Int J Clin Exp Pathol 2017;10(7):7847-7855
www.ijcep.com /ISSN:1936-2625/IJCEP0056410

Original Article

MiR-181d functions as a potential tumor suppressor in oral squamous cell carcinoma by targeting K-ras

Yuyuan Shi[1], Ying Li[2], Wei Wu[1], Bing Xu[1], Jing Ju[3]

[1]Department of Oral Medicine, Ningbo No.2 Hospital, Ningbo, China; [2]Department of Oral Medicine, The People's Hospital of Fuyang City, Hangzhou, China; [3]Department of Biochemistry and Molecular Biology, Nanjing Medical University, Nanjing, China

Received April 28, 2017; Accepted May 22, 2017; Epub July 1, 2017; Published July 15, 2017

Abstract: Oral squamous cell carcinoma (OSCC) is one of the most frequent carcinomas all over the world, and the mechanism of its progression remains poorly understood. MicroRNAs have been found to play pivotal roles in many cancers including OSCC. However, the detailed roles of miRNAs in OSCC remain to be fully elucidated. In this study, we aimed to investigate the role of miR-181d in the progression of OSCC and to further elucidate its possible regulatory mechanism. Differentially expressed miRNAs between OSCC tissues and adjacent normal tissues were identified by microarray and validated using quantitative reverse transcription PCR (qRT-PCR). Moreover, the effects of miR-181d on the cell viability and apoptosis were investigated. In addition, a direct target of miR-181a, K-ras was assessed by the luciferase reporter assay and western blot. K-ras was overexpressed to evaluate its reverse effect on miR-181d mediated tumor suppression in OSCC. A panel of 54 differentially expressed miRNAs was identified by microRNA array. Among them, miR-181d was showed to be significantly downregulated. We also found that miR-181d lowly expressed in 20 pairs of OSCC tissues and four cell lines compared with that in adjacent normal tissues and human normal oral keratinocyte cells. In vitro assays showed that upregulation of miR-181d markedly decreased cell viability and increased OSCC cell apoptosis. Furthermore, we demonstrated that K-ras was a target of miR-181d and there was a negative correlation between miR-181d and K-ras expression in OSCC tissues. Importantly, overexpression of K-ras reversed the inhibitory effects of miR-181d mimics on OSCC cells. miR-181d functions as an OSCC suppressor by targeting K-ras oncogene. Thus, miR-181d may serve as a novel therapeutic target for treating OSCC.

Keywords: microRNA-181d, K-ras, oral squamous cell carcinoma, proliferation, apoptosis

Introduction

Oral squamous cell carcinoma (OSCC), which affects hundreds of millions of people worldwide, is the sixth most common cancer worldwide, with high morbidity and mortality [1]. Although early stage OSCC are associated with high cure rates through surgery and radiotherapy, the five-year survival rates remain at less than 50% [2]. Therefore, the investigation into the pathogenesis and diagnosis of OSCC is of great significance.

MicroRNAs (miRNAs) are a class of small noncoding RNAs consisting of about 22 nucleotides that regulate gene function by targeting mRNAs for translational repression or degradation [3]. Recent observations showed that miRNAs regulated many biological processes involving in the pathogenesis of various human cancers, such as cell proliferation, differentiation, metabolism and apoptosis [4, 5]. Several miRNAs have been confirmed to participate in the initiation and progression of OSCC [6]. Yuan Hui et al found that miR-101 exerted tumor-suppressive functions by targeting CX chemokine receptor 7 (CXCR7), leading to inhibition of OSCC cell growth, invasion, and migration [7]. Recently, miR-143 and miR-137 have been reported as tumor suppressors in OSCC [8, 9]. Thus, more studies need to be performed to identify more novel miRNAs in OSCC.

Among numerous miRNAs, miR-181d,which belongs to the cluster of miR-181, has been reported to be downregulated in several cancers. For example, decreased levels of miR-181d expression was confirmed and was posi-

tively correlated with the tumor's grade in glioma [10]. This result suggests that miR-181d plays a tumor suppressive role in glioma. Recently, Li D et al showed that miR-181d also functioned as a tumor suppressor in ESCC through inversely regulating its target gene DERL1 [11]. However, the roles of miR-181d in OSCC have never been reported before.

