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Effects of miR-101 on the proliferation and apoptosis of gastric mucosal epithelial cells via Nrf2/ARE signaling pathway

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Abstract. – OBJECTIVE: The aim of this study was to investigate the effects of micro-ribonucleic acid (miR)-101 on the proliferation and apoptosis of gastric mucosal epithelial cells through nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) signaling pathway.

MATERIALS AND METHODS: Human gastric epithelial AGS (CRL-1739) cells were cultured *in vitro*. MiR-101 down-regulation or over-expression was achieved by transfection of inhibitors, or miRNA mimics, respectively. The apoptosis rate was detected by flow cytometry. Meanwhile, the targets of miR-101 were verified by the Dual-Luciferase reporter gene assay. Furthermore, the changes in protein levels were measured via Western blotting (WB).

RESULTS: Up-regulation of miR-101 significantly promoted the apoptosis of gastric mucosal epithelial cells. The 3'-untranslated region (3'-UTR) of Nrf2 was highly conserved to combine with miR-101. The Luciferase reporter gene assay showed that transfection of miR-101 mimics could remarkably inhibit the relative Luciferase activity in cells. In addition, miR-101 overexpression markedly down-regulated the messenger RNA (mRNA) and protein expressions of Nrf2 in cells.

CONCLUSIONS: MiR-101 plays an important role in regulating the proliferation and apoptosis of gastric mucosal epithelial cells by targeting the Nrf2-ARE signaling pathway.

Key Words:
MiR-101, Nrf2-ARE, Gastric mucosal epithelial cell, Proliferation, Apoptosis.

Introduction

Gastric mucosal injury, including gastritis, gastric ulcer, gastric mucosal stress injury, erosion and bleeding, is a common and refractory disease in clinic. With the improvement of people's living standards and changes in living habits, the incidence and prevalence of gastric mucosal injury have greatly increased in recent years. Reactive oxygen species (ROS) play an important role in the pathogenesis of diseases. Meanwhile, ROS participates in complex physiological processes related to oxidative stresses, such as cell signal transduction and apoptosis. Previous studies have indicated that long-term drinking is one of the most important risk factors for digestive tract diseases. Chronic and excessive drinking may eventually lead to various acute digestive tract diseases, such as gastritis, gastric ulcer and gastrorrhagia, due to subsequent gastric mucosal injury, especially those caused by oxidative stresses and apoptosis. Therefore, the specific molecular mechanism of gastric mucosal injury has already become a hotspot in current research.

Micro-ribonucleic acids (miRNAs) are a class of non-coding RNAs with about 18-25 nucleotides in length. They can regulate the 3'-untranslated regions (3'-UTRs) of target messenger RNAs (mRNAs) expressed by transcribed proteins, thus inhibiting the translation or degradation of target mRNAs. Currently, more than 474 miRNAs have been found to be associated with over 10% of

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protein-encoded mRNAs in the human genome. This indicates that miRNAs exert a crucial role in human biological processes. Research has revealed that miRNAs are abnormally expressed or mutated in cancer. Meanwhile, the analysis of miRNA spectrum helps to distinguish between cancer and normal tissues. A large number of miRNAs have been confirmed up-regulated or down-regulated in various malignant tumors[1]. In the occurrence of malignancies, miRNAs act as tumor suppressors or promoters. Previous studies have demonstrated that miR-101 is of great significance in malignant tumors. Its abnormal expression has been confirmed by multiple studies[2,3]. In addition, studies have shown that miR-101 is associated with the metastasis of the tumor. For example, inhibiting miR-101 can promote colorectal cancer metastasis[4].

Nuclear factor erythroid 2-related factor 2 (Nrf2) molecule binds to antioxidant response element (ARE), which regulates the expression of various cell protection genes involved in cell anti-oxidation and anti-inflammatory responses[5]. Under normal conditions, Nrf2 is isolated from the cytoplasm by Kelch-like ECH-associated protein-1 (Keap1)[6,7]. After activation, Keap1 releases Nrf2 and transports it into the nucleus, thus activating ARE[8,9]. The Nrf2/ARE signaling pathway regulates more than 200 endogenous protective genes involved in cell anti-oxidation and anti-inflammatory defense systems[10], including phase II antioxidant/detoxifying enzyme genes and anti-inflammatory costimulatory and molecular chaperone genes. Meanwhile, these protective genes play an important role in improving tissue antioxidant ability. They also exert antitoxin, anti-tumor, anti-inflammatory and anti-apoptosis effects[11]. Therefore, the activation of the Nrf2/ARE signaling pathway is a feasible strategy to reduce inflammation. However, the specific role of the Nrf2/ARE signaling pathway in gastric mucosal injury has not been fully elucidated. The aim of this study was to investigate the function of Nrf2/ARE in gastric mucosal injury and the possible underlying mechanism.

