European Review for Medical and Pharmacological Sciences 2018; 22: 150-157

MiR-101 promotes nasopharyngeal carcinoma cell apoptosis through inhibiting Ras/Raf/MEK/ERK signaling pathway

R.-S. WU, E.-H. QIU, J.-J. ZHU, J.-R. WANG, H.-L. LIN

Department of Otolaryngology, the 2nd Affiliated Hospital of Fujian Medical University, Quanzhou, Fujian, China

Abstract. – OBJECTIVE: Extra-cellular signal regulated kinase (ERK)/mitogen activated protein kinase (MAPK) signaling pathway is widely involved in cell proliferation and apoptosis. MAPK kinase 1 (MEK1) is the upstream protein kinase of ERK that can activate ERK/MAPK signaling pathway. microRNA-101 (MiR-101) down-regulation is found to be associated with nasopharyngeal carcinoma (NPC) pathogenesis. Bioinformatics analysis shows the complementary targeted relationship between miR-101 and the 3'-UTR of MEK1 mRNA. This study explores the role of miR-101 in regulating ME pression, ERK/MAPK signaling pathway tion, and NPC pathogenesis.

MATERIALS AND MEKHODS: Dual lucife assay confirmed the targeted relationship tween miR-101 and MEK1. MiR-101 d MEK1 e pressions were compared in i ry naso pharynx tissue and NPC tis MiR , MEK1, phosphorylated ERK 1/2 (K1/2), vivin expressions in NP69, CN ONE1 2 cell lines were det ed. ine 1 was cultured *in* and ed into four groups, including R-NC, miR si-NC and si-MEK1. Cell a was deter by flow cytometry. C pro on was ev uated by EdU staini

RESU MiR-101 tar inhibited MEK1 expres MiR-101 was sig antly down-regulate hile M was significantly elevated in NP sue c ared with inflammatory naso-phar MiR-10 as markedly declined, whereas 1, p-ER 2, and survivin were ap- tly i se CNE-1, HONE1, and C666-1 c comp th NP69 cells. MiR-101 mimic and/or si- K1 transfection significantly re MEK1, p-ERK1/2, and survivin levels, in l proliferation, and enhanced cell optosis.

CONCLUSIONS: Down-regulation of miR-101 related to NPC pathogenesis. MiR-101 elevation suppressed NPC cell proliferation and promoted apoptosis through targeted inhibiting MEK1 expression to alleviate ERK/MAPK signaling pathway and survivin expression.

Key Words:
miR-101, M s/Raf/MEK/E ptosis, Proliferation, Na l carcinoma.

Introduction

asopharynge arcinoma (NPC) is a kind of e ial malign tumor occurred in the top and side f na arynges. It is a common type of malign South-East Asia and South China with an incidence at 40-60/100,000. The incidence has been top in head and neck malignant NPC may cause various symptoms, including nasal congestion, nose blood, diplopia, headache, and cranial nerve compression based on different severity degree, leading to serious threat to the quality of life and health[2,3]. Therefore, in-depth study of NPC pathogenesis and exploration of the abnormal change of molecule expression are of great significance for NPC early diagnosis, therapeutic efficacy, and prognosis. Extra-cellular signal regulated kinase (ERK)/mitogen activated protein kinase (MAPK) signaling pathway that widely expresses in various tissues and cells, regulates multiple biological processes, including cell proliferation, cycle, apoptosis, migration, and invasion[4,5]. ERK/MAPK signaling pathway excessive activation induces cell abnormal proliferation, apoptosis, and differentiation, which are closely related to tumor pathogenesis, progression, and metastasis[6-8]. MAPK kinase 1 (MEK1) phosphorylates the residue of Tyr/Thr on ERK protein to activate ERK/MAPK signaling pathway. MEK1 elevation plays a crucial promoting role in a variety of tumor occurrence and development[9-11]. It was showed that MEK1 expression and function enhancement are related to NPC cell proliferation, apoptosis, and drug resistance[12-14]. microRNA (MiRNA) is a type of

Corresponding Author: Ruishan Wu, MD; e-mail: ruishanwuty@126.com

endogenous single stranded non-coding RNA at the length of 22-25 nt discovered from eukaryote. It plays a degrading or inhibiting role on mRNA by binding with the 3'-UTR, thus participating in cell proliferation, differentiation, and migration. The role of miRNA in tumorigenesis receives more and more attention[15-17]. Numerous studies revealed that miR-101 significantly reduced in NPC tissue and cells, suggesting that miR-101 may play a tumor suppressor role in NPC occurrence and development[18,19]. Bioinformatics analysis shows the complementary targeted relationship between miR-101 and the 3'-UTR of MEK1 mRNA. This study explores the role of miR-101 in regulating MEK1 expression, ERK/MAPK signaling pathway activation, and NPC pathogenesis.

