European Review for Medical and Pharmacological Sciences 2018; 22: 1241-1248

MiR-16 inhibits pituitary adenoma cell proliferation via the suppression of ERK/MAPK signal pathway

D.-W. WANG[1], Y.-Q. WANG[2], H.-S. SHU[1]

[1]Department of Neurosurgery, the Second Affiliated Hospital of Bengbu Me College, B Anhui, People's Republic of China
[2]Department of Endocrinology, Benxi Central Hospital, Benxi, Liaoning, le's R blic of China

Abstract. – OBJECTIVE: Extracellular signal-regulated kinase (ERK)/mitogen activated protein kinase (MAPK) signal pathway participates in cell proliferation, cycle, and apoptosis. MiR-16 is down-regulated in the pituitary tumor. This study investigated the role and related mechanism of miR-16 on pituitary tumor proliferation, cycle, and apoptosis.

PATIENTS AND METHODS: Dual-luciferase reporter assay was conducted to demonstrate the targeted regulation between miR-16 and MEK1. MiiR-16, MEK1, p-ERK1/2, Survivin and Cycli D1 expression were compared between normal onic pituitary cells, HP75 tumor cells. Flow c e try detection measured cell proliferation and e. Cultured HP75 cells were divided into four gro miR-NC, miR-16 mimic, si-NC, and si-MEK1. Expr sions of miR-16, MEK1, p-ERK1/2 in, and C clin D1 were compared, and cel n, cycle, and apoptosis were tested b w cyto ry.

RESULTS: Bioinforma analys howed complementary binding bet and MEK1. Dual luci se assay val- dated the direct re tion be miR-16 and MEK1. Compare at of norm uitary tissues, significa miR-16 exp ion, but higher MEK1 el w und in adenoma tissues. Com red to nor mbryonic pituitary cells, th el of miR-16 decreased, while the ex sions of p-ERK1/2, urvivin, and Cyclin along w cell proliferation or S or G2/M ph tio up-regulated in the group of HP75 nsfect of miR-16 mimic or si-MEK1 r ably s ressed the expressions K1, K Survivin or Cyclin D1 in H cells, d cell proliferation and induc apoptosi and cycle arrest.

CONCLUSIONS: MiR-16 inhibited ERK/MAPK pathway activity via the suppression of MEK1 expression, and further suppressed proliferation of pituitary tumor cells.

Key Words:
MiR-16, MEK1, ERK/MAPK, Pituitary tumor, Cell cycle, Proliferation, Apoptosis.

Introduction

Pituita dend PA) belongs to the group of endocrine tumors ed from anterior pituitar ior pituitary residual cells from c pharyngeal canal epithelium[1]. PA is a type common intra anial carcinoma and occupies t 10-20% of primary intracranial tumors, w represents third common one after glioma ha. Recently, PA incidence is increas ears[2-5].

Mitogen-activated protein kinase (MAPK) si- thway is widely distributed in multiple tis- cells, and plays important roles in mediating cell survival, proliferation, cycle, apoptosis, migration, and invasion[6,7]. Abnormal activation of MAPK signal pathway is closely correlated with occurrence, progression, and metastasis of various tumors[8,9]. In many cancers (e.g., melanoma), a defect in the ERK/MAPK pathway results in uncontrolled growth, suggesting its essential role in modulating life activities. Many compounds are accordingly developed to potential drugs for treating cancer via the inhibiting steps in the MAP/ERK pathway. An extracellular signal-regulated kinase (ERK) induced MAPK pathway and mainly deploy the function of MAPK. In this pathway, Ras/Raf/MEK/ERK is the major transduction module for ERK/MAPK signal pathway[10,11]. MAPK kinase 1 (MEK1) presents as a dual specific protein kinase and functions the upstream of ERK, as it can activate ERK by phosphorylation on Try/Thr amino acid residues of ERK protein. Numerous studies[12-14] showed aberrantly elevation of MEK1 protein expression in onset, progression, metastasis, and prognosis of multiple tumors. A previous study[15] also indicated the correlation between improving function of MEK1 and PA occurrence. MicroRNA (miR) is characterized as a group of endogenous sin-

Corresponding Author: Dawei Wang, MD; e-mail: liangtiji889@163.com

gle-stranded non-coding small molecule RNA with 20-24 nucleic acids length, and can bind with 3'-untranslated region (3'-UTR) of target gene mRNA to interrupt mRNA stability, leading to mRNA degradation or translation inhibition, thus giving rise to negative regulation on gene expression at post-transcriptional level[16]. Abnormal expression or dysfunction of miR has become a research focus on tumor pathogenesis[17-19]. Accumulative studies[20,21], for instance, demonstrated significant decreasing level of miR-16 in PA tumor tissues, indicating its potential tumor-suppressing role in PA pathogenesis. Thus, this investigation concerns the role of miR-16 as well as the mechanism involved in PA cell proliferation and/or apoptosis.

