

Original Article

MicroRNA-19a inhibits nasopharyngeal carcinoma CNE1 cells viability and migration, but induces apoptosis by inhibition of Bcl-2

Hongbin Xu, Long Li, Yuan Wu, Haicui He, Desheng Hu

Department of Radiation Oncology, Hubei Cancer Hospital, Wuhan, China

Received February 19, 2017; Accepted May 3, 2017; Epub June 1, 2017; Published June 15, 2017

Abstract: Nasopharyngeal carcinoma (NPC) is a highly metastatic cancer, frequently occurring in Southeast Asia and Southern China. Several microRNAs (miRNAs) have been shown to have an inhibitory effect on NPC, whereas the effect of miR-19a on NPC remains unclear. In this study, an effort has been made to study the potential effect of miR-19a on NPC cell viability, migration and apoptosis. Human NPC CNE1 cells were transfected with miR-19a mimic, miR-19a inhibitor, Bcl-2 targeted small interfering RNA (siRNA) and corresponding controls, respectively. RT-qPCR and western blot were performed to determine the expressions of miR-19a at mRNA and protein levels. Subsequently, cell viability, migration and apoptosis of the transfected cells were analyzed by Cell Counting Kit-8 (CCK-8), Transwell and flow cytometry assays. Analysis of signaling pathways were detected by western blot. Results showed that miR-19a overexpression inhibited cell viability and migration but promoted apoptosis in CNE1 cells ($P < 0.05$ or $P < 0.001$). Bcl-2 was negatively regulated by miR-19a, and Bcl-2 silence could dramatically induce apoptosis, and abolish miR-19a suppression induce increases in cell viability and migration ($P < 0.05$ or $P < 0.01$ or $P < 0.001$). Besides, Bcl-2 silence abolished miR-19a suppression induced activation of p38MAPK, p65 and I κ B α . These results indicated that miR-19a inhibited cell viability, migration and induced apoptosis by down-regulating Bcl-2 in NPC cells.

Keywords: MicroRNA-19a, nasopharyngeal carcinoma, viability, migration, apoptosis, Bcl-2

Introduction

Nasopharyngeal carcinoma (NPC) is a rare malignant tumor deriving from epithelial cells located in the nasopharynx [1]. The incidence of NPC is higher in southern China and southeast Asia than in western countries [2]. Currently, the most effective treatment for NPC is radiotherapy, whereas the 5-year overall survival rate remains less than 50% [3]. It is a complex process of NPC occurrence, development, and metastasis. Growing evidence supports that microRNAs (miRNAs) play an important role in NPC [4]. Therefore, a better understanding of the molecular mechanisms of NPC will contribute to the development of new therapeutic and diagnostic strategies.

MiRNAs are a family of small non-coding RNAs that range from 20-22 nucleotides in length [5]. An increasing number of studies have reported

that miRNAs play important roles in the regulation of biological processes, such as cell differentiation, proliferation, migration and apoptosis [6]. Abnormal expressions in miRNAs are associated with cellular functions and also have been observed in a variety of diseases, including cancer [7]. Many miRNAs target oncogenes and tumor suppressor genes with direct involvement in carcinogenesis [8]. Currently, several miRNAs, such as miR-15a, miR-24, and miR-608 have been demonstrated to target specific mRNAs to regulate NPC development and progression [3]. Thus, it seems that modulation of miRNA could be a new approach for gene therapy of NPC [9]. MiR-19a belongs to miR-17-92 cluster, is associated with the pathogenesis and development of variety of human cancers, including cervical carcinoma [10], hepatocellular carcinoma [11], lung carcinoma [12] and prostate carcinoma [13]. However details of the function and molecular mechanism of miR-19a

Effect of miR-19a in nasopharyngeal carcinoma

in NPC cell proliferation, migration and apoptosis have not been well investigated.

Bcl-2 (B-cell lymphoma 2), encoded in humans by the BCL2 gene, is the founding member of the Bcl-2 family of regulator proteins that regulate cell apoptosis [14]. It is specifically considered an important anti-apoptotic protein but it is not considered a proto-oncogene because it is not a growth signal transducer [15]. Damage to the Bcl-2 gene has been identified as a cause of a number of cancers, including melanoma, breast cancer, prostate cancer, chronic lymphocytic leukemia and lung cancer [16]. Bcl-2 gene also has been reported as a possible cause of schizophrenia and autoimmunity [17]. Despite these observations, the Bcl-2 regulation in NPC, particularly through miRNAs remains unclear.

In the present study, we intended to find out the molecular mechanism of miR-19a in NPC cells, as well as to find out whether miR-19a plays deleterious or protective role in NPC by regulating Bcl-2. Human NPC cell line CNE1 was transfected with miR-19 mimic, miR-19 inhibitor, Bcl-2 targeted small interfering RNA (siRNA) and their controls. Then cell viability, migration and apoptosis were determined by Cell Counting Kit-8 (CCK-8), Transwell and flow cytometry assays. Besides, the signal pathways of mitogen-activated protein kinase (MAPK) and nuclear factor (NF)- κ B were detected by western blot. The results could provide a new insight into the potential therapeutic target for NPC.

Materials and methods

Cell culture

The Hubei Cancer Hospital (Wuhan, China) kindly provided the human NPC cell line CNE1 for the present study. CNE1 cells were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium (HyClone, Logan City, Utah, USA) with 10% fetal bovine serum (FBS) (HyClone) at 37°C with 5% CO₂ [18].

