European Review for Medical and Pharmacological Sciences 2019; 23: 2053-2061

MiR-99a inhibits cell proliferation of nasopharyngeal carcinoma by targeting mTOR and serves as a prognostic factor

S.-H. WU[1], L. HAN[2], B.-C. LU[3], H.-Y. WANG[4], C.-P. ZHENG[2]

[1]Department of Radiation Oncology, the Second Clinical Medical College of Jinan University, Shenzhen People's Hospital, Shenzhen, China
[2]Department of Otorhinolaryngology, the Second Clinical Medical College of Jinan University, Shenzhen People's Hospital, Shenzhen, China
[3]Department of Otorhinolaryngology, First Affiliated Hospital of Xinxiang Medical University, Xinxiang, China
[4]Department of Thorax, the Second Clinical Medical College of Jinan University, Shenzhen People's Hospital, Shenzhen, China

Shihai Wu and Ling Han contributed equally to this work

Abstract. – OBJECTIVE: Nasopharyngeal carcinoma is the most common head and neck tumor in Southern China and Southeast Asia, presenting high rates of local invasion and early distant metastasis. Abnormally expressed miR-99a has been discovered in many tumors, and it is involved in nasopharyngeal carcinoma as well. This study aims to explore the molecular mechanisms of miR-99a and mTOR in regulating nasopharyngeal carcinoma.

PATIENTS AND METHODS: MiR-99a expression in nasopharyngeal carcinoma cells was detected by quantitative Real Time-Polymerase Chain Reaction (qRT-PCR). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay was performed for accessing cell proliferative capacity. Dual-Luciferase reporter gene assay was employed to verify the combination between miR-99a and mTOR.

RESULTS: We found that miR-99a was downregulated while mTOR was upregulated in nasopharyngeal carcinoma cell lines CNE1 and SUNE1. Low expression of miR-99a or high expression of mTOR predicted poor prognosis of nasopharyngeal carcinoma. MiR-99a overexpression inhibited the proliferation of CNE1 and SUNE1 cells through targeting mTOR.

CONCLUSIONS: We provided evidence that miR-99a inhibits NPC cell proliferative ability by inhibiting mTOR. The newly identified miR-99a/mTOR axis provides novel insight into the pathogenesis of NPC and represents a potential therapeutic target for NPC.

Key Words:
MiR-99a, Proliferation, Nasopharyngeal carcinoma, mTOR.

Introduction

Nasopharyngeal carcinoma (NPC), the most common head and neck tumor in Southern China and Southeast Asia, exerts high rates of local invasion and early distant metastasis. Bones, lungs and liver are frequently affected by NPC[1]. Although the 5-year survival rate of NPC has increased through radiotherapy (RT), 30% of NPC patients could not be cured due to distant metastasis[2,3]. Therefore, looking for a new molecular target of NPC is urgently needed.

MicroRNAs (miRNAs), conserved noncoding RNAs with about 22-28 nucleotides, could enhance the degradation or post-transcriptional translational repression of target genes *via* base-pairing with their 3'-untranslated region (3'-UTR)[4,5]. A large amount of evidence displayed that many miRNAs play vital roles in NPC, including miR-125b, miR-130a, miR-663 and miR-148[6-9], suggesting the potential participation of miRNAs in NPC. MiR-99a belongs to the miR-99 family, and was reported to be associated with the pathogenesis of various tumors, including esophageal squamous cell carcinoma, gastric cancer, myeloid leukemia[10-12]. Mei et al[10] has shown that miR-99a could inhibit cell proliferation, migration and invasion in esophageal squamous cell carcinoma. Besides, miR-99a is associated with poor prognosis of myeloid leukemia[12]. Wang et al[13] displayed that miR-99a could inhibit cell invasion and metastasis of NPC through HOXA1. However, the effect of

Corresponding Author: Chaopan Zheng, MD; e-mail: zhengclimb@tom.com

miR-99a on the proliferation of nasopharyngeal carcinoma cells has not been reported yet.

The mammalian target of rapamycin (mTOR) belongs to the phosphatidylinositol kinase-related kinase (PIKK) family; it is an evolutionarily conserved protein kinase, functioning as Ser/Thr kinase[14]. mTOR is an important member of the PI3K/mTOR signaling pathway, associated with tumorigenesis and metabolic disorders[15-17]. Victoria A Robb discovered that mTOR is activated in most clear cell renal cell carcinomas[18]. Guri et al[19] showed that hepatic mTORC2 promotes steatosis and tumorigenesis through *de novo* fatty acid and lipid synthesis. Guo et al[20] has reported the association between mTOR and distant metastasis of NPC. MiR-3188 regulated the proliferation and chemosensitivity by targeting mTOR in NPC[21]. However, the relationship between miR-99a and mTOR has not been studied in NPC. In our report, miR-99a was lowly expressed while mTOR was overexpressed in NPC cell lines CNE1 and SUNE1. MiR-99a inhibited NPC cell proliferation by targeting mTOR. In addition, we also detected the overall survival (OS) of NPC patients on the basis of the expressions of miR-99a and mTOR.

Patients and Methods

Patients and Clinical Samples

Our study included 56 NPC patients treated in Shenzhen People's Hospital from 2015 to 2017. Their biopsy samples were obtained for analysis. None of them received tumor biopsy before being enrolled in this experiment. Clinical characteristics of enrolled NPC patients were listed in Table I. Samples for experiments was collected with the informed consent from patients and experimental procedures were approved by the Ethics Committee of Shenzhen People's Hospital.