Materials and Methods

Cell Culture

Human gastric epithelial AGS (CRL-1739) cells were obtained from the Cell Bank of Chinese Academy of Sciences (Shanghai, China). All cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640; Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) in an incubator with 5% CO_2 at 37°C.

Cell Transfection

MiR-101/negative control (NC) mimics and miR-101/NC inhibitors were synthesized by GenePharma (Shanghai, China). Cell transfection was performed according to the manufacturer's instructions of Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA in cells was extracted in strict accordance with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Subsequently, 1 μL of RNA solution was collected, and the concentration and purity of total RNA were detected by a microplate reader. Optical density $(OD)_{260}/OD_{280}$ value should be 1.6-1.8. The remaining RNA solution was sub-packaged and stored at -80°C for subsequent use.

Complementary deoxyribonucleic acid (cDNA) was synthesized by TaKaRa PrimeScript™ Kit (TaKaRa, Otsu, Shiga, Japan). Specific operation steps were based on the instructions. In the first step, RNAs with a total mass of 2 μg was added. According to the respective concentrations, the volume of added RNA was 1 μL for Oligo (dT) primer (50 μM) and 1 μL for dNTP Mixture (10 mM), respectively. The system was supplemented with enzyme-free double distilled water to 10 μL. After they were gently mixed, the system was heated at 65°C for 5 min by a Polymerase Chain Reaction (PCR) apparatus and quickly cooled on ice. 4 μL 5× PrimeScript Buffer, 1 μL of PrimeScript RTase, 0.5 μL of RNase inhibitors and 4.5 μL of enzyme-free water were added to the above reaction system and mixed evenly. The reaction system was heated at 42°C for 45 min in a PCR apparatus, followed by heating at 95°C for 5 min. Subsequently, the reaction system was transferred onto the ice for rapid cooling to synthesize single-stranded cDNAs. Finally, the products were placed at -20°C for Polymerase Chain Reaction (PCR) amplification reaction. Primer sequences used in this study were as follows: Nrf2, F: 5'-CAACAGCCCCTGCTTACTC-3', R: 5'-GGAGGGATGTGTCCGATGGA-3'; miR-101, F: 5'-GAACTGTAAGCATCCTCCTG-3', R: 5'-CCTGCGTGTCAAGGAGTCG-3'; U6: F: 5'-GCTTC-

GGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; GAPDH: F: 5'-CGCTCTCTGCTCCTCCTGTTC-3', R: 5'-ATCCGTTGACTCCGACCTTCAC-3'.

Detection of Apoptosis via Flow Cytometry

After different treatments, the cell culture medium was collected into centrifuge tubes. Cells were washed with Phosphate-Buffered Saline (PBS; Gibco, Grand Island, NY, USA) twice, collected and moderately digested with trypsin. The digestion of all cells in each group was terminated with the culture solution collected before heating. Subsequently, the cells were collected into tubes, followed by centrifugation at 1000 rpm for 5 min. The supernatant was discarded, and 1 mL of precooled PBS solution was added to re-suspend the cells. After centrifugation at 1000 rpm for 5 min, the supernatant was discarded, and 50 μL of remaining solution was reserved. Subsequently, all cells were transferred into 1.5 mL Eppendorf (EP) tubes (Eppendorf, Hamburg, Germany) and washed with PBS solution repeatedly. The supernatant was then discarded. The apoptosis rate of cells in each group was detected according to the instructions of Annexin V-FITC (fluorescein isothiocyanate) Apoptosis Kit (BD Biosciences, Franklin Lakes, NJ, USA). Specifically, each sample was first added with 0.5 mL of freshly prepared staining buffer, 5 μL of Annexin V-FITC and 5 μL of Propidium Iodide (PI). Then, the solution was gently mixed and incubated at room temperature for 15 min in the dark. Flow cytometry was used to measure the spectral value of the excitation wavelength of cells in each group within 1 h. The apoptosis map was drawn, and the proportions of early and late apoptotic cells were analyzed. The experiment was repeated three times. Finally, the apoptosis rate in each group after different treatments was compared.