Patients and Methods

Clinical Information

A total of 39 PA patients who received treatment in the Second Affiliated Hospital of Bengbu Medical College from May 2016 to December 2016 were recruited in this study. Tumor ti... were collected during surgery. There w... males and 20 females in the patient group, ...g between 33 and 57 years (average age = 41.... ars). Another cohort of 15 normal pituitary sa... ples collected from brain contu... rgery w... recruited as the control grou... ich ...sisted o... 7 males and 8 females, agi... om 31 ...8 years (average age = 42.6 years... sampl... have obtained inform... co... om pa... pants, and this inve... tion wa... approved by the Ethical Com... e of the Se... Affiliated Hospital of Be... al College.

Major R...ent and M...ials

Hum... A cell line HP75... purchased from Scie... (Carl..., CA, USA). Dulbecco's Modif... agle'... edium (DMEM) medium, penicill... trepton... were acquired from ...o (... m, M... USA). Horse serum and ...ovin... (FBS) were obtained from Gib... (Waltha... MA, USA). miR-NC, miR-16 m... miR-16 inhibitor were designed and ...es... y Gima (Shanghai, China). Rabbit ...ti-MEK1, ERK1/2, p-ERK1/2 were procured ... Abcam (Cambridge, MA, USA). Mouse anti... in D1, Survivin, and beta-actin antibody were got from GeneTex Inc (Irvine, CA, USA). Dual-luciferase Reporter Assay System and pMIR plasmid were from Promega (Madison, WI, USA). Transfection reagent FuGENE6 was gained from Roche (Indianapolis, IN, USA). Total RNA extraction reagent TRIzol Universal was purchased from Tiangen Biochem (Beijin... Chi... na). Real-time qPCR reagent TransS... One-Step qRT-PCR SuperMix was ...ght from Quanshijin Bio (Beijing, China). ...ck-iT EdU Alexa Fluor 488 Flow Cytometry ... Kit was collected from Molecular P... es (Et... OR, USA). RIPA lysis buffer a... ll apoptosi... were from Beyotime (B... g, Chi...).

Cell Culture

Human PA c... ne H... was in... ated in DMEM medi... containing ...% ...l bovine serum (FBS... orse serum ...% penicillin-strept... cin, ...ere incubated in a 37°C chamber with 5% ... Culture medium was cha... ery 3 days, ... cells at log-growth p... with satisfactory sta...s were used for further experiments.

S...ration an... ulture of Embryonic Pitu... Cells

Six ... were collected from water balloon ...bortion at 6th gestation month, without abnormal ...levelopment. The embryo was cut into pie... s ... was digested in 0.1% type I collagenase at 37°C for 2 h. The digestion was quenched in culture medium containing 10% FBS. Undigested tissue debris was filtered out, and the filtrate was centrifuged at 1600 rpm for 8 min to obtain the precipitation, which was re-suspended in DMEM medium containing 10% FBS and 1% penicillin-streptomycin. Cells were inoculated in culture dish and were incubated at 37°C with 5% CO_2. Culture medium was changed every 3 days, and cells at log-growth phase with satisfactory status were used for further experiments.

Dual Luciferase Reporter Gene Assay

Using HEK293T cell genome as the template, full-length fragment of 3'-UTR of MEK1 gene was amplified and was ligated into luciferase reporter pMIR plasmid for transforming DH5α competent bacteria. Positive clones with correct sequences were screened out by sequencing. Those clones with correct sequences were named as pMIR-MEK1-3'-UTR-wt or pMIR-MEK1-3'-UTR-mut. FuGENE6 was used to co-transfect pMIR-MEK1-3'-UTR-wt (or pMIR-MEK1-3'-UTR-mut) and miR-16 mimic (or miR-NC or miR-16 inhibitor) into HEK293T cells. After 48 h incubation, cells were rinsed twice in PBS. Pas-

sive Lysis Buffer from Dual-Luciferase Reporter Assay System kit was added for complete lysis at 4°C. 10 μL lysate supernatant was added into 96-well plate, followed by the addition of Stop&Glo buffer. Dual luciferase activity was measured on a microplate reader.