Cell transfection

The mimic control, miR-19a mimic, inhibitor control, miR-19a inhibitor and Bcl-2 targeted siRNA were synthesized by Genepharma (Shanghai, China). CNE1 cells were seeded in 6-

well plates and cultured overnight at 37°C in a humidified atmosphere of 95% air and 5% CO₂. Subsequently, transfections were performed by using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol.

Cell viability

Cells were seeded in 96-well plate with 5000 cells/well for adherence, and then cell viability was assessed by a Cell Counting Kit-8 (CCK-8, Dojindo Molecular Technologies, Gaithersburg, MD). Briefly, the cells were cultivated in 96-well plates and transfected with miR-19a mimic, miR-19a inhibitor, Bcl-2 siRNA, mimic control and inhibitor control. Forty-eight hours later, 10 μ l of CCK-8 solution was added to the culture medium, and the cultures were incubated for 1 h at 37°C in humidified 95% air and 5% CO₂. The absorbance was measured at 450 nm using a Microplate Reader (Bio-Rad, Hercules, CA).

Migration assay

Cell migration was determined by using a modified two-chamber migration assay with a pore size of 8 μ m. In brief, cells suspended in 200 μ l of serum-free medium were seeded on the upper compartment of 24-well transwell culture chamber, and 600 μ l of complete medium was added to the lower compartment. After a 12 h-incubation at 37°C, cells were fixed with 4% methanol (NIST, USA) for 30 min. Non-traversed cells were removed from the upper surface of the filter carefully with a cotton swab. Cells that migrated to the lower side of the membrane were stained with 0.1% crystal violet (Merck, Darmstadt, Germany) for 20 min, and images were captured using an inverted microscope (Olympus, Tokyo, Japan) [19].

Apoptosis assay

Cell apoptosis was performed by using the FITC Annexin V Apoptosis Detection Kit (Beijing Biosea Biotechnology, Beijing, China). Briefly, miR-transfected cells were washed twice in PBS and stained in 5 μ l PI and 10 μ l FITC-Annexin V in the presence of 50 μ g/ml RNase A (Sigma-Aldrich), and then incubated for 1 h at room temperature in the dark. Flow cytometry analysis was done by using a FACS can (Beckman Coulter, Fullerton, CA, USA).

Effect of miR-19a in nasopharyngeal carcinoma

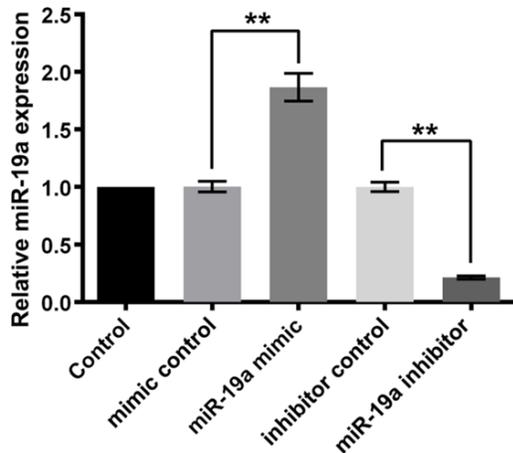


Figure 1. MiR-19a expression was up-regulated by miR-19a mimic. CNE1 cells were transfected with miR-19a mimic, miR-19a inhibitor or controls and then relative miR-19a expression in transfection cells were detected by RT-qPCR. MiR-19a: microRNA-19a; RT-qPCR: quantitative real time polymerase chain reaction; NPC: nasopharyngeal carcinoma; ** $P < 0.01$.

RNA extraction and quantitative real-time PCR (RT-qPCR)

Total RNAs of CNE1 cells were extracted from transfected cells by Trizol Reagent (Invitrogen). For miR-19a detection, reverse-transcribed complementary DNA (cDNA) was synthesized with the PrimeScript RT reagent Kit (TaKaRa, Dalian, China), and RT-qPCR was performed with SYBR Premix ExTaq (TaKaRa) with the Stratagene Mx3000P real-time PCR system (Agilent Technologies, Inc., Santa Clara, CA, USA). Expression levels were normalized against the endogenous snRNA U6 control. For mRNA analyses, cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase (Promega, Madison, WI, USA). RT-qPCR was performed with SYBR Premix ExTaq with the Stratagene Mx3000P real-time PCR system. GAPDH was used as internal controls for mRNA quantification. The relative expression ratio of mRNA was calculated by the $2^{-\Delta\Delta CT}$ method. PCR reactions for each gene were repeated three times. Independent experiments were done in triplicate.