Materials and Methods

Main Reagents and Materials

Human NPC cell line CNE-1, HONE1, and C666-1, and human nasopharynges epithelial cell line NP69 were purchased from Xinyu Biotech co., Ltd (Shanghai, China). Dulbecco's Modified Eagle Medium (DMEM) and Roswell Park Memorial Institute 1640 (RPMI-1640) medium were bought from Lonza Inc. (Allendale, NJ, USA). Fetal bovine serum (FBS), penicillin, and streptomycin were bought from Gibco BRL Co., Ltd., (Grand Island, NY, USA). MiniBEST Universal RNA Extraction Kit, PrimeScript RT reagent Kit, and SYBR Green were obtained from TaKaRa (Dalian, China). Lipofectamine 2000 was provided by Invitrogen Life Technologies (Carlsbad, CA, USA). MiR-101 mimic, miR-101 inhibitor and miR-NC were bought from Ribobio (Guangzhou, China). Mouse anti-human MEK1/2, p-ERK1/2, survivin, and β-actin primary antibodies were obtained from Abcam Biotech. (Cambridge, MA, USA). Horse radish peroxidase (HRP) conjugated secondary antibodies were derived from NeoBioscience (Shenzhen, China). Luciferase reporter gene vector pLUC was bought from Ambion Inc. (Austin, TX, USA). Luciferase activity detection kit was purchased from Promega (Madison, MI, USA). BCA and bicinchoninic (BCA) protein assay kit, and BeyoECL Plus enhanced chemiluminescent (ECL) reagent were obtained from Beyotime (Haimen, China). Annexin V-FITC/PI cell apoptosis detection kit was derived from BD Biosciences (Franklin Lakes, NJ, USA). EdU cell proliferation detection kit was purchased from Molecular Probes (Eugene, OR, USA).

Clinical Information

A total of 65 NPC patients who received treatment in the 2nd Affiliated Hospital of Fujian Medical University between March 2016 and December 2016 were enrolled, including 29 males and females with mean age at 52.3 ± 11.2 years old. All the subjects received surgery for the first time without preoperative radiotherapy or chemotherapy. Tumor tissue was resected and stored at -80°C. Another 40 cases of patients suffered from chronic nasopharyngitis were selected as control, including 18 males and 22 females with average age at 50.6 ± 10.8 years old (Table I). No statistical difference was observed on age and gender between the groups (Table I, $p>0.05$). The experimental protocol has been pre-approved by the Ethical Committee of the 2nd Affiliated Hospital of Fujian Medical University and written consents have been obtained from all patients and healthy volunteers.

Cell Culture

CNE-1, HONE1, C666-1, and NP69 cells were cultured in RPMI-1640 medium containing 10% FBS and 1% penicillin-streptomycin. The cells were passaged at 1:4.

Luciferase Reporter Gene Assay

PCR products containing the full-length of MEK1 gene 3'-UTR segment were cloned to pLUC after endonuclease SacI and XbaI cloning. Next, it was named as pLUC-MEK1-wt (or pLUC-MEK1-UTR-mut) and co-transfected to HEK293T cells using Lipofectamine 2000 together with miR-101 mimic (miR-101 inhibitor or miR-NC). The luciferase activity was detected according to the Stop&Glo Luciferase Assay manual after cultured for 48 h.

Cell Grouping and Transfection

C666-1 cells were cultured *in vitro* and divided into four groups, including miR-NC, miR-101 mimic, si-NC and si-MEK1 groups. MiR-NC or miR-101 mimic at 30 nmol/L and si-NC or si-MEK1 at 10 nmol/L were incubated with Lipofectamine at 10 μL at room temperature for 15-20 min. Then, they were added to the cells and incubated for 6 h. After changing the medium and incubation for 72 h, the cells were collected for the following experiments.

Quantitative Real-time PCR (qRT-PCR)

Total RNA was extracted using MiniBEST Universal RNA Extraction Kit and adopted for PCR reaction by PrimeScript™ RT reagent Kit

(TaKaRa Bio. Inc., Otsu, Japan). The PCR reaction was composed of 95°C pre-denaturation for 15 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. Real-time PCR was performed on Applied Biosystems QuantStudio 3 connect (PE Applied Biosystems, Foster City, CA, USA) to test the relative expression.

Western Blot

Total protein was extracted by RIPA from cells. A total of 40 μg protein was separated by sodium dodecyl sulfonate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to membrane. Next, the membrane was blocked by 5% skim milk and incubated in primary antibody at 4°C overnight (MEK1, p-ERK1/2, survivin, and β-actin at 1:3000, 1:1000, 1:2000, and 1:10000, respectively). Then, the membrane was incubated in HRP labeled secondary antibody (1:30000) for 1 h after washed by phosphate buffer solution-tween 20 (PBST) for three times. At last, the protein expression was detected by BeyoECL Plus (Beyotime Biotech., Shanghai, China).