Western Blotting (WB)

Total protein in cells was extracted, and the concentration of extracted protein was determined by the bicinchoninic acid (BCA) method (Beyotime, Shanghai, China). 40 μg of total proteins were separated by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). Then, the membranes were incubated with primary antibodies (1:2000, Abcam, Cambridge, MA, USA) overnight. On the next day, the membranes were incubated with corresponding horseradish peroxidase-labeled secondary antibody (1:1000) at room temperature for 1 h (Beyotime, Shanghai, China). Next, 200 μL of luminescent liquid was evenly added dropwise and placed in a chemiluminescence imaging analysis system for development. Image J (NIH, Bethesda, MD, USA) software was adopted to calculate the gray value of bands on the developed image.

BrdU Cell Proliferation Assay

Cell proliferation was measured according to the manufacturer's instructions of BrdU enzyme-linked immunosorbent assay (ELISA) kit (QIA58, Calbiochem, San Diego, CA, USA). 5×10^4 cells were first seeded into 96-well plates, followed by culture in an incubator with 5% CO_2. BrdU labels were added 16 h before termination of the test. BrdU incorporated into cell DNAs was measured using a microplate reader (Polarstar Omega, BMG Biotech, Ortenberg, Germany). This experiment was repeated four times, and at least three replicates were set for each sample.

Dual-Luciferase Reporter Gene Assay

On the first day, the cells were digested and inoculated (appropriate number of cells was selected according to the specific experiment) in a 35 mm cell culture dish. Then, the cells were cultured overnight in an incubator with 5% CO_2 and saturated humidity at 37°C. When the cell density reached 70%, wild-type (WT) or mutant (Mut) 3'-untranslated regions (3'-UTRs) of Nrf2 were cloned into pGL3-Basic vectors (Promega, Madison, WI, USA), respectively. Subsequently, pGL3/Nrf2-WT and pGL3/Nrf2-Mut recombinant vectors were constructed after sequencing. AGS cells cultured in 24-well plates were cotransfected with 100 nM miR-101 mimics and pGL3/Nrf2-WT or pGL3/Nrf2-Mut according to the instructions of Lipofectamine 3000. Luciferase activity at 48 h after transfection was measured through the Dual-Luciferase report analysis system (Promega, Madison, WI, USA).

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 17.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analysis. The difference between the two groups was compared *via t*-test. One-way analysis of variance was used to compare the difference among different groups, followed by Post-Hoc Test (Least Significant Dif-

ference). Bilateral 95% confidence interval (CI) was used in all tests. $p<0.05$ was considered statistically significant.

Results

Expression of MiR-101 in Cells

The relative expression level of miR-101 in placental trophoblasts transfected with miR-101/NC mimics or miR-101/NC inhibitors was detected *via* quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR). 48 h after transfection, the cells were harvested for detection. The results showed that the expression level was significantly increased after transfection of miR-101 mimics. However, miR-101 inhibitors transfection could markedly decrease the expression of miR-101 ($p<0.05$, Figure 1).

Detection of Cell Apoptosis

After AGS cells were transfected with miR-101 mimics and inhibitors, the apoptotic rates of cells were detected by flow cytometry, respectively. The results revealed that the apoptotic rates of cells in miR-101 mimics group, miR-101 inhibitor group and control group were (16.45±4.62)%, (5.57±2.35)% and (8.58±2.28)%, respectively, displaying statistically significant differences ($p<0.05$, Figure 2A-2C).

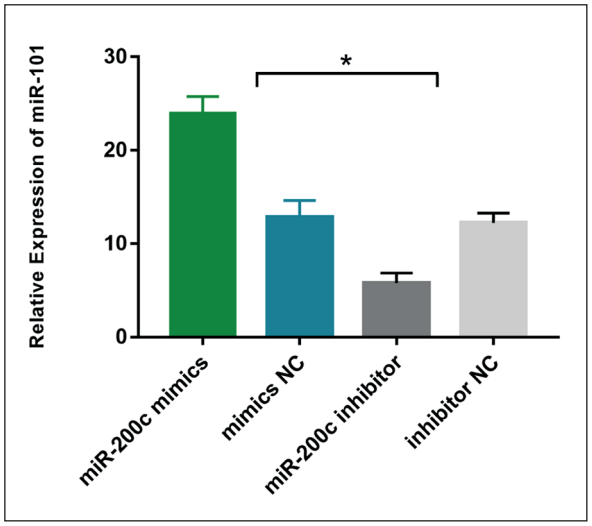

Figure 1. Expression level of miR-101 after different treatments detected via qRT-PCR. The expression level of miR-101 was significantly up-regulated after transfection with miR-101 mimics. However, the expression level of miR-101 was markedly down-regulated after transfection with miR-497 inhibitors ($p<0.05$).