Cell Transfection and Grouping

In vitro cultured HP75 cells were divided into four groups: miR-NC transfection, miR-16 mimic transfection, si-NC transfection, and si-MEK1 groups. One day before transfection, cells were sub-grouped into culture plate. At the day of transfection, cells should reach 60-70% confluence. 100 μL serum-free and dual antibiotic-free basic medium was used to dilute 10 μL FuGENE6 Reagent, followed by the addition of 20 nmol/L miR-NC, miR-161 mimi, si-NC or si-MEK1. After gentle mixture, 20 min room temperature incubation was performed. After discarding original culture medium from the plate, transfection mixture was added for gentle mixture and further incubation. 6 h later, DMEM containing 10% FBS and 1% streptomycin-penicillin was added for 72 h continuous incubation. Cells were collect[ed] for assays of cell proliferation, apoptosis, and [detection] of related indexes.

Quantitive Reverse Transcription-Polymerase Chain Reaction (qR[T-PCR]) for Gene Expression

TransScript Green One[-Step] qRT-[PCR] Super-Mix was used to test rel[ative] expres[sion] of target genes using R[NA] e[xtracted] by TR[IZOL] Universal kit as th[e] template [for] one-step qRT-PCR. In a 20 μL [reac]tion syste[m], there were 1 μg RNA temp[late], 0.4 μM forward [and] reverse primers, 10 μ[L] 2XTra[nsStart] Tip Green qPCR SuperMix, [0.4] μL RT En[zyme] Mix, 0.4 μL Dye II and [ster]ed water. qRT-P[CR] conditions were: 45°C [for] 5 min f[or] reverse transcription, followed by [95°C] 30 s, [and] 40 cycles each containing 94°C 5 s, [60°C] [for] 30 s. Gene [ex]pression was measured [by] Bio-[Rad] CFX9[6] Quantitative PCR (Hercules, [CA, U]SA) [and] analyzing fluorescent data.

Western Blot

[Cells] were lysed by RIPA lysis buffer at 4°C incubation for 30 min. After 10000 g centrifugation [for] 10 min, the supernatant was saved, and 40 μg samples were loaded and were separated in 10% separating sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separating gel (40 V for 300 min). Proteins were transferred to polyvinylidene difluoride (PVDF) membrane by electrophoresis (250 mA, 120 min). The membrane was blocked in 5% defatted milk powder for 60 min at room temperature. Primary antibody (MEK1 at 1:4000, ERK1/2 at 1:3000, p-ERK1/2 survivin at 1:3000, cyclin D1 at 1:300[0] and β-actin at 1:10000) was added for 4°C overnight incubation. The membrane was rinsed in PBST [for] three times, and horseradish peroxidase (HRP) conjugated secondary antibody (1:25000) was added for [1] h room temperature incubation. The membrane was rinsed in PBST for three times, and enhanced chemiluminescence (ECL) solution was added for 2-3 min incubation. The membrane was exposed in the dark and examined for imaging.

Cell Apoptosis Assay

Cells were digested [and] collected with trypsin. After [re-]suspension in Binding Buffer, 5 μL Annexin V-fluoresceine isothiocyanate (FITC) and 5 μL PI buffer were added for staining. Beckman Coulter CytoFLEX flow cytometry (Brea, CA, USA) was used to test cell apoptosis.

Flow Cytometry for Cell Proliferation

Cells were re-suspended in complete culture [medium]. Cell proliferation was measured by Click-iT EdU Alexa Fluor 488 Flow Cytometry Assay Kits. In brief, cells were treated in 10 μM EdU for 2 h, and were incubated for 48 h furthermore. Cells were digested with trypsin. After centrifugation, fixation, and permeabilization, reaction buffer containing Alexa Fluor 488 labels was added for 30 min dark incubation at room temperature. By centrifugation and washing, Beckman Coulter (Brea, CA, USA) CytoFLEX flow cytometry was used to test cell proliferation.