Western blot

The protein used for western blot was extracted using Radio Immunoprecipitation Assay (RIPA) lysis buffer (Beyotime Biotechnology, Shanghai,

China) supplemented with protease inhibitors (Roche, Basle, Switzerland). The proteins were quantified using the BCA™ Protein Assay Kit (Pierce, Appleton, WI, USA). Equal amounts of protein were subjected to 10-12% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) gels, and then were transferred onto polyvinylidene fluoride (PVDF) membranes (Maibio, Shanghai, China). The transfer membranes were then incubated with 5% skim milk (Nestlé, Shuangcheng, China) in Tris Buffered Saline with Tween (TBST) for 2 h and probed with the following primary antibodies overnight at 4°C at a dilution of 1:1,000: BCL2-Associated X (Bax, ab32503), Bcl-2 (ab32124), pro-caspase-3 (ab32150), cleaved caspase-3 (ab13585), pro-caspase-9 (ab32068), phosphorylated (p)-p38MAPK (ab4822), p38MAPK (ab31828), p-p65 (ab86299), p65 (ab16502), p-IkBα (ab92700), IκBα (ab32518), GAPDH (ab8245, Abcam, Cambridge, UK) and cleaved caspase-9 (#9501; Cell signaling Technology). Thereafter, the membranes were incubated with the membrane at 4°C overnight, followed by incubation with secondary antibody (1:5000, Abcam, USA) marked by horseradish peroxidase for 1 h at room temperature. After rinsing, the PVDF membrane carried blots and antibodies were transferred into the Bio-Rad ChemiDoc™ XRS system, and then 200 μl Immobilon Western Chemiluminescent HRP Substrate (Millipore, MA, US) was added to cover the membrane surface. The signals were captured and the intensity of the bands was quantified using Image Lab™ Software (Bio-Rad).

Statistical analysis

All experiments were repeated three times. The results of multiple experiments are presented as the mean \pm SD. Statistical analyses were performed using Graphpad 6.0 statistical software (GraphPad Software, San Diego, CA), and one-way analysis of variance (ANOVA). A P -value of < 0.05 was considered to indicate statistically significant results.

Results

MiR-19a inhibited cell viability and migration, but promoted apoptosis of CNE1 cells

In order to determine whether miR-19a was involved in the regulation of human NPC tumorigenesis, miR-19a mimic, miR-19a inhibitor, and

Effect of miR-19a in nasopharyngeal carcinoma

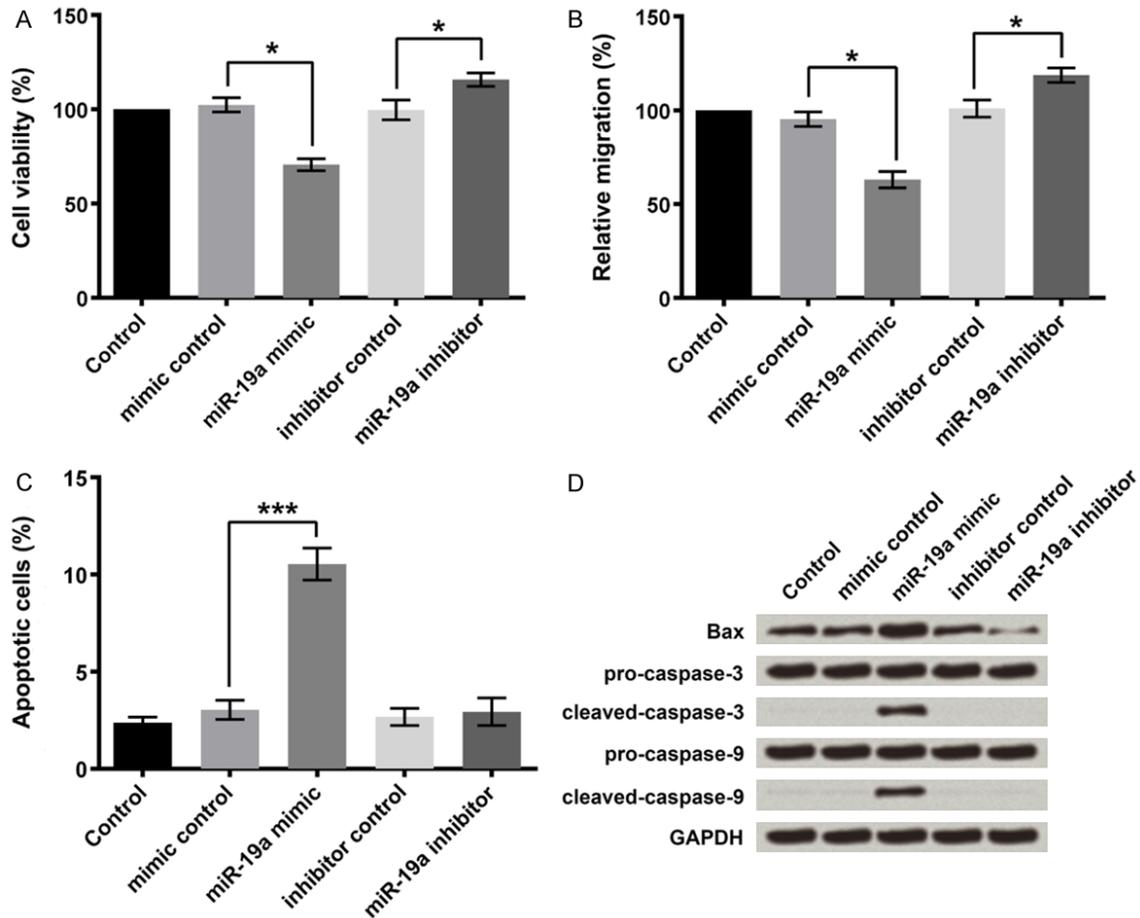


Figure 2. MiR-19a inhibited cell viability and migration, but induced apoptosis in CNE1 cells. MiR-19a mimic, miR-19a inhibitor or controls were transfected into CNE1 cells, and then (A) cell viability, (B) migration, (C) apoptosis, and (D) the protein expressions of apoptosis-related factors were respectively detected by CCK-8, Transwell, flow cytometry, and western blot assays. MiR-19a: microRNA-19a; CCK-8: Cell Counting Kit-8; NPC: nasopharyngeal carcinoma; * $P < 0.05$, *** $P < 0.001$.

their corresponding controls were respectively transfected into CNE1 cells. Transfection efficiency was examined by RT-qPCR, and results showed that miR-19a expression was significantly up-regulated by miR-19a mimic ($P < 0.01$) and down-regulated by miR-19 inhibitor ($P < 0.01$; **Figure 1**).