The expression of apoptosis-related proteins in cells of each group was detected by Western blotting, which could illustrate the effect of miR-101 on apoptosis from another aspect. The results found that miR-101 mimics transfection could significantly up-regulate the expression levels of apoptosis proteins, including Bcl-2-associated X protein (Bax) and active Caspase-3. However, the expression trend of anti-apoptosis protein b-cell lymphoma 2 (Bcl-2) was opposite (Figure 2D).

Cell Proliferation

After AGS cells were transfected with miR-101 mimics and inhibitors, the proliferation ability of cells was detected, respectively. The results manifested that the proliferation ability of cells transfected with miR-101 mimics was significantly reduced. This indicated that miR-101 could inhibit cell proliferation ($p<0.01$, Figure 3).

Dual-Luciferase Reporter Gene Assay

To further clarify the molecular mechanism of miR-101 in regulating the proliferation and apoptosis of gastric mucosal cells, miRNA target prediction program "miRanda" was applied to predict the putative targets of miR-101. Specifically, miR-101 contains complementary sites in the 3'-UTR of Nrf2 mRNAs (Figure 4A). It was inferred that Nrf2 might participate in the regulation of miR-101. To confirm this finding, Luciferase reporter gene assay was carried out. As shown in Figure 4B, miR-101 mimics transfection markedly reduced the activity of Luciferase reporter genes fused with Nrf2 3'-UTR-WT by 36%. However, the Luciferases of reporter vectors with Nrf2 3'-UTR-Mut were not affected by miR-101 mimics transfection. In addition, Western blotting analysis demonstrated that the protein level of Nrf2 was remarkably decreased after transfection with miR-101 mimics. These data indicated that Nrf2 was negatively regulated by miR-101. Furthermore, the miR-101/Nrf2 axis might play a major role in the proliferation and apoptosis of gastric mucosal epithelial cells.

MiR-101 Overexpression Inhibited the Nrf2/ARE Signaling Pathway

Overexpression of miR-101 notably down-regulated the mRNA and protein levels of Nrf2 in cells (Figure 5). After transfection with miR-101 mimics for 48 h, the expression levels of Nrf2 in different groups showed statistically significant differences ($p<0.01$).

Discussion

The balance between cell proliferation and cell death is essential for maintaining the integrity of human gastric mucosa. More and more evidence has shown that miRNAs exhibit anti-cancer or carcinogenic effects in mediating the tumorigenesis of cancers. In this work, it was observed that miR-101 could inhibit the growth of gastric mucosal cells and promote cell apoptosis *in vitro*. Our results further proved that Nrf2 was the functional target of miR-101. Moreover, miR-101 regulated cellular behaviors through the Nrf2/ARE signaling pathway targeting Nrf2.

MiR-101 is considered a tumor suppressor, which can regulate the growth, apoptosis, migration and invasion of various tumor cells. However, its exact role in gastric adenocarcinoma is still unclear. A recent study has shown that miR-101 expression in gastric cancer tissues is down-regulated when compared with normal tissues. Meanwhile, miR-101 exerts an apoptosis-promoting function in gastric cancer cells. In this work, the results demonstrated that the overexpression of miR-101 significantly inhibited the proliferation and invasion of AGS gastric adenocarcinoma cells. However, the inhibition of miR-101 could markedly promote the proliferation and invasion of cells. Our findings indicated that miR-101-3p served as an anti-cancer factor in gastric adenocarcinoma. The function of a miRNA basically depends on the function of target genes. It has been reported that miR-101 can directly target multiple tumor promoter genes. For example, in

