosis and comprehensive treatment have effectively elevated the 5- and 10-year survival rates of breast cancer patients, but breast cancer resistance is still the difficulty in the treatment of breast cancer[17,18]. According to the CCK-8 assay results, compared

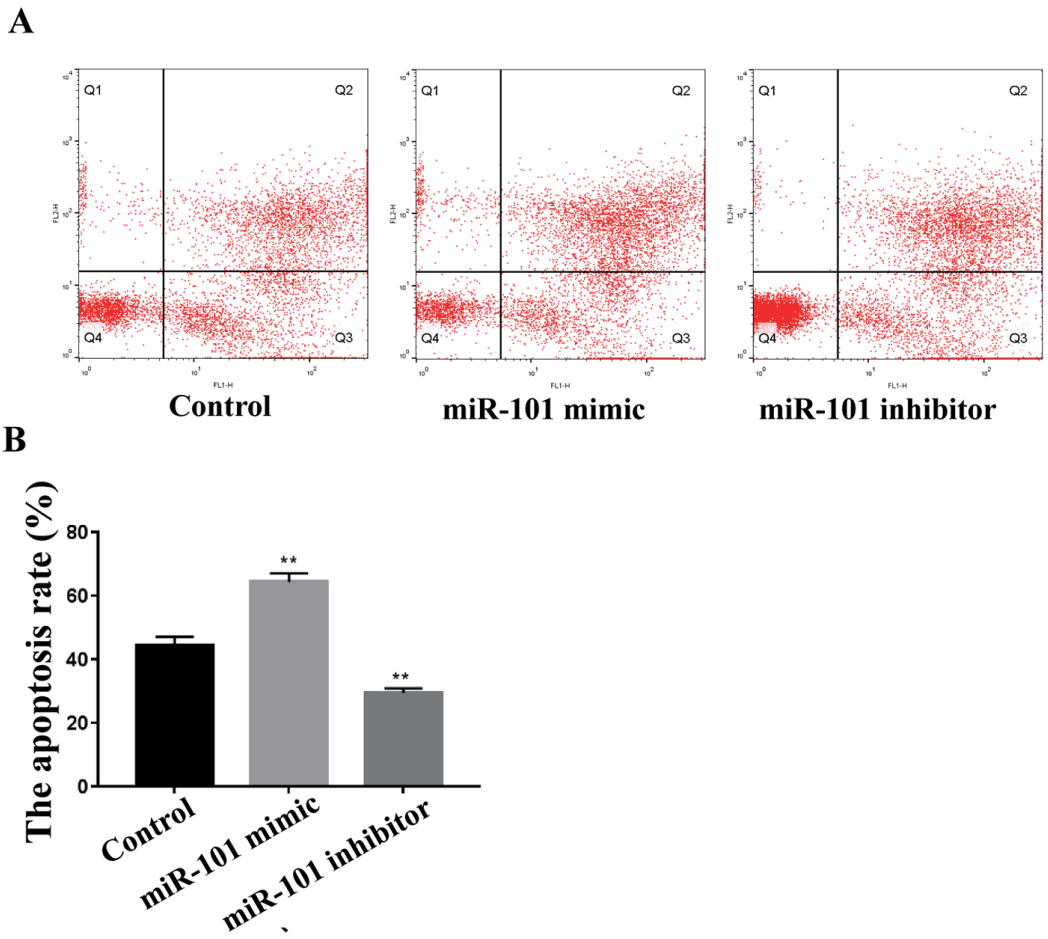

Figure 3. MiR-101 enhances the H_2O_2-induced apoptosis of MCF-7 cells. **A,** Influence of miR-101 on H_2O_2-induced MCF-7 cell apoptosis detected using the flow cytometer. **B,** Statistical graph of cell apoptosis results. **: $p<0.01$, *vs.* control group.

with that in control group, the proliferation rate of cells was notably lowered at 48, 72, and 96 h in miR-101 mimic group, with a statistically significant difference ($p<0.01$), while it was substantially elevated in miR-101 inhibitor group, displaying a statistically significant difference ($p<0.01$), which imply that the increase in the expression level of miR-101 can lower the proliferation rate of MCF-7 breast cancer cells. Moreover, to study the influence of miR-101 on the proliferation rate of MCF-7 cells, the impact of miR-101 on cell proliferation was detected *via* cell colony formation assay. The results showed that compared with control group, miR-101 mimic group exhibited a remarkably lowered colony formation rate, with a statistically significant difference ($p<0.01$), while it was substantially raised in miR-101 inhibitor group ($p<0.01$), indicating that miR-101 mimic inhibits cell proliferation, while miR-101 inhibitor promotes it, which are consistent with those of the CCK-8 assay. Subsequently, to explore the impact of miR-101 on H_2O_2-induced MCF-7 cell apoptosis, the MCF-7 cells were transfected with miR-101 mimic or miR-101 inhibitor, and after treatment with 100 nM H_2O_2 for 12 h, the changes in the apoptosis rate were detected using the flow cytometer. According to the results, compared with the procedures in control group, miR-101 mimic treatment markedly enhanced the apoptosis of MCF-7 cells, showing a statistically significant difference ($p<0.01$), while miR-101 inhibitor remarkably weakens the apoptosis ($p<0.01$).