Flow Cytometry for Cell Cycle

Cells were digested in trypsin and were fixed in 70% ethanol. Phosphate buffered solution (PBS) containing 25 μg/mL PI, 25 μg/mL RNase A, 0.1% Triton X-100 was added for 4°C dark incubation for 30 min. Beckman FC 500 MCL/MPL flow cytometry (Brea, CA, USA) was added for measuring cell cycle.

Statistical Analysis

SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. Measurement data were presented as mean±standard deviation (SD). Student *t*-test was used to compare measurement data between groups. A statistical significance was defined when $p<0.05$.

Results

Targeted Regulation Between miR-16 and MEK1

Bioinformatics analysis revealed complementary binding sites between miR-16 and 3'-UTR of MEK1 (Figure 1A). Dual luciferase gene reporter assay further validated that transfection of miR-16 mimics significantly suppressed relative luciferase activity in HEK293T cells, whilst transfection of miR-16 inhibitor elevated relative luciferase activity (Figure 1B), indicating the regulating effect of miR-16 on MEK1 mRNA.

Abnormal Expression of miR-16 and MEK1 in PA Tumor Tissues

qRT-PCR showed that, compared to that in normal pituitary tissues, the level of miR-16 was significantly decreased while MEK expression was statistically up-regulated in PA tumors ($p<0.05$) (Figure 2A). Western blot results also showed significantly higher MEK1 protein expression in PA tumor tissue compared to that in normal pituitary tissues (Figure 2B), indicating potential role of miR-16 in regulation of MEK1 protein ex sion and PA occurrence.

MiR-16 Down-Regulation and MEK1 Up-regulation in PA Tumor Cells

We also determine the level iR-16 a MEK1 in PA tumor cells results showed that, compared to nal em onic pituitary cells, in PA cells miR stically reduced with ain el M , Cyclin D1, and Su in mR vels (Figure 3A). Concomita he expressi f MEK1, p-ERK1/2, Cy d Survivin e remarkably elevated protei by Western blotting detection (Figure 3B). Flow cytometry showed that HP75 cells performed significantly higher proliferation potency (Figure 3C) and S or G2/M phase ratio (Figure 3D) than normal emb ic pituitary cells did, whilst G0/G1 phas getting lower correspondingly. T result showed that down-regulation of n 6 might be involved in the rise of MEK1 prot pression and PA pathogenesis.

MiR-16 Over-expres Inhi ed PA Tumor Cells Prolif on a nduced Cell Apoptosis a Arresti

We further date verexp ion of miR-16 on th oliferation A or cells. Intriguingly demonstra at the transfection o R-16 ic or si-MEK1 significantly suppressed levels K1, p-ERK1/2, Cyclin D1 vivin (Figu and 4B), and impede ell proliferation poten cy (Figure 4E), with itating effect cell apoptosis (Figure 4C) or arresting a /G1 phase (Figure 4D).

Discussion

PK signal transduction pathway is widely st ed in eukaryotic cells with important signal transduction function and further activates multiple transcriptional factors in nucleus and protein kinase, to regulate transcription and expression of related target genes, eventually mediating various pathological and biological processes including cell survival, proliferation, migration, apoptosis, angiogenesis, and immune response[22,23]. ERK-induced MAPK signal pathway is the classical signal transduction pathway, and is the major pathway for MAPK to exert its

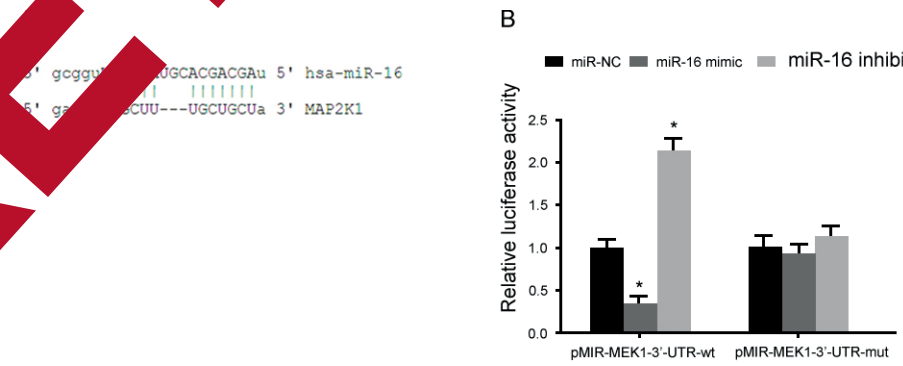

Figure 1. Targeted regulation between miR-16 and MEK1. ***(A)*** Binding sites between miR-16 and 3'-UTR of MEK1 mRNA; ***(B)*** Dual luciferase reporter gene assay. *, $p<0.05$ compared to miR-NC group.