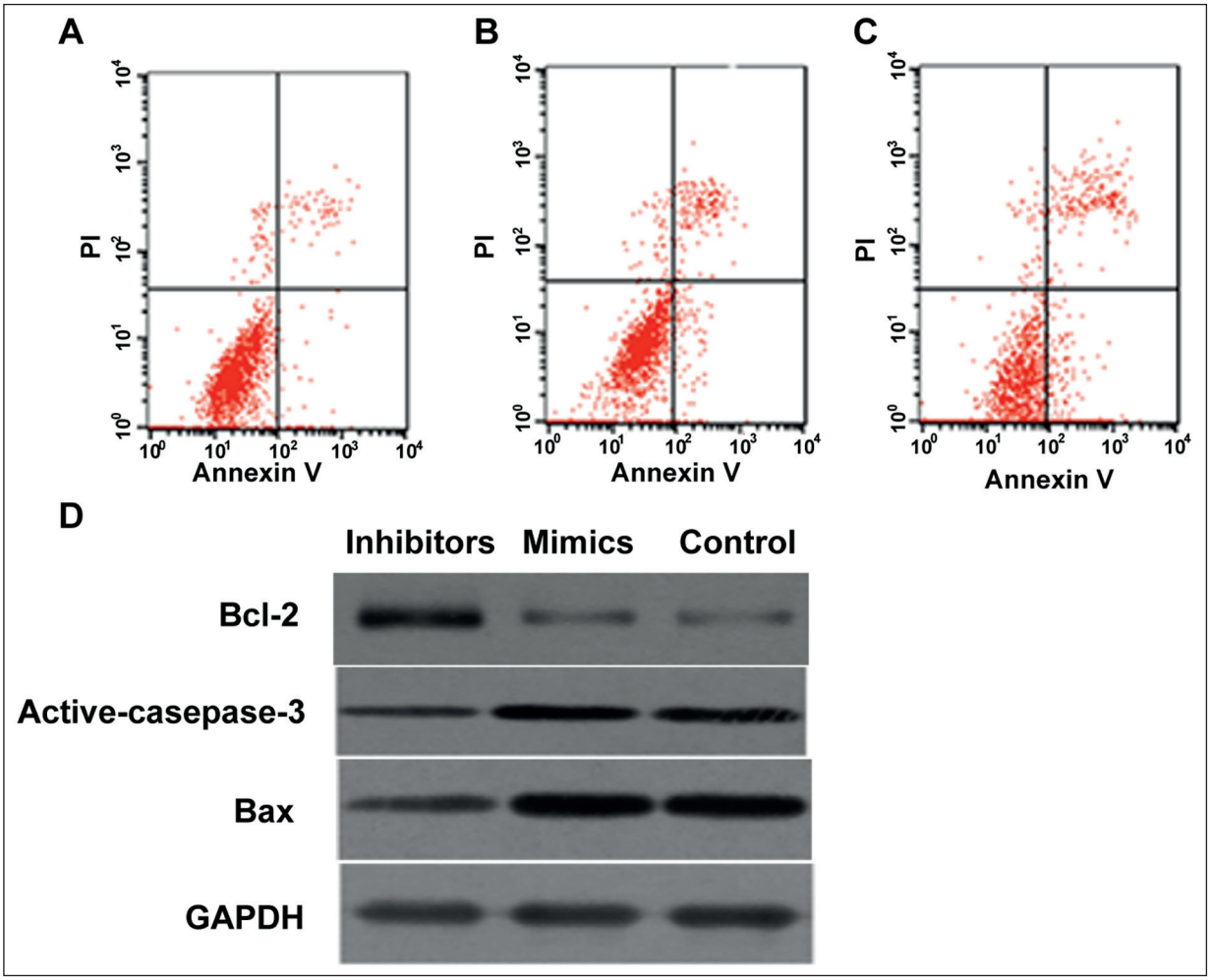

Figure 2. Apoptosis rate detected by flow cytometry. ***A,*** Apoptosis rate in miR-101 inhibitor group [(5.57±2.35) %]. ***B,*** Apoptosis rate in the control group [(8.58±2.28) %]. ***C,*** Apoptosis rate in the miR-101 mimics group [(16.45±4.62) %]. ***D,*** Expression of apoptosis-related proteins detected *via* Western blotting. Transfection with miR-101 mimics remarkably increased the expressions of Bax and active Casepase-3, whereas decreased Bcl-2.