Additionally, to reveal the related mechanism in the promotion of H_2O_2-induced MCF-7 cell apoptosis and inhibition of their proliferation by miR-101, the influence of miR-101 on the expression level of Nrf2 was detected *via* qRT-PCR. According to the experimental findings, the mRNA

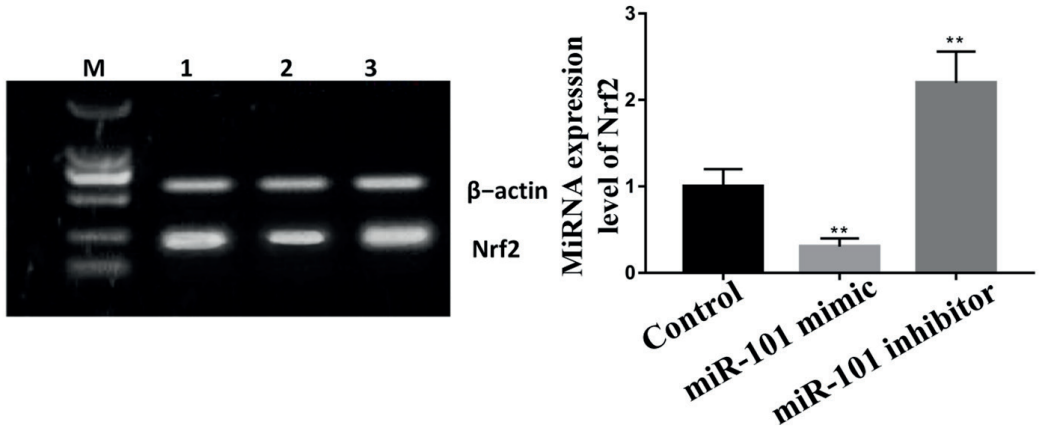

Figure 4. MiR-101 remarkably lowers the mRNA expression level of Nrf2. **A,** MRNA expression level of Nrf2 determined *via* RT-PCR. **B,** Quantitative expressions according to the experimental results. **: $p<0.01$, *vs.* control group.

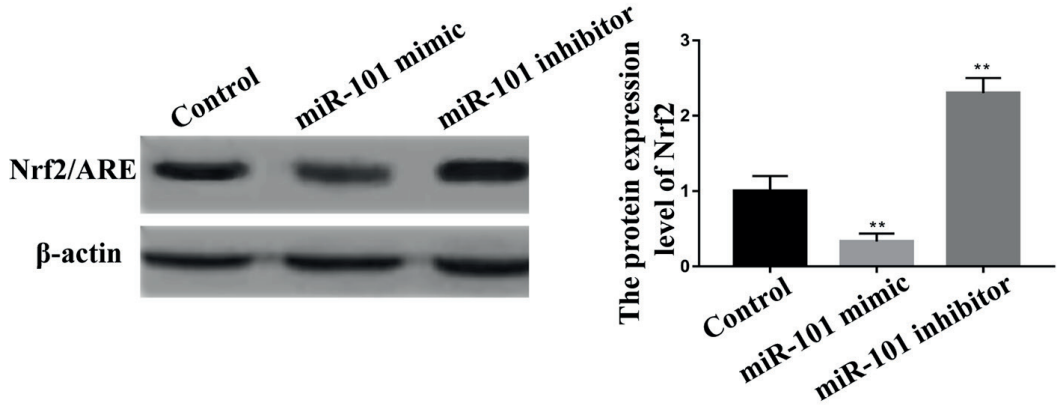

Figure 5. MiR-101 can remarkably lower the nucleoprotein expression level of Nrf2. **A,** Influence of miR-101 on the protein expression level of Nrf2 detected *via* Western blotting. **B,** Quantitative results of Western blotting. **: $p<0.01$, *vs.* control group

expression of Nrf2 was notably lower in miR-101 mimic group than that in control group ($p<0.01$), while it was raised in miR-101 inhibitor group ($p<0.01$). Finally, to further verify the results of RT-PCR, the protein level of Nrf2 was measured *via* Western blotting. It was found that miR-101 mimic group had a substantially lower protein expression level of Nrf2 in the cell nucleus than control group, with a statistically significant difference ($p<0.01$), while it was notably raised in miR-101 inhibitor group compared with that in control group, showing a statistically significant difference ($p<0.01$), which indicate that miR-101 can remarkably lower the nucleoprotein expression level of Nrf2. According to the above data, the increase in the expression level of miR-101 can reduce the protein expression level of Nrf2 in the cell nucleus.