Cell Apoptosis Detection

The cells were digested by trypsi[n] resuspended in binding buffer. Next, the [cells] were incubated in 5 μl Annexin V-FITC and 5 μl propidium iodide (PI). At last, the cells were tested on Beckman Coulter CytoFLEX flow cytometry to evaluate cell apoptosis.

EdU Staining

Cell proliferation was assesse[d] Click-iT® EdU Alexa Fluor 488 Flow Cyto[metry] Assay Kits. The cells were adde[d] with 1[0] EdU solution for 2 h. After th[ey] were incub[ated] for 48 h, the cells were dige[sted] and c[ollected]. [They] washed by PBS, fixe[d] and pen[etrated], the cells were incubated i[n] re[agent] and co[unterstaining] Alexa Fluor 48[8] room [temperature] voiding light for 30 [min]. The cell[s] [were] and tested by [Beckman] Coulter [CytoFLEX] flow cytometr[y] (Beck[man] Coulter Inc., Brea, CA, USA).

Statistical Analysis

[A]ll data analyses were performed on SPSS [22.0] (SPSS Inc., Chicago, IL, USA) software. T[he] measurement data were depicted as mean ± standard deviation and compared by *t*-test. $p<0.05$ was considered as statistical significance.

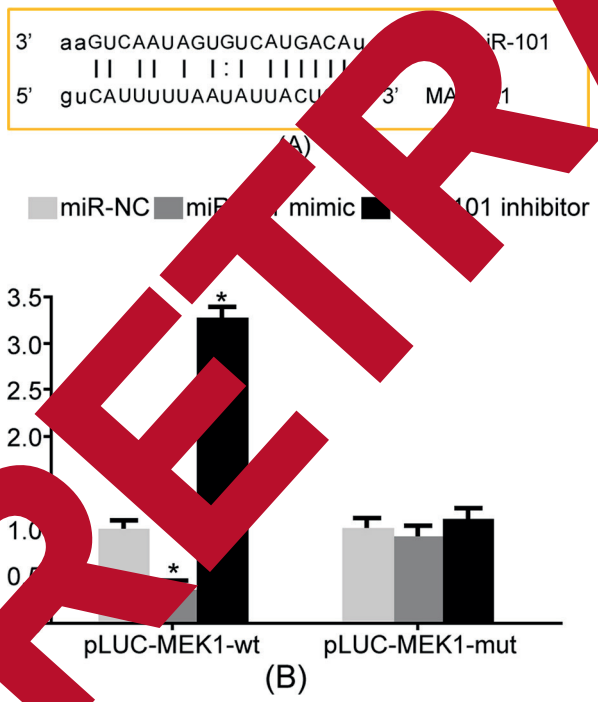

Figure 1. MiR-101 targeted inhibited MEK1 expression. ***(A)*** The binding site between miR-101 the 3'-UTR of MEK1 mRNA. ***(B)*** Dual luciferase assay. $^{*}p<0.05$, compared with mimic NC.

Results

MiR-101 Targeted Inhibited MEK1 Expression

Bioinformatics analysis showed the targeted binding site between miR-101 and 3'-UTR of MEK1 mRNA (Figure 1A). Dual luciferase assay revealed that miR-101 mimic transfection significantly declined, while miR-101 inhibitor transfection significantly elevated the relative luciferase activity of HEK293 cells, indicating the regulatory relationship between miR-101 and MEK1 mRNA (Figure 1B).

MiR-101 Down-regulated, While MEK1 Increased in NPC Tissue

qRT-PCR revealed that miR-101 significantly declined, whereas MEK1 mRNA obviously up-regulated in NPC tissue compared with inflammatory nasopharyngeal tissue (Figure 2A). MiR-101 was markedly correlated with tumor size and clinical staging. Western blot demonstrated that MEK1 protein apparently enhanced in NPC tissue compared with inflammatory nasopharyngeal tissue (Figure 2B).