Cell Lines and Culture Condition

Human nasopharyngeal carcinoma cell lines CNE1 and SUNE1 and immortalized nasopharyngeal epithelial cell NP69 were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). All cell lines were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium supplemented with 10% fetal bovine serum (FBS; Gibco Life Technologies, Rockville, MD, USA) at 37°C with 5% CO_2.

RNA Isolation and Quantitative Real Time-Polymerase Chain Reaction (qRT-PCR)

TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) or MIRcute and Separation of miRNAs Kit (Tiangen, Beijing, China) were used to extract total mRNAs and miRNAs from NPC cells. Firstly, RNA was reversely transcribed into complementary Deoxyribose Nucleic Acid (cDNA) using PrimeScript™ II 1st Strand cDNA Synthesis Kit (TaKaRa, Otsu, Shiga, Japan). SYBR Prime Script miRNA RT-PCR Kit or SYBR Premix Kit (TaKaRa, Otsu, Shiga, Japan) was utilized to perform quantitative Real-Time PCR on ABI PRISM 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Relative expression levels of mRNA and miRNA were calculated using $2^{-\Delta\Delta Ct}$ method. β-actin and U6 were used as internal control of mRNAs and miRNAs, respectively. Primer sequences used in this study were as follows: miR-99a, F: 5'-AGCAGCAGAACCAGGAGTAACAAG-3', R: 5'-GGCGTAGGTGGCGATCT-3'; mTOR, F: 5'-CTGGGACTCAAATGTGTGCAGTTC-3', R: 5'-GAACAATAGGGTGAATGATCCGGG-3'; U6: F: 5'-GCTTCGGCAGCACATATACTAAAAT-3', R: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; β-actin: F: 5'-CCTGGCACCCAGCACAAT-3', R: 5'-GCCGATCCACACGGAGTACT-3'.

Protein Extraction and Western Blotting

For Western blot assays, adherent NPC cells were firstly washed by phosphate-buffered saline (PBS). Total proteins were lysed using radioimmunoprecipitation assay (RIPA) buffer containing with 1% protease inhibitor (PMSF) (Beyotime, Shanghai, China) on ice. Bicinchoninic acid (BCA) Protein Assay Kit (Thermo Scientific, Waltham, MA, USA) was employed to determine protein concentration. Equal quality of proteins was separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred the blots onto a polyvinylidene difluoride (PVDF) membrane (Bio-Rad, Hercules, CA, USA). We blocked the non-specific binding sites by incubating the membrane with 5% nonfat dried milk for 1 h, which was dissolved by Tris-Buffered Saline and Tween 20 (TBST). After that, membranes were incubated with rabbit polyclonal antibody against mTOR (1:1000, ab25880, Abcam, Cambridge, MA,

USA) and β-actin mouse monoclonal antibody (1:2000, AA128, Beyotime, Shanghai, China) at 4°C overnight. At the other day, membranes were incubated by anti-rabbit (1:5000; Sigma-Aldrich, St Louis, MO, USA) or anti-mouse (1:5000; sc-362280, Santa Cruz Biotechnology, Santa Cruz, CA, USA) secondary antibody for 2 hours. Enhanced Chemiluminescence (ECL) Detection Kit (GE Healthcare, Waukesha, WI, USA) was used to determine the interest proteins signals on Odyssey CLx Western Blot Detection System (LI-COR Biosciences, Lincoln, NE, USA).

Cell Proliferation Assay

3-(4,5-dimethyl-2-thia-zolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and dimethyl sulfoxide (DMSO) solutions (Sigma-Aldrich, St. Louis, MO, USA) were utilized to detect the cell proliferative activity. First, cells were seeded into the 96-well plate with 5×10^3 cells per well. After cell culture for 24, 48, 72 and 96 hours at 37°C, 10 μL MTT solution was added and incubated for 4 h. Subsequently, 150 μL DMSO solution was added in each well and shaken for 10 min on a shaker. The absorbance in each well was detected using a microplate reader (Bio Tek, Winooski, VT, USA) with 490 nm wavelength.

Transfection

MiR-99a mimic, inhibitor, pcDNA3.1-mTOR and negative control vectors were obtained from GenePharma Biotechnology Co., Ltd. (Shanghai, China). CNE1 and SUNE1 cells were seeded into 6-well plate and transfection was performed using Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) until 80% of cell density. The pcDNA3.1-mTOR or pcDNA3.1-NC was used to co-transfect with miR-99a mimic into CNE1 and SUNE1 cells. Cells were harvested 48 h after transfection.

Plasmid Construction and Luciferase Reporter Assay

Target genes of miR-99a were predicted in TargetScan (http://www.targetscan.org/vert_71/) and confirmed through searching literature. The 3'-UTR of the target gene containing the binding sequences of miR-99a was amplified and inserted into the pmirGlo vector (WT) immediately. Subsequently, we mutated the binding sequences and cloned into pmirGlo vector (MUT) by the QuickChange Multi Site-Directed Mutagenesis Kit (Stratagene, Santa Clara, CA, USA). Meanwhile, miR-99a mimic or scramble mimic (NC) was also inserted into the pmirGlo vector. Cells were co-transfected with miR-99a mimic or scramble mimic and WT or MUT using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Luciferase activity of firefly was detected using Dual-Luciferase Reporter Gene Assay (Promega, Madison, WI, USA) and normalized by Renilla Luciferase.