Studies[19,20] have suggested that once the activity of Nrf2/ARE, an important antioxidant signaling pathway in the body, is weakened, it negatively regulates the downstream antioxidant proteins, causing reactive oxygen species damage to the body and oxidative stress response, which is one of major causes of breast diseases, such as breast cancer, mastitis, breast tuberculosis and fat necrosis of breast[21,22]. Yao et al[23] has manifested that the Nrf2/ARE signaling pathway plays an important role in breast cancer, and when the protein expression of Nrf2 is inhibited in breast cancer cells, the oxidation and anti-oxidation of cancer cells are out of balance, with more apoptotic breast cancer cells, which are consistent with the findings in the present study.

Some works[24,25] have demonstrated that miR-101 is lowly expressed in multiple tumors, such as

cervical cancer, small cell lung cancer, and breast cancer, and the low expression of miR-101 is considered to be a risk factor for the occurrence and development of tumors. Wang et al[26] transfected lentiviruses into cells to raise the expression level of miR-101 in breast cancer cells and found that enhancing the expression of miR-101 inhibits the proliferation and activation of cancer cells and accelerates their apoptosis. Guo et al[27] discovered through *in vitro* culture of different hepatoma cells that the over-expression of miR-101 significantly affects the proliferation, colony formation, invasion and metastasis abilities of hepatoma cells in G0/G1 phases and increases apoptotic cancer cells, which are in line with the findings in this study.

Conclusions

Lowering the expression of miR-101 in breast cancer cells can raise the expression of Nrf2, enhance their proliferation ability and weaken their sensitivity to oxidative stress, whereas the increase in the expression of miR-101 can reverse the above effects.

Conflicts of interest

The authors declare no conflicts of interest.

References

1) Zhao ZM, Yost SE, Hutchinson KE, Li SM, Yuan YC, Noorbakhsh J, Liu Z, Warden C, Johnson RM, Wu X, Chuang JH, Yuan Y. CCNE1 amplification is associated with poor prognosis in patients with triple negative breast cancer. BMC Cancer 2019; 19: 96.
2) Resnicow K, Patel MR, Mcleod MC, Katz SJ, Jagsi R. Physician attitudes about cost consciousness for breast cancer treatment: differences by cancer sub-specialty. Breast Cancer Res Treat 2019; 173: 31-36.
3) Raghunath A, Desai K, Ahluwalia MS. Current treatment options for breast cancer brain metastases. Curr Treat Options Oncol 2019; 20: 19.
4) Mortimer J, Di Palma J, Schmid K, Ye Y, Jahanzeb M. Patterns of occurrence and implications of neratinib-associated diarrhea in patients with HER2-positive breast cancer: analyses from the randomized phase III ExteNET trial. Breast Cancer Res 2019; 21: 32.
5) Ayala de la Peña F, Andrés R, Garcia-Sáenz JA, Manso L, Margelí M, Dalmau E6, Pernas S, Prat A, Servitja S, Ciruelos E. SEOM clinical guidelines in early stage breast cancer (2018). Clin Transl Oncol 2019; 21: 18-30.
6) O' Halloran N, McVeigh T, Martin J, Keane M, Lowery A, Kerin M. Neoadjuvant chemoradiation and breast reconstruction: the potential for improved outcomes in the treatment of breast cancer. Ir J Med Sci 2019; 188: 75-83.
7) Khodabandehlou N, Mostafaei S, Etemadi A, Ghasemi A, Payandeh M, Hadifar S, Norooznezhad AH, Kazemnejad A, Moghoofei M. Human papilloma virus and breast cancer: the role of inflammation and viral expressed proteins. BMC Cancer 2019; 19: 61.
8) Xiao Y, Xia J, Li L, Ke Y, Cheng J, Xie Y, Chu W, Cheung P, Kim JH, Colditz GA, Tamimi RM, Su X. Associations between dietary patterns and the risk of breast cancer: a systematic review and meta-analysis of observational studies. Breast Cancer Res 2019; 21: 16.
9) Maselli-Schoueri JH, Affonso-Kaufman FA, de Melo Sette CV, Dos Santos Figueiredo FW, Adami F. Time trend of breast cancer mortality in Brazilian men: 10-year data analysis from 2005 to 2015. BMC Cancer 2019; 19: 23.
10) Armer JM, Ballman KV, McCall L, Armer NC, Sun Y, Udmuangpia T, Hunt KK, Mittendorf EA, Byrd DR, Julian TB, Boughey JC. Lymphedema symptoms and limb measurement changes in breast cancer survivors treated with neoadjuvant chemotherapy and axillary dissection: results of American College of Surgeons Oncology Group (ACOSOG) Z1071 (Alliance) substudy. Support Care Cancer 2019; 27: 495-503.
11) Yim K, Park HS, Kim DM, Lee YS, Lee A. Image analysis of HER2 immunohistochemical staining of surgical breast cancer specimens. Yonsei Med J 2019; 60: 158-162.
12) Chang J, Zhang Y, Li Y, Lu K, Shen Y, Guo Y, Qi Q, Wang M, Zhang S. NrF2/ARE and NF- κB pathway regulation may be the mechanism for lutein inhibition of human breast cancer cell. Future Oncol 2018; 14: 719-726.
13) Black E, Richmond R. Improving early detection of breast cancer in sub-Saharan Africa: why mammography may not be the way forward. Global Health 2019; 15: 3.
14) Mechera R, Soysal SD, Piscuoglio S, Ng C, Zeindler J, Mujagic E, Daster S, Glauser P, Hoffmann H, Kilic E, Droeser RA, Weber WP, Muenst S. Expression of RET is associated with Oestrogen receptor expression but lacks prognostic significance in breast cancer. BMC Cancer 2019; 19: 41.
15) Nayar U, Cohen O, Kapstad C, Cuoco MS, Waks AG, Wander SA, Painter C, Freeman S, Persky NS, Marini L, Helvie K, Oliver N, Rozenblatt-Rosen O, Ma CX, Regev A, Winer EP, Lin NU, Wagle N. Acquired HER2 mutations in ER(+) metastatic breast cancer confer resistance to estrogen receptor-directed therapies. Nat Genet 2019; 51: 207-216.
16) Xu P, Liu Q, Xie Y, Shi X, Li Y, Peng M, Guo H, Sun R, Li J, Hong Y, Liu X, Xu G. Breast cancer susceptibility protein 1 (BRCA1) rescues neurons from cerebral ischemia/reperfusion injury through NRF2-mediated antioxidant pathway. Redox Biol 2018; 18: 158-172.