In this study, we analyzed the expression of miRNAs in OSCC tissues using microRNA array and found that miR-181d was deregulated. We also demonstrated that miR-181d suppressed the proliferation and promoted the apoptosis in OSCC cells. Moreover, K-ras was identified as a direct target of miR-181d in OSCC. Our findings provided new insights into the molecular function of miR-181d in OSCC and suggested miR-181d could serve as a potential therapeutic target of OSCC.

Materials and methods

Cell lines and tissue samples

Four cell lines (SCC-4, SCC-9, CAL-27 and Ca9-22) were maintained in our lab and cultured in Dulbecco's Modified Eagle Media (DMEM). The medium was supplemented with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA), 100 U/mL penicillin and 100 μg/mL streptomycin at 37°C in a 5% CO_2 atmosphere. A normal oral mucosa cell line (Human Oral Keratinocyte, HOK, Invitrogen) was used as control. HOK-16B cells were maintained in oral keratinocyte media, supplemented with 1% keratinocyte growth factor plus epithelial growth factor mixture (Invitrogen). Twenty freshly frozen OSCC samples and twenty normal samples (exfoliated epithelial cells, directly scraping of oral mucosa) were obtained from the Department of Oral Medical, Ningbo No.2 Hospital, Ningbo, Zhejiang Province, China. None of the patients with OSCC had received radiotherapy or chemotherapy before surgery. This study was approved by the Institutional Ethical Review Boards of our institute, and written informed consent was obtained from each patient.

MiRNA microarray

The miRNA microarray was performed as described previously [12, 13]. Briefly, total RNA of the mice brain was isolated by a miRNAeasy mini kit (Qiagen), and followed by labeling and hybridization with the miRCURY™ LNA Array (v.16.0, Exiqon). The feature extraction software (Agilent Technologies) was used to quantify the fluorescent intensity of each spot of microarray images, and signal intensities >10 were considered positive expression. The statistical significance of upregulated or downregulated miRNAs was analyzed by t-test. MEV software (v4.6, TIGR) was used to perform hierarchical clustering.

Cell transfection

MiR-181d mimic, miR-181d inhibitor and the corresponding controls were purchased from GenePharma (Shanghai, China). For constructing K-ras plasmids, the K-ras sequence was amplified and inserted into the pcDNA3.1 vector (Invitrogen, USA). The oligonucleotides and plasmids were transfected into SCC-4 and Ca9-22 cells with Lipofectamine™ RNAiMAX (Life Technologies, Grand Island, NY, USA) according to the manufacturer's instructions. 48 hours after transfection, cells were collected for further studies.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA of the cultured cells and the tissues was extracted using a mirVana miRNA isolation kit (Ambion, Carlsbad, CA, USA). miR-181d was reverse transcribed using the PrimeScript RT reagent Kit (TaKaRa, Tokyo, Japan) and quantified by real-time PCR with the TaqMan MicroRNA assay kit (Applied Biosystems). qRT-PCR analyses for K-ras and the normalization control gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were performed using SYBR Premix Ex Taq (TaKaRa) on an ABI PRISM 7500 Sequence Detection System (Applied Biosystems). The relative expression of each gene was calculated and normalized using the $2^{-\Delta\Delta}$Ct method relative to RNU6B or GAPDH. All reactions were conducted in triplicate.

Cell proliferation assays

To evaluate the effect of miR-181d on proliferative ability of OSCC cells, A cell-counting kit-8 (CCK-8, Dojindo, Kumamoto, Japan) assay was applied. Briefly, the transfected SCC-4 and Ca9-22 cells (5×10^3 cells) were added to 96-well plates. After transfection for 1 d, 2 d, 3 d and 4 d, 10 μl CCK-8 solutions were added into each well of the plate. Subsequently, these

cells were incubated for 1 h at 37°C, and the absorbance at 450 nm was measured. Experiments were independently repeated in triplicate.