Statistical Analysis

All statistical analyses were carried out using Statistical Product and Service Solutions (SPSS) 19.0 (SPSS, Chicago, IL, USA). The differences between the two groups were assessed by Student's *t*-test or Pearson's χ^2-test. The comparison of difference among multiple groups was analyzed using one-way ANOVA, followed by post-hoc test for subsequent comparison in the individual group. p-value <0.05 was considered statistically significant.

Results

Expressions of MiR-99a and mTOR in NPC Cell Lines

Real Time-Polymerase Chain Reaction was employed to determine miR-99a expression in an immortalized nasopharyngeal epithelial cell line NP69 and two NPC cell lines CNE1 and SUNE1. MiR-99a expression was lowly expressed in CNE1 (p=0.0013) and SUNE1 (p=0.0010) cells versus NP69 cells (Figure 1A). We also measured the mRNA level of mTOR in NPC cell lines CNE1 and SUNE1, as well as immortalized cell NP69. On the contrary, mRNA level of mTOR was overexpressed in CNE1 (p=0.0007) and SUNE1 (p=0.0008) cells versus NP69 cells (Figure 1B). Our results showed that miR-99a was downregulated while mTOR was upregulated in NPC cells.

Low Expression of MiR-99a or High Expression of mTOR Predicted Poor Prognosis of NPC

Fifty-six NPC patients were divided into miR-99a(+) group and miR-99a(-) group based on their miR-99a expressions. A total of 22 NPC was included in miR-99a(+) group. Meanwhile, we also divided the NPC patients into mTOR(+) group and mTOR(-)group, containing 30 and

26 patients, respectively. MiR-99a expression was negatively associated with TNM stage (p=0.047), lymph-node metastasis (p=0.041), local invasion (p=0.034) and mTOR expression (p=0.042) in NPC patients. However, miR-99a had no relationship with age (p=0.922) and gender (p=0.813) of NPC patients, but may be correlated with positive expression of Ki-67 (p=0.063) (Table I).

In addition, the OS of NPC patients was employed to Kaplan-Meier based on their miR-99a expressions. Higher OS was found in miR-99a(+) group than that of miR-99a(-) group (Log-rank p=0.0306) (Figure 1C). On the contrary, lower OS was found in mTOR(+) group versus mTOR(-) group (Log-rank p=0.125) (Figure 1D).

MiR-99a Inhibited Proliferation of NPC Cells

To detect the effect of miR-99a on proliferative ability of human NPC cells, we transfected miR-99a mimics or inhibitor in CNE1 and SUNE1 cells. MTT assay revealed that CNE1 cells transfected with miR-9a mimics showed lower proliferative rate than controls (p=0.0177, Figure 2A). Similar results were also obtained in SUNE1 cells (p=0.0226, Figure 2B). On the contrary, the transfection of miR-99a inhibitor in CNE1 (p=0.0041) and SUNE1 cells (p=0.0039) increased proliferation (Figure 2C and 2D). These findings illuminated that miR-99a could suppress the proliferation of NPC cells.