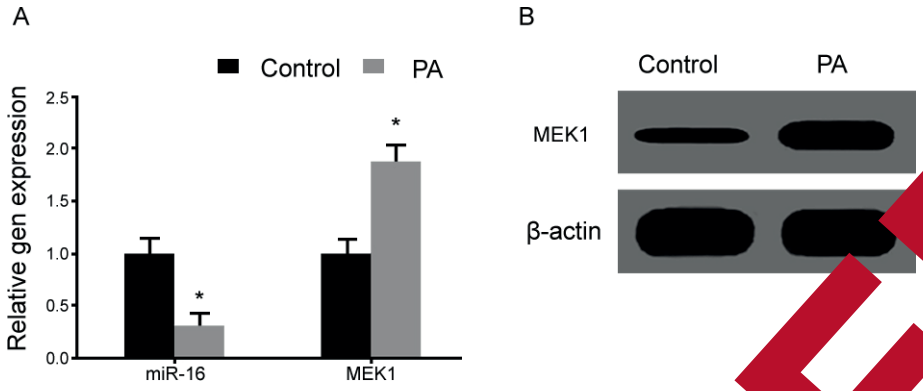

Figure 2. Abnormal expression of miR-16 and MEK1 in PA tumor tissues. ***(A)*** qRT-P[illegible] for[illegible]ession; ***(B)*** [illegible]estern blot for protein expression. *, $p<0.05$ compared to control group.

functions[9,24]. Accumulative evidence showed that abnormal activation of ERK/MAPK signal pathway was correlated with occurrence and progression of multiple tumors including gastric cancer[25], gallbladder carcinoma[6], and colon cancer[22]. Therefore, in this paper, we identified the possible mechanism of miR-16 on PA tumor that was also associated with ERK/MAPK signal pathway. MEK1 represents a type of dual-sp[illegible] protein k[illegible], a[illegible]ctions at the upstream of ERK protein to phos[illegible]late and activate tyrosine/[illegible]ne (Tyr/Thr) [illegible]lues of ERK protein, th[illegible]ctivating ERK/MAP[illegible] signal pathway. The [illegible]ious study[15] [illegible]owed the correlation between [illegible]K1 and PA [illegible]nogenesis. Interestingly, our *in [illegible]o* bioinfo[illegible]tics analysis showed complemen[illegible]targ[illegible]sites between miR-16 and 3'-UTR o[illegible]mRNA, the relation of which was