Cell apoptosis detection by flow cytometry

After transfection for 48 h, SCC-4 and Ca9-22 cells were collected, and then washed twice with PBS. The cells were resuspended in 500 μl binding buffer and then mixed with 10 μl Annexin V (Bio-Science, Co. Ltd, Shanghai, China) for 10 min in the dark at room temperature (RT), followed by the addition of 5 μl PI (Bio-Science, Co. Ltd., Shanghai, China). After incubation at RT in the dark for 5 min, samples were analyzed by a FACS Aria flow cytometry (BD Biosciences, San Jose, CA, USA). Experiments were repeated in triplicate.

Western blot

For western blot analysis, cells were lysed with RIPA lysis buffer (Beyotime, Jiangsu, China). 50 μg proteins were fractionated on a 15% SDS-polyacrylamide gel. After electrophoretically transferring to a Pure Nitrocellulose Blotting membrane, the blots were probed with anti-K-ras (1:1000, Santa Cruz Biotechnology, USA) and then with horseradish peroxidase-conjugated secondary antibodies. Anti-β-actin antibody was used as an internal control. Signals were visualized by ECL chemiluminescence. Equal protein loading was assessed by the expression of β-actin. The bands were semi-quantified using Image J software.

Luciferase reporter assays

A whole fragment of 3'UTR K-ras mRNA and a mutant form were cloned into pGL-3-Luc. The HEK 293T cells were seeded in 12-well plates and co-transfected with pGL-3-K-ras wild-type or mutant portion and TK100 Renilla combined with miR-181d mimic, miR-181d inhibitor or NC control using Lipofectamine 2000 (Invitrogen). After 48 h of incubation, cells were collected for application in the Dual-Luciferase Reporter System (Promega, Madison, WI) following the manufacturer's recommendations. All of the dual-luciferase reporter assays were done in triplicate within each experiment, and three independent experiments were conducted.

Statistical analysis

Data are reported as mean ± standard deviation. Statistical significance among different groups was determined by Two-way ANOVA test of variance using the GraphPad Prism software (version 5.0). A p value of less than 0.05 was considered to be significant.

Results

miR-181d is downregulated in OSCC tissues and cell lines

To evaluate the expression of miRNAs involved in OSCC, miRNA microarray was performed. Based on microarray analysis, we identified 54 miRNAs differentially expressed between OSCC tissues and adjacent normal tissues (**Figure 1A**). Among the aberrantly expressed miRNAs, miR-181d was chosen as the candidate for further study because of its suppressive effects in several types of human cancer [10]. Subsequently, the expression of miR-181d was assessed in 20 pairs of OSCC and adjacent normal oral tissues using qRT-PCR. As shown in **Figure 1B**, the expression level of miR-181d was significantly decreased in OSCC tissues compared with normal tissues. Next, we analyzed the expression of miR-181d in four OSCC cell lines (i.e., Cal-27, Ca9-22, SCC-9, and SCC-4). A normal oral mucosa cell line HOK-16B was used as control. MiR-181d was considerably lower in OSCC cells than in HOK-16B, especially in SCC-4 and Ca9-22 cells (**Figure 1C**). These finding suggests that downregulation of miR-181d may be involved in the progression of OSCC.

Tumor suppressive effect of miR-181d on OSCC

Given the down-regulation of miR-181d in OSCC tissues, we predicted that miR-181d may function as a tumor suppressor. To verify our hypothesis, the miR-181d mimics were transfected into two OSCC cell lines, SCC-4 and Ca9-22, then the cell viability was assessed by CCK-8 assay. As shown in **Figure 2A** and **2B**, transfection of miR-181d mimics into OSCC cells significantly inhibited cell viabilitycompared with the mimics NC group. Further, we asked whether miR-181d overexpression affected cell apoptosis regulation. As indicated in **Figure 2C** and