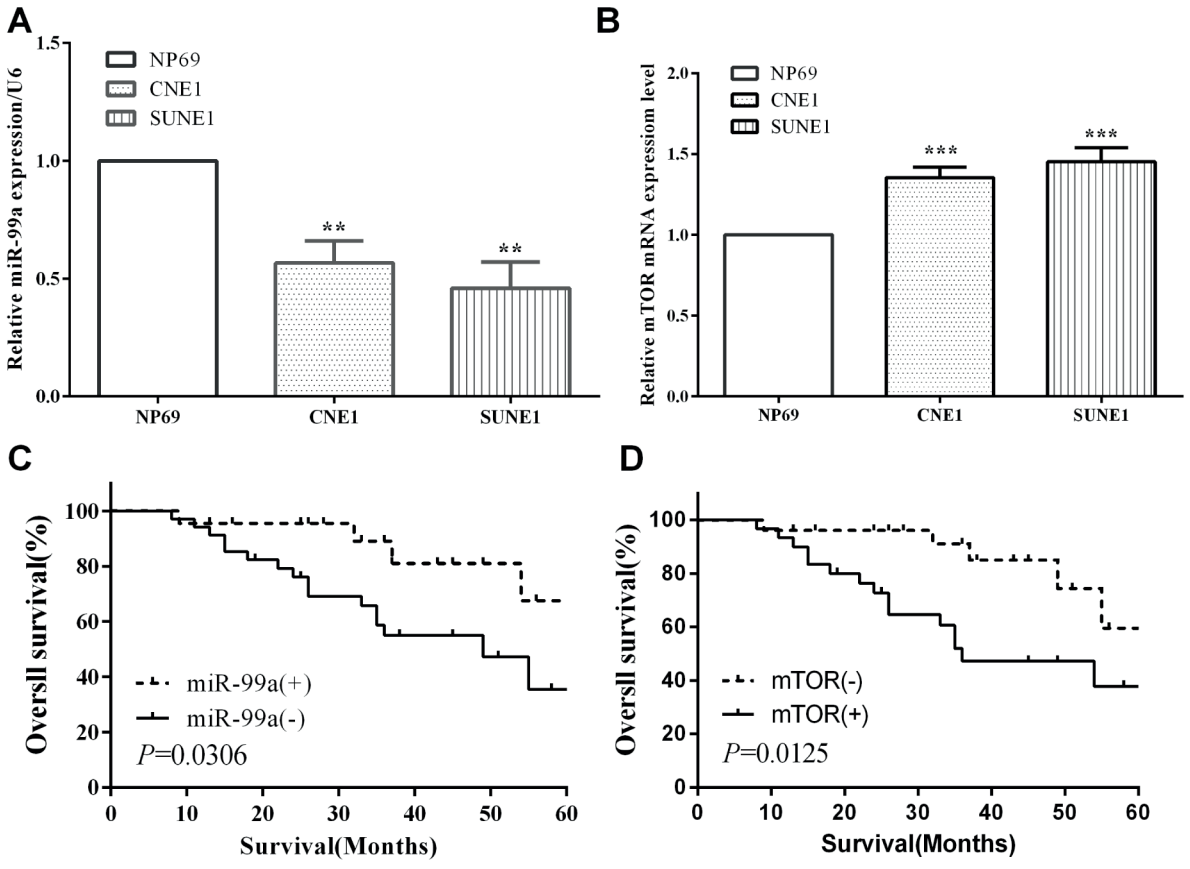

Figure 1. MiR-99a and mTOR were correlated with survival in nasopharyngeal carcinoma. ***A,*** Low expression of miR-99a was detected in nasopharyngeal carcinoma cell lines CNE1 and SUNE1 compared with immortalized nasopharyngeal epithelial cell NP69. ***B,*** mTOR was highly expressed in nasopharyngeal carcinoma cells versus immortalized nasopharyngeal epithelial cell. ***C,*** Effect of miR-99a on overall survival of nasopharyngeal carcinoma. ***D,*** OS of nasopharyngeal carcinoma patients on the basis of mTOR expression. **, p<0.01; ***, p<0.001.

Table I. miR-99a expression and clinicopathological features in 56 paired NPC.

Clinicopathological features	Cases (n=56)	miR-99a expression		*p*-value*
		22 High (%)	34 Low (%)	
Age (years)				0.922
<50	25	10 (40.0)	15 (60.0)	
≥50	31	12 (38.7)	19 (61.3)	
Gender				
Male	32	13 (40.6)	19 (59.4)	0.813
Female	24	9 (37.5)	15 (62.5)	
TNM stage				
I-II	25	13 (52.0)	12 (48.0)	0.047*
III-IV	31	9 (29.0)	22 (71.0)	
Lymph-node metastasis				0.041*
NO	19	11 (57.9)	8 (42.1)	
YES	37	11 (29.7)	26 (70.3)	
Local invasion				0.034*
T1-T2	21	12 (57.1)	9 (42.9)	
T3-T4	35	10 (28.6)	25 (71.4)	
Ki-67				0.063
<14%	27	14 (51.9)	13 (48.1)	
≥14%	29	8 (27.6)	21 (72.4)	
mTOR				0.042*
Negative	26	14 (53.8)	12 (46.2)	
Positive	30	8 (26.7)	22 (73.3)	

*Statistically significant difference ($p<0.05$).

MiR-99a Targeted to mTOR and Inhibited its Expression

To inquire how miR-99a affects the proliferation of NPC cells, TargetScan software was utilized to predict its target genes. We found mTOR was a putative target of miR-99a and the binding sequence was UACGGGU located at 295 to 301 on mRNA 3'-UTR. Binding sequence was mutated from 5'-…CCAUAACUUUAGAAAUACGGGUU…3' to 5'-…CCAUAACUUUAGAAAAUGCCCAU…3' (Figure 3A).

After co-transfection of mTOR WT and miR-99a mimic in NPC cells, the Luciferase activity reduced compared with those transfected with mTOR WT and scramble mimic in CNE1 (p=0.0028) and SUNE1 cells (p=0.0013). Whereas, there is no significant difference between NPC cells co-transfected mTOR MUT and miR-99a mimic or scramble mimic in both CNE1 (p=0.8094) and SUNE1 cells (p=0.9564) (Figure 3B).

To investigate whether miR-99a could regulate mTOR expression, we detected mTOR expression after transfection with miR-99a mimic or inhibitor. We found that miR-99a overexpression decreased the mRNA level of mTOR in both CNE1 (p=0.0068) and SUNE1 cells (p=0.0028). In addition, the inhibition of miR-99a increased mRNA level of mTOR in CNE1 (p=0.0072) and SUNE1 cells (p=0.0007) (Figure 3C). Taken together, these results suggested that mTOR is a direct target of miR-99a.

mTOR Could Reverse Partial Function of MiR-99a

To confirm that miR-99a inhibits the proliferative activity by targeting mTOR, co-transfection of miR-99a mimics/inhibitor and pcDNA3.1-mTOR/pcDNA3.1-NC in NPC cells was performed. As expected, both mRNA and protein expressions of mTOR decreased after miR-99a overexpression in CNE1 (p=0.0004) and SUNE1 (p=0.0004) cells. However, mTOR expression was reversed after co-transfection in CNE1 (p=0.0270) and SUNE1 cells (p=0.0056) (Figure 4A). In addition, compared with individual transfection of miR-99a, the proliferative rate increased in co-transfected CNE1