Next, CCK-8, Transwell and flow cytometry assays were conducted to investigate the biological functions of miR-19a on CNE1 cells. As results shown in **Figure 2A** and **2B**, cell viability and migration rates in miR-19a-overexpressing cells were much lower than those in mimic control ($P < 0.05$), while these two rates in miR-19a-suppressing cells were higher than in inhibitor control ($P < 0.05$). Results in **Figure 2C** showed an increase of apoptotic cells percent-

age in miR-19a-overexpressing cells compared to mimic control ($P < 0.001$). However, miR-19a suppression had no obvious impact on cell apoptosis. In addition, western blot analysis was performed to detect the protein expression levels of apoptosis-related factors. Results showed that miR-19a overexpression up-regulated Bax expression and activated cleaved caspase-3 and cleaved caspase-9 expressions, while miR-19a suppression showed an opposite impact on these factors expressions. The protein levels of pro-caspase-3 and pro-caspase-9 were not significantly regulated by miR-19a overexpression or suppression (**Figure 2D**). These results indicated that miR-19a might be act as a tumor suppressor gene by inhibiting NPC cells viability and migration, as well as improving cell apoptosis.

Effect of miR-19a in nasopharyngeal carcinoma

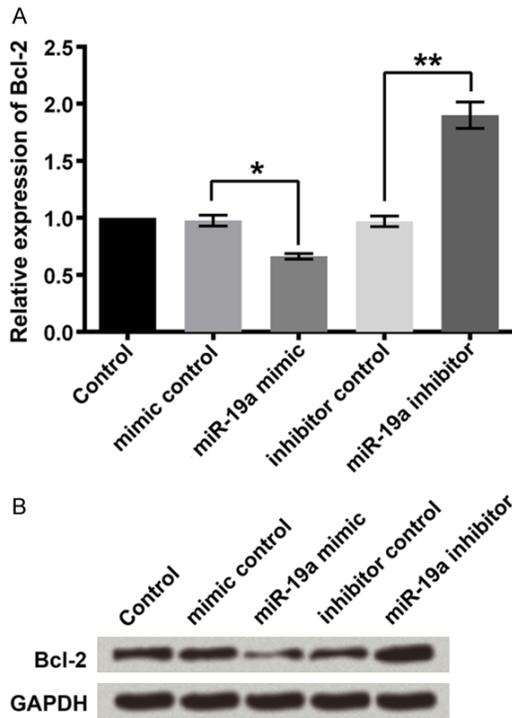


Figure 3. Bcl-2 was negatively regulated by miR-19a. CNE1 cells were transfected with miR-19a mimic, miR-19a inhibitor or controls. After transfection for 48 h, the (A) mRNA level and (B) protein level of Bcl-2 in miR-19a-overexpressing cells and miR-19a-suppressing cells were examined by RT-qPCR and western blot. Bcl-2: B-cell lymphoma 2; miR-19a: microRNA-19a; RT-qPCR: quantitative real time polymerase chain reaction; NPC: nasopharyngeal carcinoma; * $P < 0.05$, ** $P < 0.01$.

Bcl-2 was negatively regulated by miR-19a

To further explore the effect of miR-19a on the regulation of NPC cells, miR-19a mimic, miR-19a inhibitor and their corresponding controls were transfected into CNE1 cells, and the mRNA and protein levels of Bcl-2 were detected by RT-qPCR and western blot. Results showed that the expression of Bcl-2 was significantly down-regulated in miR-19a-overexpressing cells, while was obviously up-regulated in miR-19a-suppressing cells ($P < 0.05$, $P < 0.01$) (Figure 3A and 3B). Thus, we inferred that Bcl-2 was negatively regulated by miR-19a.

Bcl-2 silence induced apoptosis and abolished the promoting effect of miR-19a suppression on cell viability and migration

To investigate whether Bcl-2 was involved in the biological functions underlied the effects

of miR-19a on CNE1 cells; Bcl-2 was silenced by using its specific siRNA. As shown in Figure 4A-C, miR-19a suppression together with Bcl-2 silence significantly reduced cell viability and migration, while promoted apoptotic cells rate when compared with miR-19a suppression ($P < 0.01$ or $P < 0.001$). In addition, western blot analytical results in Figure 4D showed that miR-19a suppression together with Bcl-2 silence up-regulated the protein levels of Bax and activated cleaved caspase-3 and cleaved caspase-9 expressions compared with miR-19a suppression. The protein level of pro-caspase-3 and pro-caspase-9 were not significantly change. These results indicated that Bcl-2 silence could abolish the promoting effect of miR-19a suppression on cell viability and migration, and could promote apoptosis.