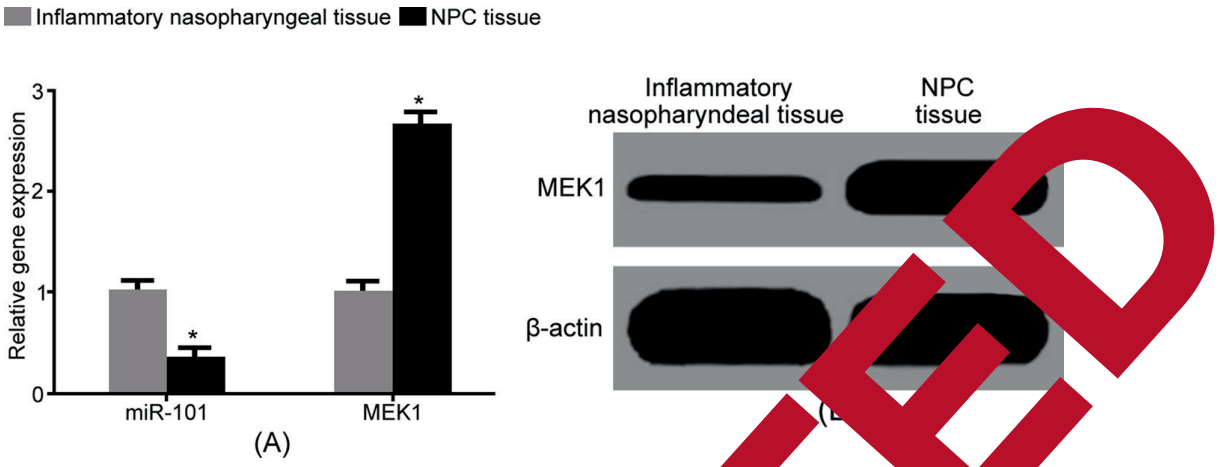

Figure 2. MiR-101 down-regulated, while MEK1 increased in NPC tissue. ***(A)*** qRT-PCR detection of miR-101 expression. ***(B)*** Western blot detection of MEK1 protein expression. *$p<0.05$, compared with inflammatory nasopharyngeal tissue.

MiR-101 Reduced, Whereas MEK1 Enhanced in NPC Cells

qRT-PCR revealed that miR-101 significantly declined, whereas MEK1 mRNA obviously up-regulated in NPC cell lines CNE-1, HONE1, and C666-1 compared with NP69 cells (Figure 3A and B). Western blot demonstrated that MEK1 was markedly increased in NPC cell lines CNE-1, HONE1, and C666-1 compared with NP69 cells (Figure 3C).

MiR-101 Over-expression Mimic Down-regulation Suppressed NPC Cell Proliferation and Induced Cell

MiR-101 mimic and siRNA transfection significantly reduced MEK1, ERK1/2, and survivin levels in C666-1 cells (Figure 4A and B), attenuated proliferation (Figure 4C), and enhanced cell apoptosis (Figure 4D).

Discussion

Globally, there are more than 100,000 patients diagnosed as NPC each year. More than 80% new cases are from South East Asia, China, and other Asian countries[1,2,21]. The morbidity peak is between 40-50 years old. It may cause nasal congestion, nose bleeding, aural fullness, hearing loss, diplopia, and facial numbness, and may induce cranial nerve because of peripheral tissue infiltration, which seriously threats to life and health[2,3].

MAPK signaling pathway is an important signal transduction system that widely exists in eukaryotes. It regulates various target genes expression and activation mediated by intracellular receptor tyrosine kinase, G-protein coupled receptor, and cytokine receptor under the effect of cytokines, growth factors, neurotransmitter, and G-protein coupled receptor, thus participating in

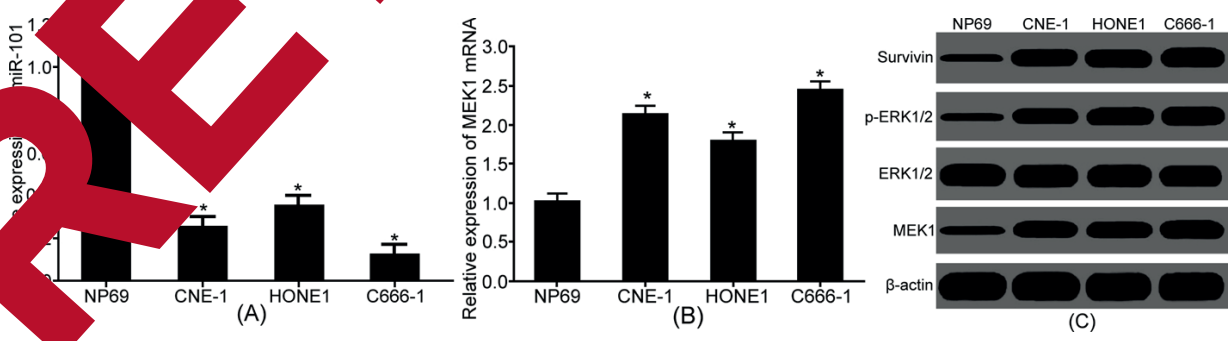

Figure 3. MiR-101 reduced, whereas MEK1 enhanced in NPC cells. ***(A)*** qRT-PCR detection of miR-101 expression. ***(B)*** qRT-PCR detection of MEK1 mRNA expression. ***(C)*** Western blot detection of protein expression. *$p<0.05$, compared with NP69 cells.