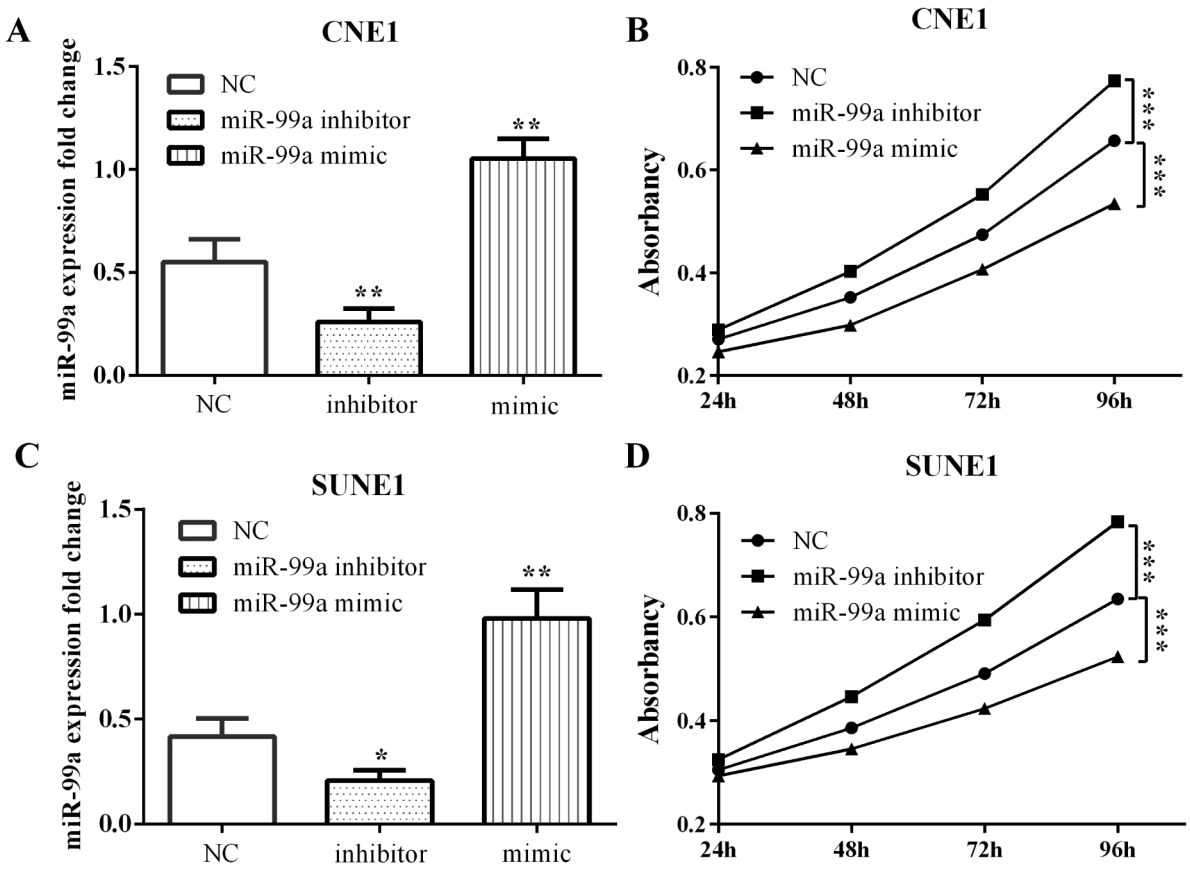

Figure 2. MiR-99a suppressed proliferation of NPC cells. ***A,*** and ***C,*** Transfection efficacy of miR-99a mimic or inhibitor in CNE1 and SUNE1 cells. ***B,*** and ***D,*** The proliferative ability was reduced after miR-99a overexpression, whereas miR-99a knockdown increased the proliferative ability in CNE1 and SUNE1 cells. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

($p<0.0001$) and SUNE1 cells ($p<0.0001$). It is suggested that mTOR reversed partial function of miR-99a (Figure 4B).

Discussion

Nasopharyngeal carcinoma is the most common head and neck tumor in Southern China and Southeast Asia[1]. Looking for new biomarkers of NPC treatment is necessarily required. MicroRNAs are conserved noncoding RNAs, which regulate target gene expression *via* base-pairing with the 3'-UTR of target gene[4,5]. Some miRNAs are closely related to the proliferation and prognosis of NPC[6-9]. MiR-99a is served as a pathogenic factor in many tumors[10-12]. In esophageal squamous cell carcinoma cell, miR-99a suppresses cell proliferation, migration and invasion *via* targeting the IGF1R signaling pathway[10]. MiR-99a is lowly expressed in breast cancer side population cells, which suppresses cell migration and invasion, as well as sphere formation[22]. Wang et al[23] reported that miR-99a inhibited cervical cancer cell proliferation and invasion by inhibiting mTOR expression. The previous study has demonstrated that miR-99a could affect cell metastasis and invasion. However, the impact of miR-99a on the proliferation of NPC cells is still unknown. In this work, miR-99a was lowly expressed in NPC cell lines CNE1 and SUNE1 measured by RT-PCR. We found that miR-99a overexpression inhibited the proliferation of NPC cells. Additionally, many target genes of miR-99a have been reported, including IGF1R, CAPNS1, AKT1 and mTOR[10,11,22,24]. In our research, we discovered that mTOR was a target of miR-99a. MiR-99a was able to downregulate mTOR expression. It was the first time to pro-

pose that miR-99a could affect proliferation of NPC cells by directly targeting mTOR. MiR-99a expression was closely correlated to the prognosis of NPC patients.