MiR-19a regulated the expression of Bcl-2 via MAPK and NF- κ B pathways

MAPKs and NF- κ B pathways have been reported to involve in different biological and pathological processes [20]. In our study, we analyzed the MAPK and NF- κ B pathways by detection the expressions of p38MAPK, p65 and I κ B α . Western blot results showed that the expression of p-p38MAPK, p-p65 and p-I κ B α were significantly promoted by miR-19a suppression, whereas these activations were remarkably abolished by Bcl-2 silence (Figure 5). Results indicated that miR-19a regulated the expression of Bcl-2 possibly via MAPK and NF- κ B signaling pathways, and further regulated cell viability, migration and apoptosis.

Discussion

More and more evidences indicated that miRNAs might contribute to the pathogenesis of tumor, and their functions and potential application have become hot research areas [21]. To date, approximate 32 miRNAs were studied in NPC [22], whereas the effect of miR-19a on NPC remains unclear. In the present study, we demonstrated that overexpression of miR-19a inhibited CNE1 cells viability and migration, but promoted apoptosis. In addition, the suppressive effect of miR-19a in CNE1 cells possibly via inhibition of Bcl-2. Further, miR-19a suppression promoted the activations of p38MAPK, p65 and I κ B α , while these activations were remarkably abolished by Bcl-2 silence.