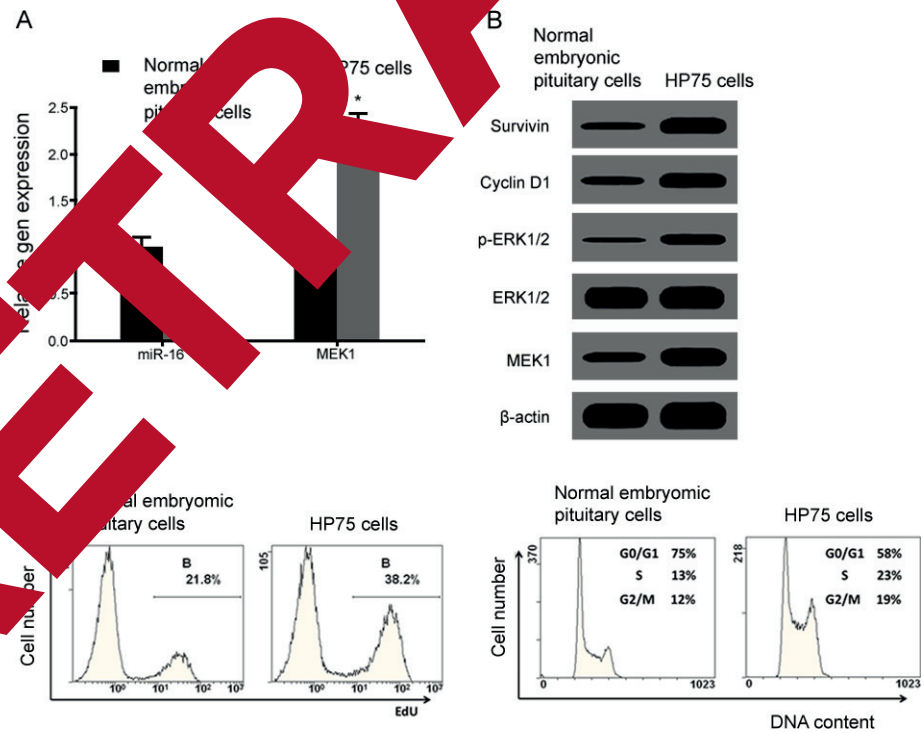

Figure 3. MiR-16 down-regulation and MEK1 up-regulation in PA tumor cells. ***(A)*** RT-PCR for gene expression; ***(B)*** Western blot for protein expression; ***(C)*** Flow cytometry for cell proliferation; ***(D)*** Flow cytometry for cell cycle. *, $p<0.05$ compared to normal embryonic pituitary cells.

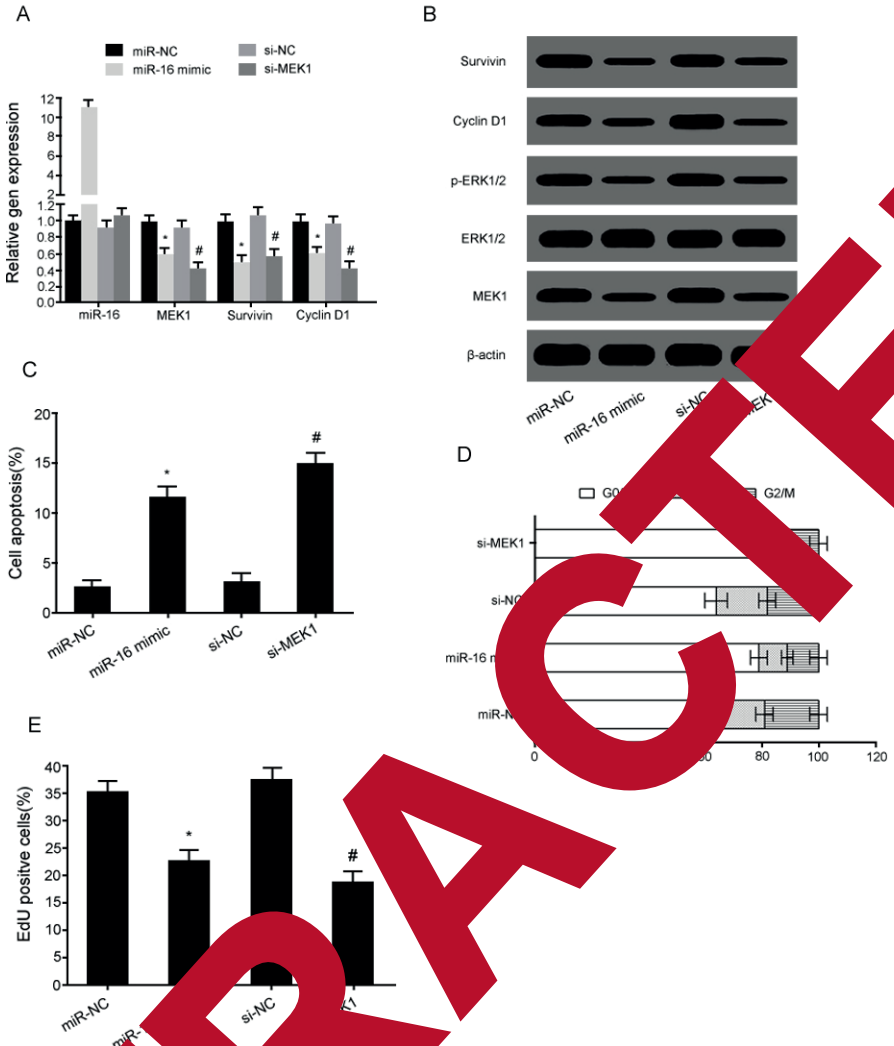

Figure 4. MiR-16 over-e sion PA cell proliferation and induced cell apoptosis or cell cycle arrest. ***(A)*** qRT-PCR for gene expression; ***(B)*** tern blot in expression; ***(C)*** Flow cytometry for cell apoptosis; ***(D)*** Flow cytometry for cell cycle; ***(E)*** Flow cyto for cell prolif otency. a, $p<0.05$ comparing between miR-16 mimic and miR-NC; b, $p<0.05$ comparing betwe nd si-NC.

further ied by dual luci e gene reporter assa our p iminary data showed that the mi expr n in PA patient tumor tissues was s dy de ed with growing level MEK ng w e evidence that miR-16 xpres ited PA tumor cells proli-fera n, we sp ated the involvement of ERK/M nal pathway in the regulation of miR-1 lopment.