In our study, miR-99a mediated mTOR expression in NPC cell lines CNEA and SUNE1. mTOR is a member of the PI3K family, exerting a crucial role in PI3K/mTOR signaling pathway[14-17]. Guri et al[19] showed that hepatic mTORC2 promotes steatosis and tumorigenesis through de novo fatty acid and lipid synthesis. In cervical cancer cells, miR-99a could inhibit cell proliferation and invasion. Considering these findings, we strongly believed that microRNA-99a impacted the proliferation of NPC cells *via* regulating mTOR. We confirmed that mTOR was a target of miR-99a by Dual-Luciferase reporter gene assay. Moreover, mTOR expression was negatively regulated by miR-99a. In addition, the inhibited proliferative activity by miR-99a overexpression was partially reversed by mTOR overexpression, suggesting that miR-99a inhibited the proliferation of NPC cells by targeting mTOR. We first found that miR-99a regulated mTOR expression in NPC cells. The previous study has pointed out that

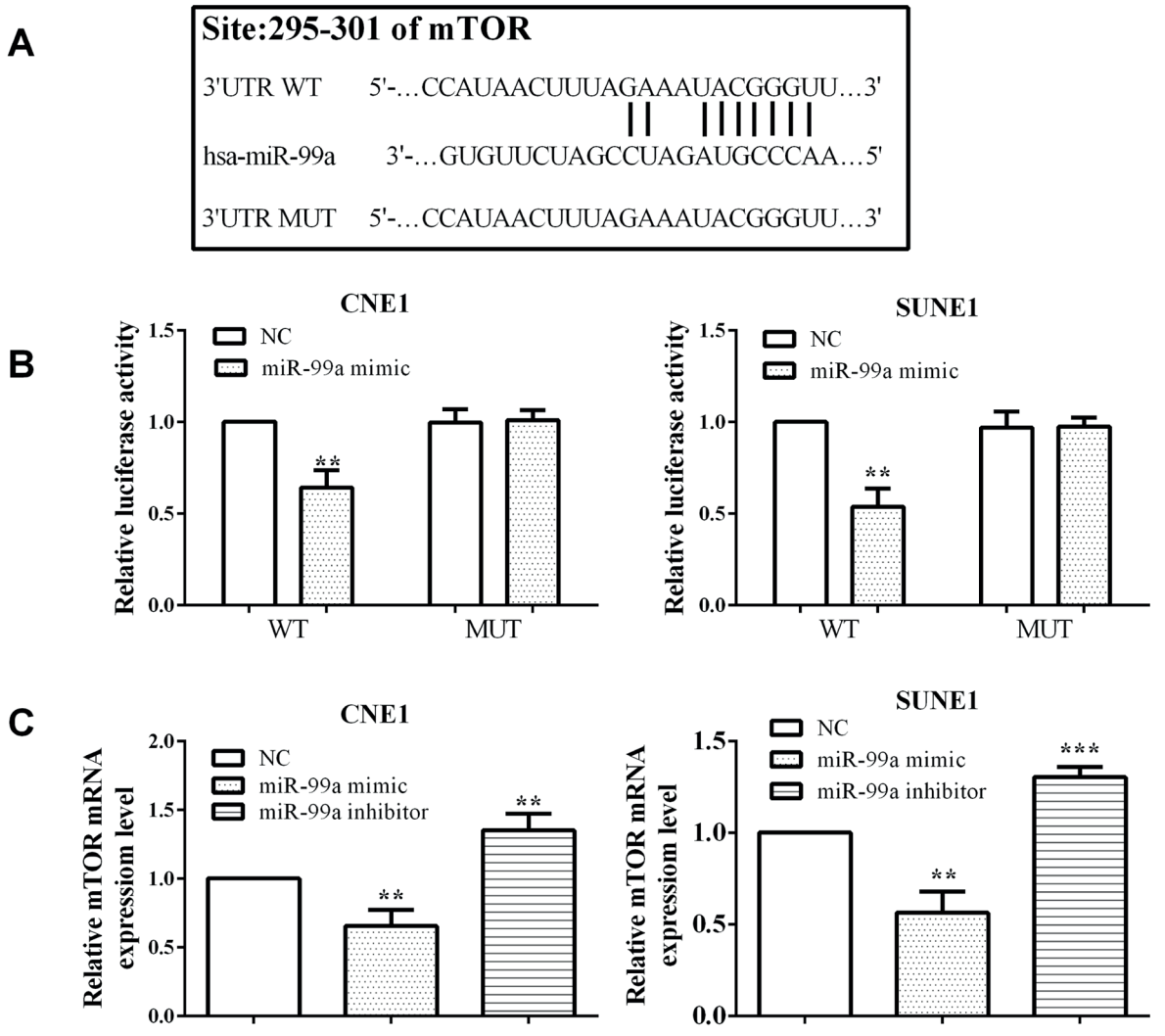

Figure 3. MiR-99a targeted mTOR and inhibited its expression. ***A,*** Predicted targeting sequence of miR-99a at the mTOR 3'-UTR. ***B,*** The wild-type of mTOR binding to miR-99a. ***C,*** mTOR expression was negatively regulated by miR-99a expression in NPC cells. ***, $p<0.001$; NC, negative control; WT, wide-type of USP39 3'UTR; MUT, mutant-type of USP39 3'UTR.