Effect of miR-19a in nasopharyngeal carcinoma

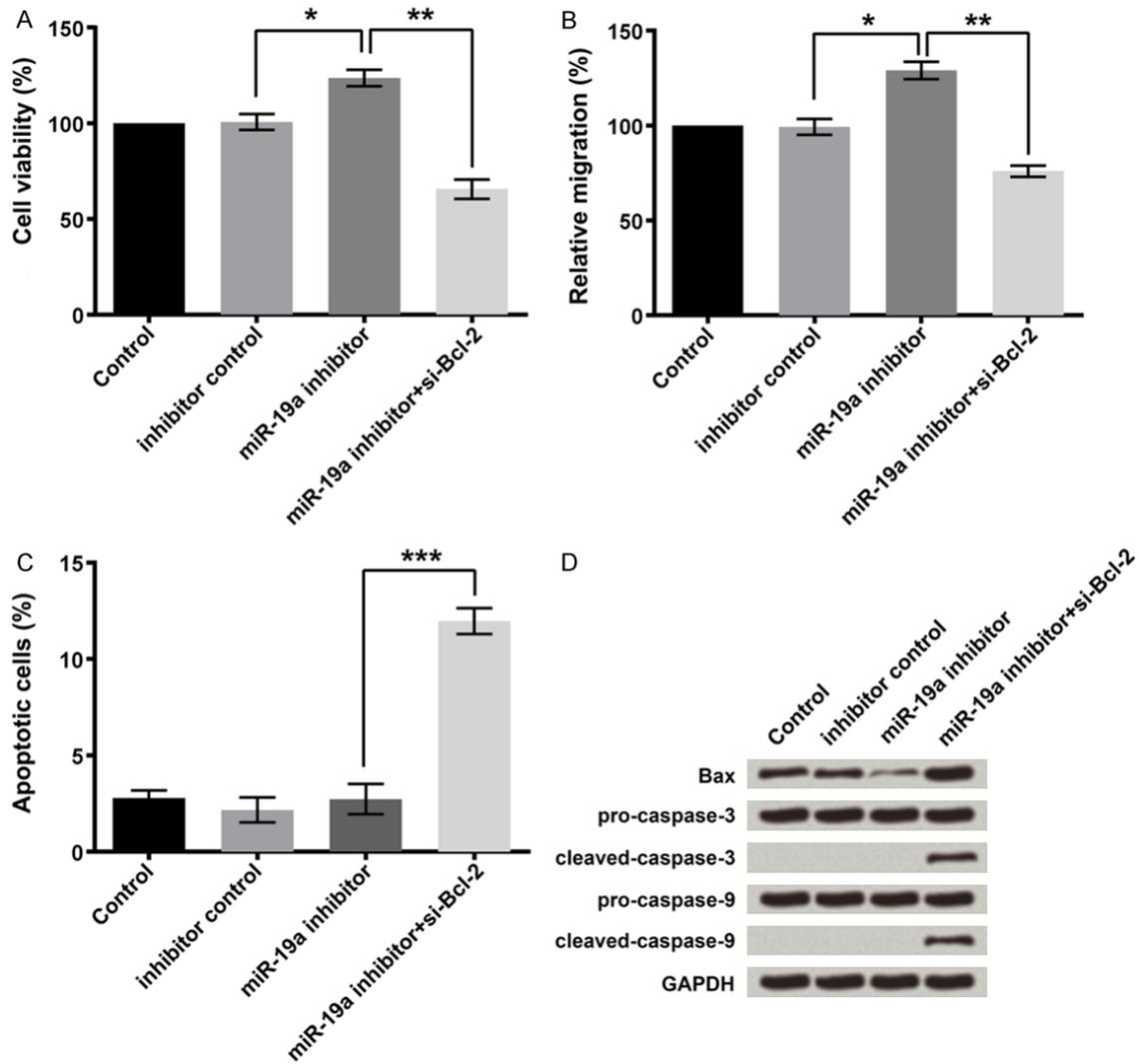


Figure 4. Bcl-2 silence induced apoptosis and abolished the promoting effect of miR-19a suppression on cell viability and migration. CNE1 cells were transfected with miR-19a inhibitor, miR-19a inhibitor + si-Bcl-2 and their corresponding controls, and then (A) cell viability, (B) cell migration, (C) apoptosis and (D) the protein expressions of apoptosis-related factors were respectively detected by CCK-8, Transwell, flow cytometry, and western blot assays. Bcl-2: B-cell lymphoma 2; miR-19a: microRNA-19a; siRNA: small interfering RNA; CCK-8: Cell Counting Kit-8; NPC: nasopharyngeal carcinoma; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Aberrant expression of miR-19a have been reported frequently in cancers, such as lung cancer [23], gastric cancer [24], thyroid cancer and cervical cancer [25]. In addition, miR-19a regulated key genes related to cell viability, migration and apoptosis. Specifically, miR-19a was up-regulated in lung cancer and exhibited promoting effects on cell proliferation and migration [23]. Besides, up-regulation of miR-19a enhanced cell proliferation and augmented the tumorigenesis of HepG2 cells *in vitro* [26]. Similarly, results of our study showed that overexpression of miR-19a inhibited cell viability

and migration, but promoted cell apoptosis in CNE1 cells. We inferred that miR-19a might act as a tumor suppressor of NPC.

Bcl-2 gene is originally identified at a breakpoint of translocations commonly occurring in follicular B cell lymphomas and was later found to play a key role in cell survival and inhibition of apoptosis [27, 28]. Many studies have already showed that Bcl-2 could be regulated by several miRNAs [29]. For instance, Bcl-2 has been reported as a direct target of miR-15 and miR-16 in chronic lymphocytic leukemia [30]. In

Effect of miR-19a in nasopharyngeal carcinoma

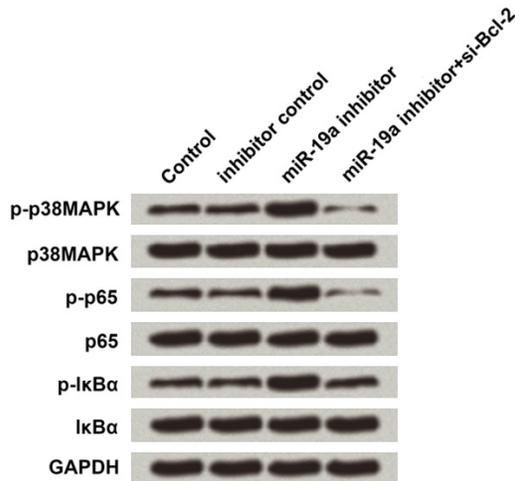


Figure 5. MiR-19a regulated the expression of Bcl-2 via MAPK and NF- κ B pathways. CNE1 cells were transfected with miR-19a inhibitor, miR-19a inhibitor + si-Bcl-2 and their corresponding controls, then the protein levels of MAPK and NF- κ B pathways factors were detected by western blot. MiR-19a: microRNA-19a; Bcl-2: B-cell lymphoma 2; siRNA: small interfering RNA; MAPK: mitogen-activated protein kinase; NF- κ B: nuclear factor- κ B.

addition, Su *et al.* reported that Bcl-2 was negatively regulated by miR-429 in NPC cells [31]. However, there was not adequate information to prove the effect of Bcl-2 via regulating miR-19a on NPC. Thus, our study for the first time uncovered the underlying relationship between miR-19a and Bcl-2 in NPC. These results demonstrated that Bcl-2 was negatively regulated by miR-19a in NPC. Moreover, Bcl-2 silence could abolish the promoting effect of miR-19a suppression on cell viability and migration, and could promote apoptosis. Thus it could be seen that Bcl-2 plays a key role in cell viability, migration and apoptosis through regulation of miR-19a.

MAPKs (including ERK, JNK and p38) are serine-threonine kinases that regulate intracellular signaling associated with several cellular activities, such as cell proliferation, gene expression, differentiation, mitosis, cell survival, and apoptosis [32]. The transcription factors of NF- κ B family are common and pleiotropic molecules that regulate the expression of more than 150 genes involved in a wide range of processes, including inflammation, immunity, cell proliferation, differentiation, and survival [33]. Many studies have established that MAPKs and NF- κ B pathways play important roles in dif-

ferent biological and pathological processes [34]. Kutlu *et al.* found that up-regulation of miR-1182 activated MAPK signal pathway, thereby inducing melanoma cells apoptosis [35]. Moreover, Kim *et al.* demonstrated that miR-125a/b induced diffuse large B-cell lymphoma apoptosis via activating NF- κ B signal pathway [36]. However, to our knowledge, none of studies have reported the effect of Bcl-2 on MAPK and NF- κ B signaling pathways by regulation of miR-19a. Herein, our study analyzed the activation of MAPK and NF- κ B pathways by the expression of p38, p65 and I κ B α . These results suggested that miR-19a suppression promoted the activations of p38MAPK, p65 and I κ B α , while these activations were remarkably abolished by Bcl-2 silence which indicating miR-19a regulated the expression of Bcl-2 via MAPK and NF- κ B pathways.