MicroRNAs exert essential regulatory function roliferation and apoptosis of pituitary tumor c Amaral et al[20] showed that, compared to that in normal pituitary tissues, significantly lower miR-16 expression was found in PA tumor tissues. Bottoni et al[21] found remarkably lower miR-16 expression in PA tissues, and its correlation with tumor lesion size, as lower miR-16 expression was accompanied with larger tumor volume. Renjie et al[27] also showed that, compared to normal pituitary tissues, PA lesion gave rise to significantly lower expression of miR-16 in GH3 and MNQ cells. Compared to non-invasive pituitary tissues, dramatically lower miR-16 expression was detected in invasive PA tumor tissues, indicating that miR-16 down-regulation was correlated with PA progression and peripheral invasion. Consistently, in this study, reduction of miR-16 level was observed in both PA patient tumor tissues and PA cell line HP75, supporting studies performed by Amaral et al[20], Bottoni et al[21], and Renjie et al[27].

Dworakowska et al[15] found that, compared to normal pituitary tissues, in PA tumor tissues, the level of MEK1 was significantly increased, which resulted in the induction of ERK/MAPK signal pathway. In this study, we observed that in PA tumor cells, MEK1 expression and ERK/MAPK pathway activity were abnormally potentiated, as similar with Dworakowska et al[15]. Further study showed that transfection of miR-16 mimic or si-MEK1 significantly suppressed MEK1, p-ERK1/2, Cyclin D1, and Survivin expression in HP75 cells, inhibited the cell proliferation potency, indicating that miR-16 could weaken malignant biological property of PA cells via targeted inhibition on MEK1 expression based on the finding of in silico prediction and luciferase gene reporter assay. Moreover, Renjie et al[27] showed that over-expression of miR-16 could weaken clonal formation or invasion potency of PA cells, which were similar with our observation that miR-16 antagonized malignant biological characteristics of PA cells. However, *in vivo* study is further required for the evaluation of miR-16 on PA cell growth or tumor formation potency within animal model.

Conclusions

MiR-16 is down-regulated, while ERK/MA signal pathway is activated in pa sis of PA Over-expression of miR-16 d ta e proliferation potency of PA tu cells, i ced cell cycle arrest, and cell ap via t bition of MEK1 expre n.

Conflict of In s

The Authors dec e that th no conflict of interest.

eferences

1) L GELLA C GELO D, MUSSNICH P, WIERINCKX ACCIANO AVEROT G, LACHUER J, ROTONDI JAFF OUILLAS J, FUSCO A. Mir-23b and R-130 ion is downregulated in pituitary enomas. Cell Endocrinol 2014; 390: 1-7.

2) DE MARTINO M, D'ANGELO D, MUSSNICH P, JAFFRAIN-REA ML, FRAGGETTA F, TROUILLAS J, FUSCO A. HMGA1-pseudogene expression is induced in human pituitary tumors. Cell Cycle 2015; : 1471-1475.

3) GRUPPETTA M, VASSALLO J. Epidemiology and radiological geometric assessment of pituitary macroadenomas: population-based study. Clin Endocrinol (Oxf) 2016; 85: 223-231.

4) AMLASHI FG, TRITOS NA. Thyrotropin-secreting pituitary adenomas: epidemiology, diagnosis, and management. Endocrine 2016; 52: 427-440.

5) AGUSTSSON TT, BALDVINSDOTTIR T, JONASSON JG, OLAFSDOTTIR E, STEINTHORSDOTTIR V, SIGURDSSON G AV, CARROLL PV, KORBONITS M, BENEDI epidemiology of pituitary adenom n Iceland, 1955-2012: a nationwide popula ased study. Eur J Endocrinol 2015; 173: 655-

6) BUCHEGGER K, SILVA R, LÓPEZ LI C, LEAL P, BREBI P, RIQUELME I, ROA he ERK/ pa thway is overexpresse activated in g der cancer. Pathol R act 20 13: 476-

7) LIAO T, WEN D, MA JQ N, SHI R LIU L, GUAN Q, LI DS O ociated ein 1 promotes pa y thyr ncer c roliferation by ac g the ER K naling pathway. O t 2017; 8: 11 28.