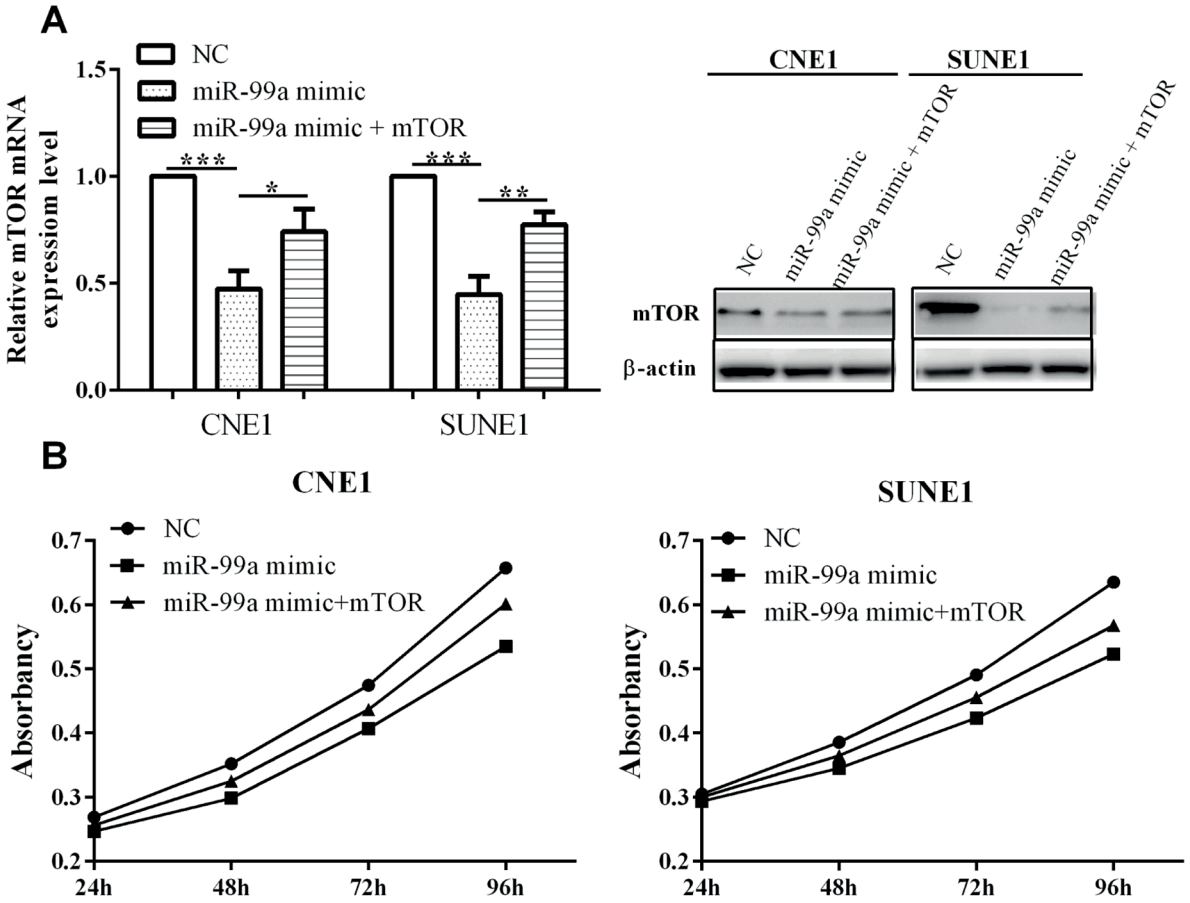

Figure 4. mTOR reversed partial function of miR-99a. ***A,*** mTOR expression was measured in CNE1 and SUNE1 cells after co-transfection with miR-99a mimic and pcDNA3.1-mTOR or individual transfection with miR-99a mimic by qRT-PCR and Western blot. ***B,*** The proliferative ability decreased when transfected with miR-133a mimic, which was reversed after overexpression of mTOR. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; NC, negative control.

miR-99a is a prognostic predictor of acute myeloid leukemia and breast cancer[12,25]. Additionally, the mTOR pathway is associated with the prognosis of NPC patients[26]. Our findings identically found that miR-99a knockdown or mTOR overexpression predicted poor prognosis of NPC patients.

Conclusions

Collectively, we provided evidence that miR-99a could inhibit the proliferative ability of NPC cells by inhibiting mTOR. MiR-99a negatively regulated mTOR expression in NPC cells. The newly identified miR-99a/mTOR axis provides a novel insight into the pathogenesis of NPC and represents a potential therapeutic target for NPC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Bensouda Y, Kaikani W, Ahbeddou N, Rahhali R, Jabri M, Mrabti H, Boussen H, Errihani H. Treatment for metastatic nasopharyngeal carcinoma. Eur Ann Otorhinolaryngol Head Neck Dis 2011; 128: 79-85.
2) Lee AW, Ma BB, Ng WT, Chan AT. Management of nasopharyngeal carcinoma: current practice and future perspective. J Clin Oncol 2015; 33: 3356-3364.
3) Razak AR, Siu LL, Liu FF, Ito E, O'Sullivan B, Chan K. Nasopharyngeal carcinoma: the next challenges. Eur J Cancer 2010; 46: 1967-1978.

4) Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.
5) Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.
6) Li LN, Xiao T, Yi HM, Zheng Z, Qu JQ, Huang W, Ye X, Yi H, Lu SS, Li XH, Xiao ZQ. MiR-125b increases nasopharyngeal carcinoma radioresistance by targeting A20/NF-kappaB signaling pathway. Mol Cancer Ther 2017; 16: 2094-2106.
7) Qu R, Sun Y, Li Y, Hu C, Shi G, Tang Y, Guo D. MicroRNA-130a-3p suppresses cell viability, proliferation and invasion in nasopharyngeal carcinoma by inhibiting CXCL12. Am J Transl Res 2017; 9: 3586-3598.
8) Liang S, Zhang N, Deng Y, Chen L, Zhang Y, Zheng Z, Luo W, Lv Z, Li S, Xu T. miR-663 promotes NPC cell proliferation by directly targeting CDKN2A. Mol Med Rep 2017; 16: 4863-4870.
9) Wu M, Ye X, Wang S, Li Q, Lai Y, Yi Y. MicroRNA-148b suppresses proliferation, migration, and invasion of nasopharyngeal carcinoma cells by targeting metastasis-associated gene 2. Onco Targets Ther 2017; 10: 2815-2822.
10) Mei LL, Qiu YT, Huang MB, Wang WJ, Bai J, Shi ZZ. MiR-99a suppresses proliferation, migration and invasion of esophageal squamous cell carcinoma cells through inhibiting the IGF1R signaling pathway. Cancer Biomark 2017; 20: 527-537.
11) Zhang Y, Xu W, Ni P, Li A, Zhou J, Xu S. MiR-99a and MiR-491 regulate cisplatin resistance in human gastric cancer cells by targeting CAPNS1. Int J Biol Sci 2016; 12: 1437-1447.
12) Si X, Zhang X, Hao X, Li Y, Chen Z, Ding Y, Shi H, Bai J, Gao Y, Cheng T, Yang FC, Zhou Y. Upregulation of miR-99a is associated with poor prognosis of acute myeloid leukemia and promotes myeloid leukemia cell expansion. Oncotarget 2016; 7: 78095-78109.
13) Wang JG, Tang WP, Liao MC, Liu YP, Ai XH. MiR-99a suppresses cell invasion and metastasis in nasopharyngeal carcinoma through targeting HOXA1. Onco Targets Ther 2017; 10: 753-761.
14) Avruch J, Hara K, Lin Y, Liu M, Long X, Ortiz-Vega S, Yonezawa K. Insulin and amino-acid regulation of mTOR signaling and kinase activity through the Rheb GTPase. Oncogene 2006; 25: 6361-6372.
15) Wang F, Tan WH, Liu W, Jin YX, Dong DD, Zhao XJ, Liu Q. Effects of miR-214 on cervical cancer cell proliferation, apoptosis and invasion via modulating PI3K/AKT/mTOR signal pathway. Eur Rev Med Pharmacol Sci 2018; 22: 1891-1898.
16) Liu J, Liu X, Ma W, Kou W, Li C, Zhao J. Anticancer activity of cucurbitacin-A in ovarian cancer cell line SKOV3 involves cell cycle arrest, apoptosis and inhibition of mTOR/PI3K/Akt signaling pathway. J BUON 2018; 23: 124-128.
17) Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cell 2006; 124: 471-484.
18) Robb VA, Karbowniczek M, Klein-Szanto AJ, Henske EP. Activation of the mTOR signaling pathway in renal clear cell carcinoma. J Urol 2007; 177: 346-352.
19) Guri Y, Colombi M, Dazert E, Hindupur SK, Roszik J, Moes S, Jenoe P, Heim MH, Riezman I, Riezman H, Hall MN. mTORC2 promotes tumorigenesis via lipid synthesis. Cancer Cell 2017; 32: 807-823.
20) Guo Q, Lu T, Chen Y, Su Y, Zheng Y, Chen Z, Chen C, Lin S, Pan J, Yuan X. Genetic variations in the PI3K-PTEN-AKT-mTOR pathway are associated with distant metastasis in nasopharyngeal carcinoma patients treated with intensity-modulated radiation therapy. Sci Rep 2016; 6: 37576.
21) Zhao M, Luo R, Liu Y, Gao L, Fu Z, Fu Q, Luo X, Chen Y, Deng X, Liang Z, Li X, Cheng C, Liu Z, Fang W. miR-3188 regulates nasopharyngeal carcinoma proliferation and chemosensitivity through a FOXO1-modulated positive feedback loop with mTOR-p-PI3K/AKT-c-JUN. Nat Commun 2016; 7: 11309.
22) Yang Z, Han Y, Cheng K, Zhang G, Wang X. miR-99a directly targets the mTOR signalling pathway in breast cancer side population cells. Cell Prolif 2014; 47: 587-595.
23) Wang L, Chang L, Li Z, Gao Q, Cai D, Tian Y, Zeng L, Li M. miR-99a and -99b inhibit cervical cancer cell proliferation and invasion by targeting mTOR signaling pathway. Med Oncol 2014; 31: 934.
24) Yu SH, Zhang CL, Dong FS, Zhang YM. miR-99a suppresses the metastasis of human non-small cell lung cancer cells by targeting AKT1 signaling pathway. J Cell Biochem 2015; 116: 268-276.
25) Li J, Song ZJ, Wang YY, Yin Y, Liu Y, Nan X. Low levels of serum miR-99a is a predictor of poor prognosis in breast cancer. Genet Mol Res 2016; 15. doi: 10.4238/gmr.15038338.
26) Wang W, Wen Q, Xu L, Xie G, Li J, Luo J, Chu S, Shi L, Huang D, Li J, Fan S. Activation of Akt/mTOR pathway is associated with poor prognosis of nasopharyngeal carcinoma. PLoS One 2014; 9: e106098.