To sum up, this study demonstrated that miR-19a inhibited cell viability and migration, but induced apoptosis in CNE1 cells, which indicating miR-19a may be a tumor suppressor gene of NPC. Besides, the tumor suppressive effects of miR-19a on CNE1 cells possibly via inhibition of Bcl-2, and inactivation of MAPK and I κ B α pathways. These results provides basic information for the potential usages of miR-19a in treating NPC, but further studies on the regulatory mechanisms remain necessary.

Disclosure of conflict of interest

None.

Address correspondence to: Desheng Hu, Department of Radiation Oncology, Hubei Cancer Hospital, 116 South Zhuodaoquan Road, Wuhan 430-079, China. E-mail: hudesheng1122@126.com

References

- [1] Kontny U, Franzen S, Behrends U, Böhrlen M, Christiansen H, Delecluse H, Eble M, Feuchtinger T, Gademann G and Granzen B. Diagnosis and treatment of nasopharyngeal carcinoma in children and adolescents-recommendations of the GPOH-NPC study group. *Klin Pädiatr* 2016; 228: 105-12.
- [2] Kang M, Xiao J, Wang J, Zhou P, Wei T, Zhao T and Wang R. MiR-24 enhances radiosensitivity in nasopharyngeal carcinoma by targeting SP1. *Cancer Med* 2016; 5: 1163-73.
- [3] Zhu K, He Y, Xia C, Yan J, Hou J, Kong D, Yang Y and Zheng G. MicroRNA-15a inhibits prolifera-

Effect of miR-19a in nasopharyngeal carcinoma

- tion and induces apoptosis in CNE1 nasopharyngeal carcinoma cells. *Oncol Res* 2016; 24: 145-151.
- [4] Huang T, Yin L, Wu J, Gu JJ, Wu JZ, Chen D, Yu HL, Ding K, Zhang N and Du MY. MicroRNA-19b-3p regulates nasopharyngeal carcinoma radiosensitivity by targeting TNFAIP3/NF- κ B axis. *J Exp Clin Cancer Res* 2016; 35: 188.
- [5] Murakami Y and Kawada N. MicroRNAs in hepatic pathophysiology. *Hepatol Res* 2017; 47: 60-69.
- [6] Eriksen AH, Andersen RF, Pallisgaard N, Sørensen FB, Jakobsen A, Hansen TF. MicroRNA expression profiling to identify and validate reference genes for the relative quantification of microRNA in rectal cancer. *PLoS One* 2016; 11: e0150593.
- [7] Riordan AM, Thomas MK, Ronnekleiv-Kelly S, Warner T, Geiger PG and Kennedy GD. Utility of micro-ribonucleic acid profile for predicting recurrence of rectal cancer. *J Surg Res* 2012; 177: 87-92.
- [8] Mestdagh P, Vlierberghe PV, An DW, Muth D, Westermann F, Speleman F and Vandesompele J. A novel and universal method for microRNA RT-qPCR data normalization. *Genome Biol* 2009; 10: R64.
- [9] Von BM, Pandarakalam JJ, Kroon L, Loeser H, Herden J, Braun G, Wendland K, Dienes HP, Engelmann U and Engelmann U. MicroRNA 15a, inversely correlated to PKC α , is a potential marker to differentiate between benign and malignant renal tumors in biopsy and urine samples. *Am J Pathol* 2012; 180: 1787-1797.
- [10] Xu XM, Wang XB, Chen MM, Liu T, Li YX, Jia WH, Liu M, Li X and Tang H. MicroRNA-19a and -19b regulate cervical carcinoma cell proliferation and invasion by targeting CUL5. *Cancer Lett* 2012; 322: 148-158.
- [11] Baik SH, Lee J, Lee YS, Jang JY and Kim CW. ANT2 shRNA downregulates miR-19a and miR-96 through the PI3K/Akt pathway and suppresses tumor growth in hepatocellular carcinoma cells. *Exp Mol Med* 2016; 48: e222.
- [12] Wang X and Chen Z. MicroRNA-19a functions as an oncogenic microRNA in non-small cell lung cancer by targeting the suppressor of cytokine signaling 1 and mediating STAT3 activation. *Int J Mol Med* 2015; 35: 839-46.
- [13] Wang SY, Shiboski S, Belair CD, Cooperberg MR, Simko JP, Stoppler H, Cowan J, Carroll PR and Blleloch R. miR-19, miR-345, miR-519c-5p serum levels predict adverse pathology in prostate cancer patients eligible for active surveillance. *PLoS One* 2014; 9: e98597.
- [14] Anderson MA, Deng J, Seymour JF, Tam C, Kim SY, Fein J, Yu L, Brown JR, Westerman D and Si EG. The BCL2 selective inhibitor venetoclax induces rapid onset apoptosis of CLL cells in patients via a TP53 independent mechanism. *Blood* 2016; 127: 3215-24.
- [15] Dai G. Myricanol induces apoptotic cell death and anti-tumor activity in non-small cell lung carcinoma in vivo. *Int J Mol Sci* 2015; 16: 2717-2731.
- [16] Tzifi F, Economopoulou C, Gourgiotis D, Ardanian A, Papageorgiou S and Scorilas A. The role of BCL2 family of apoptosis regulator proteins in acute and chronic leukemias. *Adv Hematol* 2012; 2012: 524308.
- [17] Glantz LA, Gilmore JH, Lieberman JA and Jarskog LF. Apoptotic mechanisms and the synaptic pathology of schizophrenia. *Schizophr Res* 2006; 81: 47-63.
- [18] Wang Z, Zeng Q, Chen T, Liao K, Bu Y, Hong S and Hu G. Silencing NFBD1/MDC1 enhances the radiosensitivity of human nasopharyngeal cancer CNE1 cells and results in tumor growth inhibition. *Cell Death Dis* 2015; 6: e1849.
- [19] Lv X, Li Y, Qian M, Ma C, Jing H, Wen Z and Qian D. ADAM17 silencing suppresses the migration and invasion of non-small cell lung cancer. *Mol Med Rep* 2014; 9: 1935-1940.
- [20] Sun R, Zhang Y, Ma S, Qi H, Wang M, Duan J, Ma S, Zhu X, Li G and Wang H. Down-regulation of mitogen-activated protein kinases and nuclear factor- κ B signaling is involved in rapamycin suppression of TLR2-induced inflammatory response in monocytic THP-1 cells. *Microbiol Immunol* 2015; 59: 614-622.
- [21] Zhang J, Kong X, Li J, Luo Q, Li X, Shen L, Chen L and Fang L. miR-96 promotes tumor proliferation and invasion by targeting RECK in breast cancer. *Oncol Rep* 2013; 31: 1357-63.
- [22] Tan G, Tang X and Tang F. The role of microRNAs in nasopharyngeal carcinoma. *Tumor Biol* 2015; 36: 69-79.
- [23] Hu W, Jin P, Ding C and Liu W. miR-19a/b modulates lung cancer cells metastasis through suppression of MXD1 expression. *Oncol Lett* 2016; 12: 1901-1905.
- [24] Qin S, Ai F, Ji WF, Rao W, Zhang HC and Yao WJ. miR-19a promotes cell growth and tumorigenesis through targeting SOCS1 in gastric cancer. *Asian Pac J Cancer Prev* 2013; 14: 835-840.
- [25] Wu TY, Zhang TH, Qu LM, Feng JP, Tian LL, Zhang BH, Li DD, Sun YN and Liu M. MiR-19a is correlated with prognosis and apoptosis of laryngeal squamous cell carcinoma by regulating TIMP-2 expression. *Int J Clin Exp Pathol* 2013; 7: 56-63.
- [26] Baik SH, Lee J, Lee YS, Jang JY and Kim CW. ANT2 shRNA downregulates miR-19a and miR-96 through the PI3K/Akt pathway and suppresses tumor growth in hepatocellular carcinoma cells. *Exp Mol Med* 2016; 48: e222.
- [27] Szegezdi E, Macdonald DC, Ní CT, Gupta S and Samali A. Bcl-2 family on guard at the ER. *Am J Physiol Cell Physiol* 2009; 296: C941-53.