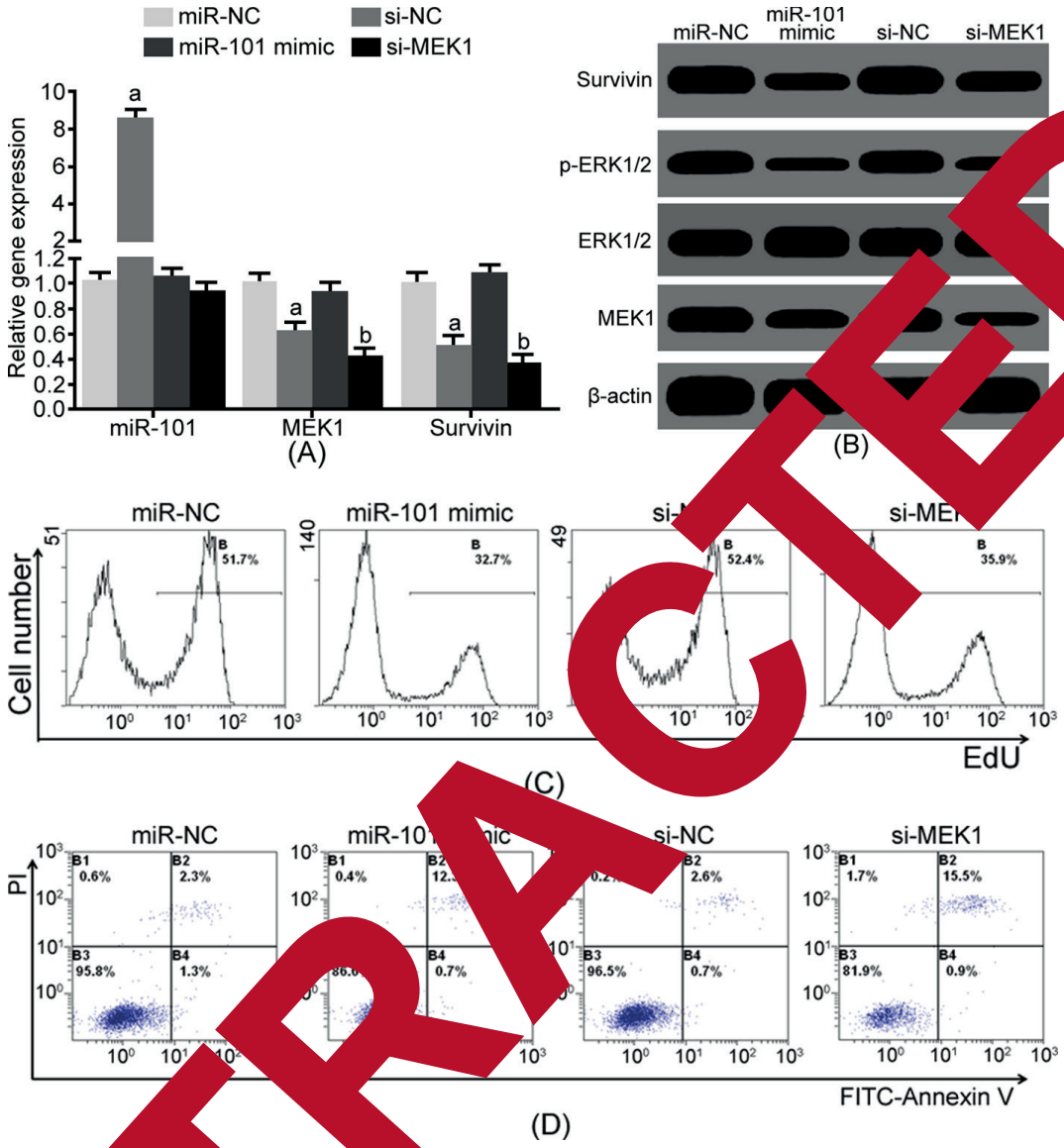

Figure 4. MiR[illegible]ver[illegible]ion or MEK1 down-regulation suppressed NPC cell proliferation and induced cell apoptosis. ***(A)*** qRT-PCR detection of ge[illegible]ression. (B) Western blot detection of protein expression. ***(C)*** EdU staining detection of cell proliferatio[illegible] Flow cytometri[illegible]tion of cell apoptosis. [a]$p< 0.05$, compared with miR-NC; [b]$p< 0.05$, compared with si-NC.