17) Zhao J, Jiang GQ. MiR-4282 inhibits proliferation, invasion and metastasis of human breast cancer by targeting Myc. Eur Rev Med Pharmacol Sci 2018; 22: 8763-8771.

18) Wu CF, Lee MG, El-Shazly M, Lai KH, Ke SC, Su CW, Shih SP, Sung PJ, Hong MC, Wen ZH, Lu MC. Isoaaptamine induces T-47D cells apoptosis and autophagy via oxidative stress. Mar Drugs 2018; 16. pii: E18.

19) Choi BH, Ryu DY, Ryoo IG, Kwak MK. NFE2L2/NRF2 silencing-inducible miR-206 targets c-MET/EGFR and suppresses BCRP/ABCG2 in cancer cells. Oncotarget 2017; 8: 107188-107205.

20) Wang Y, Zhang S. Berberine suppresses growth and metastasis of endometrial cancer cells via miR-101/COX-2. Biomed Pharmacother 2018; 103: 1287-1293.

21) Wang H, Wang L, Zhang G, Lu C, Chu H, Yang R, Zhao G. MALAT1/miR-101-3p/MCL1 axis mediates cisplatin resistance in lung cancer. Oncotarget 2017; 9: 7501-7512.

22) Liang C, Zhuang H, Zhou Z, Zhang H. SNHG3 promotes proliferation and invasion by regulating the miR-101/ZEB1 axis in breast cancer. Rsc Advances, 2018; 8: 15229-15240.

23) Yao ZS, Li C, Liang D, Jiang XB, Tang JJ, Ye LQ, Yuan K, Ren H, Yang ZD, Jin DX, Zhang SC, Ding JY, Tang YC, Xu JX, Chen K, Xie WX, Guo DQ, Cui JC. Diagnostic and prognostic implications of serum miR-101 in osteosarcoma. Cancer Biomark 2018; 22: 127-133.

24) Cai T, Liu Y, Xiao J. Long noncoding RNA MALAT1 knockdown reverses chemoresistance to temozolomide via promoting microRNA-101 in glioblastoma. Cancer Med 2018; 7: 1404-1415.

25) Fan B, Shi S, Shen X, Yang X, Liu N, Wu G, Guo X, Huang N. Effect of HMGN2 on proliferation and apoptosis of MCF-7 breast cancer cells. Oncol Lett 2019; 17: 1160-1166.

26) Wang CM, Yang WH, Liu R, Wang L, Yang WH. FOXP3 activates SUMO-conjugating UBC9 gene in MCF7 breast cancer cells. Int J Mol Sci 2018; 19. pii: E2036.

27) Guo L, Li F, Wang M, Xu Y, Wang B, Ran D, Zhang X. 17beta-estradiol regulates the malignancy of cancer stem-like cells derived from the MCF7 cell line partially through Sox2. Oncol Lett 2018; 15: 3790-3795.