Effect of miR-19a in nasopharyngeal carcinoma

- [28] Zhang Y, Liu C, Barbier O, Smalling R, Tsuchiya H, Lee S, Delker D, Zou A, Hagedorn CH and Wang L. Bcl2 is a critical regulator of bile acid homeostasis by dictating Shp and lncRNA H19 function. *Sci Rep* 2016; 6: 20559.
- [29] Zhu W, Shan X, Wang T, Shu Y and Liu P. miR-181b modulates multidrug resistance by targeting BCL2 in human cancer cell lines. *Int J Cancer* 2010; 127: 2520-2529.
- [30] Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, Wojcik SE, Aqeilan RI, Zupo S and Dono M. miR-15 and miR-16 induce apoptosis by targeting BCL2. *Proc Natl Acad Sci U S A* 2005; 102: 13944-13949.
- [31] Su W, Lin Y, Wu F, Guo H, Li L, Zhu S, Lai Z, Liang R and Yang Z. Bcl-2 regulation by miR-429 in human nasopharyngeal carcinoma in vivo. 2016.
- [32] Irrera N, Bitto A, Interdonato M, Squadrito F, Altavilla D. Evidence for a role of mitogen-activated protein kinases in the treatment of experimental acute pancreatitis. *World J Gastroenterol* 2011; 20: 16535-43.
- [33] Panday A, Inda ME, Bagam P, Sahoo MK, Osorio D and Batra S. Transcription factor NF- κ B: an update on intervention strategies. *Arch Immunol Ther Expe (Warsz)* 2016; 64: 463-483.
- [34] Chai W, Zhang J, Yan D, Pan D, Liu W, Li Y, Xue Y and Chen B. Pseudomonas pyocyanin stimulates IL-8 expression through MAPK and NF- κ B pathways in differentiated U937 cells. *BMC Microbiol* 2014; 14: 26.
- [35] Kutlu K, Taheri H and Yilmazer A. Upregulation of MicroRNA-1182 activates the apoptotic pathway p38/MAPK in MDA-MB-435 melanoma cells following reprogramming with transcription factors Oct3/4, Klf-4, Sox-2, c-Myc. 2017.
- [36] Kim SW, Ramasamy K, Bouamar H, Lin AP, Jiang D and Aguiar RC. MicroRNAs miR-125a and miR-125b constitutively activate the NF- κ B pathway by targeting the tumor necrosis factor alpha-induced protein 3 (TNFAIP3, A20). *Proc Natl Acad Sci U S A* 2012; 109: 7865-7870.