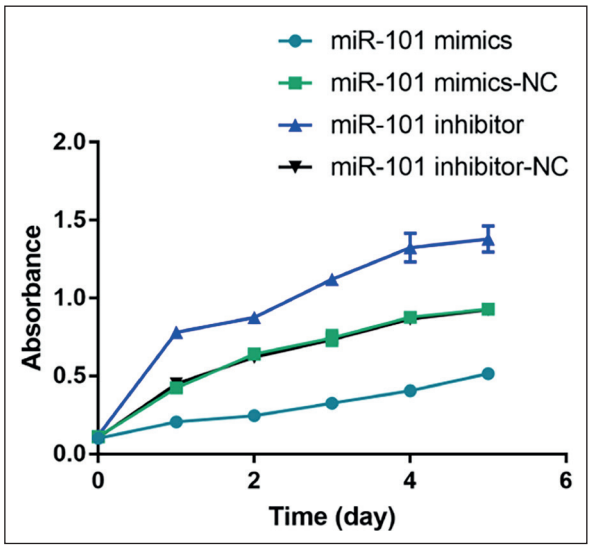

Figure 3. Cell proliferation ability detection. Cell proliferation ability was markedly reduced after transfection with miR-101 mimics (p<0.01).

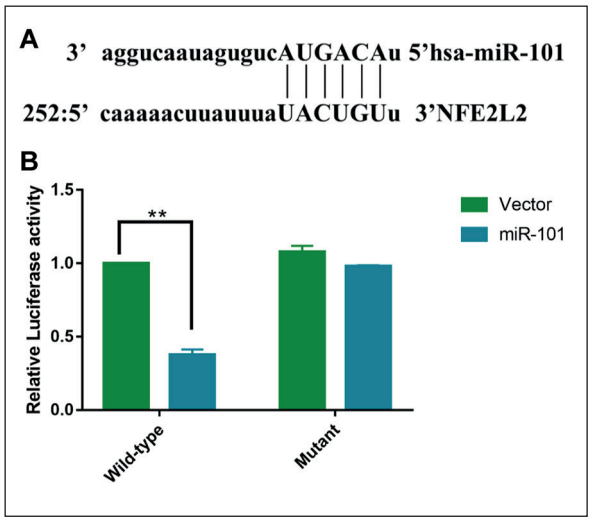

Figure 4. ***A,*** MiR-101 directly targets the 3'-UTR of Nrf2: predicted binding sites of Wnt1 and miR-101 in the 3'-untranslated region (3'UTR). ***B,*** Interaction between miR-101 and Nrf2 determined by Luciferase reporter gene assay.

triple negative breast cancer, miR-101 directly targets AMP-activated protein kinase (AMPK) and regulates tumor metabolism[12]. MiR-101 can also directly target and inhibit gene enhancer EZH2. Meanwhile, it can suppress the expression of mitosis-initiated proto-oncogenes[13,14]. Wang et al[15] have reported that miR-101 inhibits lung cancer progression by targeting the PTEN/AKT signaling pathway. Recently, Wu et al[16] have found that miR-101-3p inhibits HOX transcription RNA (HOTAIR)-induced proliferation and invasion by directly targeting SRF in gastric cancer cells. Interestingly, miR-101 has also been found to be associated with liposarcoma. MiR-101 blocks the autophagy of osteosarcoma cells, thus enhancing chemo-sensitivity of osteosarcoma cells[17]. In this work, we reported the importance of miR-101 in the pathogenesis of gastric mucosal injury for the first time. Meanwhile, Nrf2 could serve as a new target gene for miR-101-3p. Nrf2 is a transcription factor widely expressed and involved in the progression of various cancers[18].

Nrf2 is a redox-sensitive transcription factor, which can be located in the cytoplasm and interact with Keap1 under normal conditions[19]. Once activated, Nrf2 will be transferred to the nucleus and bind to the ARE of the target gene promoter. This may result in the transcription of various