8) RIVERS MO I V, STECCA L KLF4 is regulated by RAS/R K/ERK signaling through E d promotes noma cell growth. Oncol 2017; 36: 3322 3.

9) LI L, ZHAO GD, SHI Z, QI LL, ZHOU LY, FU ZX. The Ras/Raf/MEK K signaling pathway and its role in the occurre and development of HCC. Oncol Lett 2016 3045-3050.

10) ZHAO X, QIN Y, GONG X. Bicyclol ind cycle arrest and autophagy in HepG2 human hepatocellular carcinoma cells through the PI3K/AKT and Ras/Raf/MEK/ERK pathways. Cancer 2016; 16: 742.

11) XIN L, MA X, XIAO Z, YAO H, LIU Z. Coxsackievirus B3 induces autophagy in HeLa cells via the AMPK/MEK/ERK and Ras/Raf/MEK/ERK signaling pathways. Infect Genet Evol 2015; 36: 46-54.

12) PENZVALTO Z, LANCZKY A, LENART J, MEGGYESHAZI N, KRENACS T, SZOBOSZLAI N, DENKERT C, PETE I, GYORFFY B. MEK1 is associated with carboplatin resistance and is a prognostic biomarker in epithelial ovarian cancer. BMC Cancer 2014; 14: 837.

13) LI L, WANG J, ZHANG Y, 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 tumorigenesis in liver cancer. FEBS Lett 2013; 587: 3921-3927.

14) GYSIN S, PAQUETTE J, MCMAHON M. Analysis of mRNA profiles after MEK1/2 inhibition in human pancreatic cancer cell lines reveals pathways involved in drug sensitivity. Mol Cancer Res 2012; 10: 1607-1619.

15) DWORAKOWSKA D, WLODEK E, LEONTIOU CA, IGREJA S, CAKIR M, TENG M, PRODROMOU N, GOTH MI, GROZINSKY-GLASBERG S, GUEORGUIEV M, KOLA B, KORBONITS M, GROSSMAN AB. Activation of RAF/MEK/ERK and PI3K/AKT/mTOR pathways in pituitary adenomas and their effects on downstream effectors. Endocr Relat Cancer 2009; 16: 1329-1338.

16) HE J, ZHAO J, ZHU W, QI D, WANG L, SUN J, WANG B, MA X, DAI Q, YU X. MicroRNA biogenesis pathway genes polymorphisms and cancer risk: a systematic review and meta-analysis. PeerJ 2016; 4: e2706.

17) Shrestha S, Hsu SD, Huang WY, Huang HY, Chen W, Weng SL, Huang HD. A systematic review of microRNA expression profiling studies in human gastric cancer. Cancer Med 2014; 3: 878-888.

18) 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.

19) 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.

20) Amaral FC, Torres N, Saggioro F, Neder L, Machado HR, Silva WA, Jr., Moreira AC, Castro M. MicroRNAs differentially expressed in ACTH-secreting pituitary tumors. J Clin Endocrinol Metab 2009; 94: 320-323.

21) Bottoni A, Piccin D, Tagliati F, Luchin A, Zatelli MC, degli Uberti EC. miR-15a and miR-16-1 down-regulation in pituitary adenomas. J Cell Physiol 2005; 204: 280-285.

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 cancer cell proliferation by activating MAPK pathway. Oncotarget 2017; 8: 1-1554.

24) Knight T, Irving JA. Ras/Raf/MEK/ERK pathway activation in childhood acute lymphoblastic leukemia and its therapeutic targeting. Front Oncol 2014; 4: 160.

25) Jia S, Lu J, Qu T, Feng Y, X, Liu C, inhibits migration and invasion via blocking MAPK/ERK signaling pathway in gastric cancer. Chin J Cancer Res 2017; 29: 3-13.

26) Cui M, Zhang JP. Effect of microRNA-21 targeting PTEN on proliferation and apoptosis of pituitary tumor cells. Rev Med Pharmacol Sci 2017; 21: 2995-

27) Renjie , Haiqia iR-132, miR-15a and miR-16 synergistically inhibit pituitary tumor cell proliferation, invasion and migration by targeting Sox5. Oncol Lett 2015; 356: -578.