aff[illegible] cel[illegible]rvival, proliferation, migration, apopt[illegible]ogenes[illegible]nd immune response [illegible] ER[illegible]APK[illegible]aling pathway excessive a[illegible]ion [illegible]cell abnormal proliferation, apo[illegible]sis, an[illegible]ifferentiation, and promote m[illegible]transformation. It is closely associated [illegible] tumors pathogenesis, progression, [illegible]d metastasis, such as oral cancer[6], lung cancer[7], [illegible]esophageal cancer[8]. ERK/MAPK signaling pa[illegible]y mainly includes small G protein Ras, Raf kinase, MEK, and ERK. ERK/MAPK signaling pathway conforms to the classic three-step enzymatic cascade, and presents the same activation mode under different stimulus. As a member of MAP2K family, MEK1 phosphorylates the Tyr/Thr residue of ERK protein, thus activating ERK/MAPK signaling pathway. MEK1 expression and functional activity are related to a variety of cancers pathogenesis, progression, metastasis, and drug resistance, including pancreatic cancer[24], bile duct cancer[25], and hepatic cancer[9]. It was showed that MEK1 over-expression is related to NPC cell abnormal proliferation, apoptosis reduction, and drug resistance[12-14]. Multiple studies revealed that miR-101 expression reduced in NPC tissue and cell line, suggesting that miR-101 may play a tumor

Table I. MiR-101 expression in NPC tissue with different characteristics.

Group	Cases	miR-101 expression	*p*-value
Age			0.067
≤ 45 years old	25	1.62±0.25	
>45 years old	40	1.53±0.22	
Gender			0.182
Male	29	1.49±0.24	
Female	36	1.55±0.28	
T stage			<0.001
T1-T2	41	1.87±0.26	
T3-T4	24	1.23±0.21	
N stage			0.173
N0-N1	31	1.57±0.27	
N2-N3	34	1.63±0.24	
M stage			0.228
M0	13	1.61±0.23	
M1	52	1.55±0.26	
Clinical stage			<0.001
I-II	28	1.92±0.29	
III-IV	37	1.18±0.25	

suppressor role in the occurrence and development of NPC[18,19]. Bioinformatics analysis shows the complementary targeted relationship between miR-101 and the 3'-UTR of MEK1 mRNA. This study explores the role of miR-101 in regulating MEK1 expression, ERK/MAPK signaling pathway activation, and NPC pathogenesis.

Dual luciferase assay revealed that miR-101 mimic transfection significantly decreased, while miR-101 inhibitor transfection elevated relative luciferase activity of HEK293 cells, indicating the regulatory relationship between miR-101 and MEK1 mRNA. MiR-101 significantly declined in NPC tissue compared with inflammatory nasopharyngeal tissues. MiR-101 was markedly correlated with tumor size and clinical staging. It indicated that miR-101 down-regulation may play a role in increasing MEK1 expression and facilitating NPC tumorigenesis. Tang et al[19] reported that miR-101 level declined in NPC tissue compared with normal nasopharynx epithelium, showing high metastasis. In this study, miR-101 expression reduced in NPC tissue, which was according with Tang et al[19]. MiR-101 mimic declined, while MEK1, p-ERK1/2, survivin apparently enhanced in NPC cell lines CNE-1, HONE1, and C666-1 compared with NP69 cells, revealing that MEK1 elevation mediates ERK/MAPK signaling pathway and survivin expression, while miR-101 decrease is its promoting factor. Sun et al[18] showed that miR-101 level significantly declined in NPC cell lines CNE-1, CNE-2, 5-8F, and 6-10B compared with NP69 cells. Tang et al[19] demonstrated that miR-101 down-regulated in NPC cell lines CNE-2, 5-8F, and C666-1 compared with NP69 cells. This study found that miR-101 abnormally [illegible] NPC cells, which was similar to Sun et al[18] and Tang et al[19]. Further analysis revealed that miR-101 mimic and/or si-MEK1 transfection significantly reduced MEK1, p-ERK1/2, and survivin levels, attenuated cell proliferation and enhanced apoptosis. Alajez[26] reported that pre-miR-101 transfection upregulated miR-101 expression, attenuated C666-1 cell proliferation and survival, and enhanced radiotherapeutic sensitivity through targeting EZH2. Tang et al[19] found that miR-101 mimic transfection weakened NPC cell migration and invasion *in vitro*, and inhibited NPC cell pulmonary metastasis in mice model via down-regulated suppressing TGA3 expression[19]. MiR-101 inhibitor transfection enhanced NPC cell migration and invasion. Sun et al[18] presented that miR-101 overexpression markedly alleviated NPC cell proliferation and survival, and reduced radiotherapy resistance in CNE-2 and 5-8F cells. It confirmed that miR-101 declined malignant characteristic of NPC cells, which supported our results. Wong et al[27] showed that NPC cell proliferation attenuated, while cell apoptosis enhanced after treated by AZD6244 to suppress MEK1 function and ERK/MAPK signaling pathway activity, indicating that MEK1 mediated ERK/MAPK signaling pathway is related to NPC pathogenesis. Yang et al[14] presented that MEK1 suppression inhibited ERK1/2 phosphorylation and ERK/MAPK signaling pathway, arrested cell cycle, and attenuated cell proliferation in NPC cells. Cheung et al[12] exhibited that application of PD098059 blocked MEK1 activity, induced NPC cell apoptosis, and enhanced sensitivity to taxol. Wang et al[28] demonstrated that MEK1 siRNA or U0126 treatment markedly attenuated NPC cell migration and motility, whereas MEK1 over-expression enhanced cell invasion. It suggested that MEK1 mediated ERK/MAPK signaling pathway enhancement is a promoting factor of NPC pathogenesis, while reducing MEK1 expression may inhibit NPC. This study revealed that down-regulation of miR-101 increased MEK1, enhanced ERK/MAPK signaling pathway, elevated survivin expression, and promoted NPC pathogenesis. This study only used C666-1 cells to investigate the regulatory role of miR-101 on MEK1, ERK/MAPK signaling pathway, NPC cell proliferation and apoptosis. However, we did