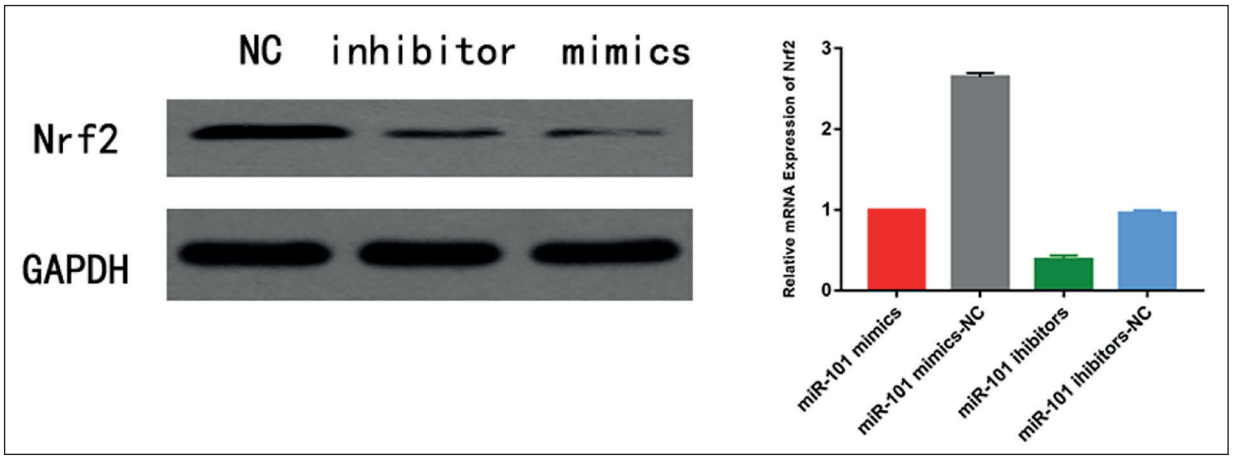

Figure 5. MiR-101 down-regulated Nrf2 expression in cells. After miR-101 overexpression, the mRNA and protein levels of Nrf2 were significantly decreased (p<0.01).

antioxidant and detoxifying enzymes, eventually showing cell protection. The activation of the Nrf2 pathway is a therapeutic target for gastric diseases in this study. Besides, in the past few decades, researchers have found that enhancing Nrf2 in the nucleus or improving its downstream genes can effectively protect cells from oxidative stress-induced cell injury. For example, red pigment inhibits oxidative stress-induced damage in human umbilical vein endothelial cells (HUVECs) by activating the Nrf2 signaling pathway[20]. Moreover, chlorogenic acid protects mouse osteoblast MC3T3-E1 from H_2O_2-induced oxidative stress *via* phosphatidylinositol-3-kinase (PI3K)/protein kinase B (Akt)-mediated Nrf2/HO-1 signaling pathway[21]. Furthermore, the PI3K/Akt signal is considered an important upstream event of the activation of the Nrf2 signaling pathway. In addition, the PI3K/Akt signaling pathway regulates the expression of antioxidant genes in different cells in Nrf2-dependent transcription mode to cope with oxidative stresses.

Conclusions

Our findings first confirmed that the biological behaviors of gastric mucosal epithelial cells were regulated through miR-101/Nrf2-related apoptosis pathway. Therefore, miR-101 might potentially combine with Nrf2 to regulate the injury response of gastric mucosal epithelial cells.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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References

1) Slaby O, Svoboda M, Michalek J, Vyzula R. MicroRNAs in colorectal cancer: translation of molecular biology into clinical application. Mol Cancer 2009; 8: 102.
2) Wu RS, Qiu EH, Zhu JJ, Wang JR, Lin HL. MiR-101 promotes nasopharyngeal carcinoma cell apoptosis through inhibiting Ras/Raf/MEK/ERK signaling pathway. Eur Rev Med Pharmacol Sci 2018; 22: 150-157.
3) Zheng HB, Zheng XG, Liu BP. miRNA-101 inhibits ovarian cancer cells proliferation and invasion by down-regulating expression of SOCS-2. Int J Clin Exp Med 2015; 8: 20263-20270.
4) Strillacci A, Valerii MC, Sansone P, Caggiano C, Sgromo A, Vittori L, Fiorentino M, Poggioli G, Rizzello F, Campieri M, Spisni E. Loss of miR-101 expression promotes Wnt/beta-catenin signalling pathway activation and malignancy in colon cancer cells. J Pathol 2013; 229: 379-389.
5) Lee JM, Johnson JA. An important role of Nrf2-ARE pathway in the cellular defense mechanism. J Biochem Mol Biol 2004; 37: 139-143.
6) Zhao X, Sun G, Zhang J, Ting SM, Gonzales N, Aronowski J. Dimethyl fumarate protects brain from damage produced by intracerebral hemorrhage by mechanism involving Nrf2. Stroke 2015; 46: 1923-1928.
7) Zhao X, Sun G, Zhang J, Strong R, Dash PK, Kan YW, Grotta JC, Aronowski J. Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage. Stroke 2007; 38: 3280-3286.
8) McMahon M, Thomas N, Itoh K, Yamamoto M, Hayes JD. Dimerization of substrate adaptors can facilitate cullin-mediated ubiquitylation of proteins by a "tethering" mechanism: a two-site interaction model for the Nrf2-Keap1 complex. J Biol Chem 2006; 281: 24756-24768.
9) Luo Y, Eggler AL, Liu D, Liu G, Mesecar AD, van Breemen RB. Sites of alkylation of human Keap1 by natural chemoprevention agents. J Am Soc Mass Spectrom 2007; 18: 2226-2232.
10) Vargas MR, Johnson DA, Sirkis DW, Messing A, Johnson JA. Nrf2 activation in astrocytes protects against neurodegeneration in mouse models of familial amyotrophic lateral sclerosis. J Neurosci 2008; 28: 13574-13581.
11) Kim SK, Yang JW, Kim MR, Roh SH, Kim HG, Lee KY, Jeong HG, Kang KW. Increased expression of Nrf2/ARE-dependent anti-oxidant proteins in tamoxifen-resistant breast cancer cells. Free Radic Biol Med 2008; 45: 537-546.
12) Liu P, Ye F, Xie X, Li X, Tang H, Li S, Huang X, Song C, Wei W, Xie X. mir-101-3p is a key regulator of tumor metabolism in triple negative breast cancer targeting AMPK. Oncotarget 2016; 7: 35188-35198.
13) Liu D, Li Y, Luo G, Xiao X, Tao D, Wu X, Wang M, Huang C, Wang L, Zeng F, Jiang G. LncRNA SPRY4-IT1 sponges miR-101-3p to promote proliferation and metastasis of bladder cancer cells through up-regulating EZH2. Cancer Lett 2017; 388: 281-291.
14) Liu XY, Liu ZJ, He H, Zhang C, Wang YL. MicroRNA-101-3p suppresses cell proliferation, invasion and enhances chemotherapeutic sensitivity in salivary gland adenoid cystic carcinoma by targeting Pim-1. Am J Cancer Res 2015; 5: 3015-3029.
15) Liu Z, Wang J, Mao Y, Zou B, Fan X. MicroRNA-101 suppresses migration and invasion via targeting

vascular endothelial growth factor-C in hepatocellular carcinoma cells. Oncol Lett 2016; 11: 433-438.

16) Wu X, Zhou J, Wu Z, Chen C, Liu J, Wu G, Zhai J, Liu F, Li G. miR-101-3p suppresses HOX transcript antisense RNA (HOTAIR)-induced proliferation and invasion through directly targeting SRF in gastric carcinoma cells. Oncol Res 2017; 25: 1383-1390.

17) Chang Z, Huo L, Li K, Wu Y, Hu Z. Blocked autophagy by miR-101 enhances osteosarcoma cell chemosensitivity *in vitro*. ScientificWorldJournal 2014; 2014: 794756.

18) Yu R, Chen C, Mo YY, Hebbar V, Owuor ED, Tan TH, Kong AN. Activation of mitogen-activated protein kinase pathways induces antioxidant response element-mediated gene expression via a Nrf2-dependent mechanism. J Biol Chem 2000; 275: 39907-39913.

19) Kim YJ, Choi WI, Jeon BN, Choi KC, Kim K, Kim TJ, Ham J, Jang HJ, Kang KS, Ko H. Stereospecific effects of ginsenoside 20-Rg3 inhibits TGF-beta1-induced epithelial-mesenchymal transition and suppresses lung cancer migration, invasion and anoikis resistance. Toxicology 2014; 322: 23-33.

20) Hu R, Shen G, Yerramilli UR, Lin W, Xu C, Nair S, Kong AN. In vivo pharmacokinetics, activation of MAPK signaling and induction of phase II/III drug metabolizing enzymes/transporters by cancer chemopreventive compound BHA in the mice. Arch Pharm Res 2006; 29: 911-920.

21) Jeon WK, Hong HY, Kim BC. Genipin up-regulates heme oxygenase-1 via PI3-kinase-JNK1/2-Nrf2 signaling pathway to enhance the anti-inflammatory capacity in RAW264.7 macrophages. Arch Biochem Biophys 2011; 512: 119-125.