not test the corresponding mechanism in other NPC cell lines, such as CNE-1 and HONE1.

Conclusions

Down-regulation of miR-101 was related to NPC pathogenesis. MiR-101 elevation suppressed NPC cell proliferation and promoted apoptosis through targeted inhibiting MEK1 expression to alleviate ERK/MAPK signaling pathway and survivin expression.

Acknowledgments

This work was supported by Fujian Provincial Natural Science Foundation (No. 2016J01441).

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Shen ZH, Zhao KM, Du T. HOXA10 p... nasopharyngeal carcinoma cell proliferati... invation via inducing the expression of ZIC... Rev Med Pharmacol Sci 2017; 21: 945-952.
2) Hu Y, Fu JT, Shi D, Feng B, Shi Z. Clinical effica... and safety of gemcitabine pl... atin in th... treatment of advanced naso... ynge... rcinoma. J Cancer Res Ther 2016: ...252-C2...
3) Tan WL, Tan EH, Lim ... Ng QS ... A, Ang MK. Advan... li... ... nasopharyngeal ... inoma... Clin Oncol 2016; 5: 21.
4) Buchegger K... opez J, Ili C, ... C, Leal P, Brebi P, Ric... I, ... The ERK/M... K pathway is over...pressed a... tivated in gallbladder canc... thol Res Prac... 213: 476-482.
5) Lia... Wen D, Ma B, Hu J... N, Shi RL, Liu L, ... Q, Li ... QH. Yes-associated protein 1 ...notes ... ary thyroid cancer cell proliferation ... the ER... MAPK signaling pathway. On... 2017: ... 19-11728.
... oyama ... K, Kasamatsu A, Okamoto A, ...sama H... wa Y, Shimada K, Yokoe H, Shiiba ... Tanzawa ... Uzawa K. ANGPTL3 is a novel ...ker as it activates ERK/MAPK pathway in ... Cancer Med 2015; 4: 759-769.
... Lu Z, Ding L, Hong H, Hoggard J, Lu Q, Chen YH. Claudin-7 inhibits human lung cancer cell migration ...d invasion through ERK/MAPK signaling ...athway. Exp Cell Res 2011; 317: 1935-1946.
8) Zheng ST, Huo Q, Tuerxun A, Ma WJ, Lv GD, Huang CG, Liu Q, Wang X, Lin RY, Sheyhidin I, Lu XM. The expression and activation of ERK/MAPK pathway in human esophageal cancer cell line EC9706. Mol Biol Rep 2011; 38: 865-872.
9) Li L, Wang J, Zhang Y, Ma L, Weng W, Qiao Y, Xiao W, Wang H, Yu W, Pan Q, He Y, Sun F. MEK1 promotes YAP and their interaction is critical for tum... in liver cancer. FEBS Lett 2013; 587: 3... ...27.
10) Penzvalto Z, Lanczky A, Lenart J... ...gyeshazi N, Krenacs T, Szoboszlai N, Denkert... ...r I, Gyorffy B. MEK1 is associated with carbop... ...sistance and is a prognostic biomarl... in epit... ...varian cancer. BMC Cancer 201... ...: 837.
11) Gysin S, Paquette J, Mc... ...n M. An...lysis of ... profiles after MEK1/2... ...bition in... ...an pancre... cancer cell lines re... ...pathw... ...involved in drug sensitivity. Mol ...nce... ...; 10: 16... ...19.
12) Cheung HW... MT, Ts... ...Y, Wo... ...C, Wang X. Id-1-in... Raf/MEK ... ...tivation is essenti... protective ... against taxol-induc... apop... nasopharyn...geal carcinoma cells. Carcinogen... 2004; 25: 881-887.
13) ...u WJ. Advan... he study on p38 MAPK ...ated drug resistan... in leukemia. Eur Rev Med Pharmacol Sci 2016; 20: 1064-1070.
... Yang Y, Liao ... Wei F, Li X, Zhang W, Fan S, Shi ..., Gong Z, M... Zhou M, Xiang J, Peng S, Xiang ... Deng H, L... iong W, Zeng Z, Li G. LPLUNC1 ...s, na... aryngeal carcinoma cell growth vi... ...ulation of the MAP kinase and cyclin D1/E2F pathways. PLoS One 2013; 8: e62869.
... urestha S, Hsu SD, Huang WY, Huang HY, Chen ...eng SL, Huang HD. A systematic review of ...roRNA expression profiling studies in human gastric cancer. Cancer Med 2014; 3: 878-888.
16) Catto JW, Alcaraz A, Bjartell AS, De Vere White R, Evans CP, Fussel S, Hamdy FC, Kallioniemi O, Mengual L, Schlomm T, Visakorpi T. MicroRNA in prostate, bladder, and kidney cancer: a systematic review. Eur Urol 2011; 59: 671-681.
17) Li X, Abdel-Mageed AB, Mondal D, Kandil E. MicroRNA expression profiles in differentiated thyroid cancer, a review. Int J Clin Exp Med 2013; 6: 74-80.
18) Sun Q, Liu T, Zhang T, Du S, Xie GX, Lin X, Chen L, Yuan Y. MiR-101 sensitizes human nasopharyngeal carcinoma cells to radiation by targeting stathmin 1. Mol Med Rep 2015; 11: 3330-3336.
19) Tang XR, Wen X, He QM, Li YQ, Ren XY, Yang XJ, Zhang J, Wang YQ, Ma J, Liu N. MicroRNA-101 inhibits invasion and angiogenesis through targeting ITGA3 and its systemic delivery inhibits lung metastasis in nasopharyngeal carcinoma. Cell Death Dis 2017; 8: e2566.
20) Liu Z, Chang ET, Liu Q, Cai Y, Zhang Z, Chen G, Huang QH, Xie SH, Cao SM, Shao JY, Jia WH, Zheng Y, Liao J, Chen Y, Lin L, Liang L, Ernberg I, Vaughan TL, Adami HO, Huang G, Zeng Y, Zeng YX, Ye W. Quantification of familial risk of nasopharyngeal carcinoma in a high-incidence area. Cancer 2017; 123: 2716-2725.
21) Tang LL, Chen WQ, Xue WQ, He YQ, Zheng RS, Zeng YX, Jia WH. Global trends in incidence and mortality of nasopharyngeal carcinoma. Cancer Lett 2016; 374: 22-30.

22) Pancione M, Giordano G, Parcesepe P, Cerulo L, Coppola L, Curatolo AD, Conciatori F, Milella M, Porras A. Emerging insight into MAPK inhibitors and immunotherapy in colorectal cancer. Curr Med Chem 2017; 24: 1383-1402.

23) Zhang X, Liu K, Zhang T, Wang Z, Qin X, Jing X, Wu H, Ji X, He Y, Zhao R. Cortactin promotes colorectal cancer cell proliferation by activating the EGFR-MAPK pathway. Oncotarget 2017; 8: 1541-1554.

24) Vena F, Li Causi E, Rodriguez-Justo M, Goodstal S, Hagemann T, Hartley JA, Hochhauser D. The MEK1/2 inhibitor pimasertib enhances gemcitabine efficacy in pancreatic cancer models by altering Ribonucleotide Reductase Subunit-1 (RRM1). Clin Cancer Res 2015; 21: 5563-5577.

25) Furuse J, Nagashima F. Inhibitor of MEK1/2, selumetinib, for biliary tract cancer. Expert Rev Gastroenterol Hepatol 2011; 5: 579-581.

26) Alajez NM, Shi W, Hui AB, Bruce J, Lenarduzzi M, Ito E, Yue S, O'Sullivan B, Liu FF. Enhancer of Zeste homolog 2 (EZH2) is overexpressed in recurrent nasopharyngeal carcinoma and is regulated by miR-26a, miR-101, and miR-98. Cell D... 2010; 1: e85.

27) Wong CH, Ma BB, Cheong HT, H... W, Hui EP, Chan AT. Preclinical evaluation ...3K inhibitor BYL719 as a single agent and ...rgism in combination with cisplati... MEI...itor in nasopharyngeal carcino...PC). An...ce... Res 2015; 5: 1496-150...

28) Wan XB, Long ZJ, Ya... Xu J, X... Liu L, Zh..., Huang XF, Wang XR, ... Hon..., Liu Q. Inhibition of Aurora-A s... ...ial-mes...ymal transition and ...sion b... regulat...MAPK in nasopharyn... carcinoma... C...nogenesis 2008; 29... 937.