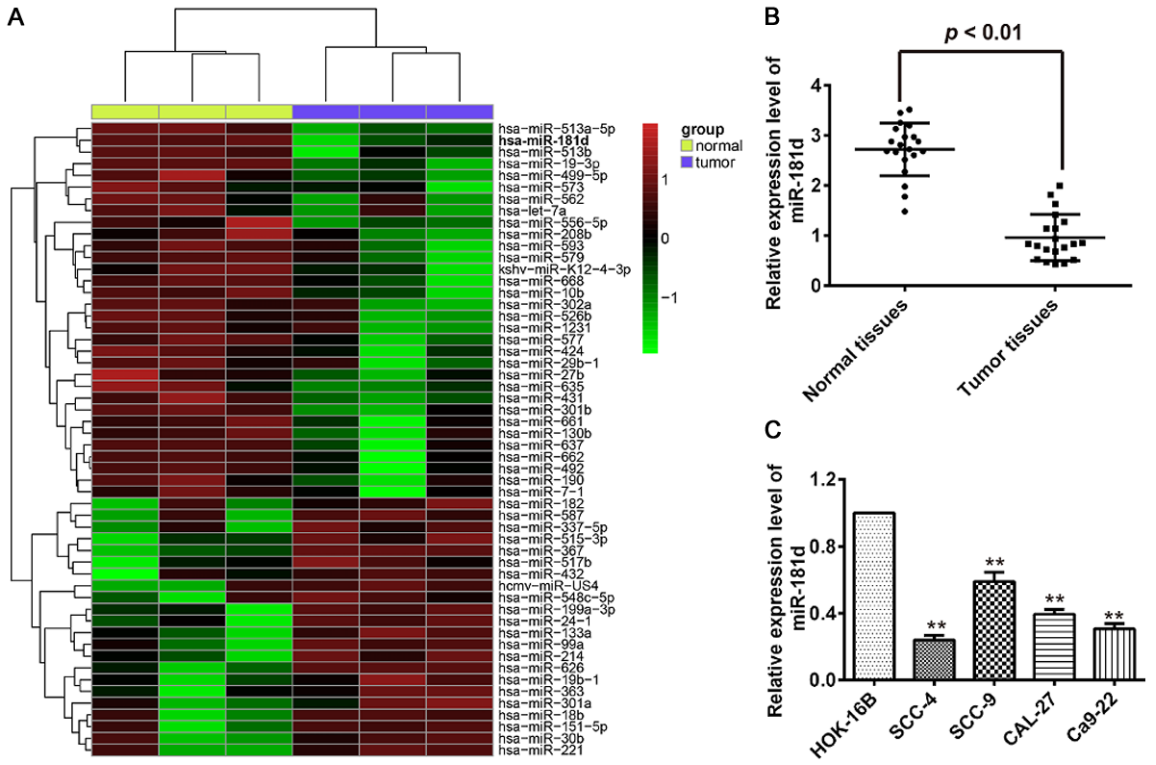

Figure 1. miR-181d is significantly downregulated in OSCC tissues and cell lines. A. Differentially expressed miRNAs in tumor tissues group and normal tissues group. B. miR-181d mRNA level was decreased in 20 pairs of OSCC tissues. P < 0.01 vs normal tissues. C. qRT-PCR assay was performed to determine miR-181d expression in the four OSCC cell lines (SCC-4, SCC-9, CAL-27 and Ca9-22) and HOK-16B cells. U6 was used as endogenous control. Data are shown as means ± SD of three separate experiments. **P < 0.01 vs HOK-16B cells.

2D, we found that overexpression of miR-181d promoted cell apoptosis compared with the control group. All these results suggested that miR-181d executed a tumor suppressive effect on OSCC cells.

K-ras is a direct target of miR-181d

To explore the molecular mechanism by which miR-181d functions in OSCC, TargetScan and PicTar algorithms were usedto identify the potential target genes of miR-181d. Previous studies proven that activation of an oncogenic K-ras allele can initiate a variety of cancer including OSCC [14, 15]. Interestingly, the data of two public databases showed that K-ras was a potential target of miR-181d. As suggested in **Figure 3A**, the binding sites between miR-181d and K-ras were illustrated. To confirm the direct binding relationship between miR-181d and K-ras, a luciferase activity assay was conducted. As shown in **Figure 3B**, co-transfection of miR-181d mimic and pGL-3-K-ras-wt significantly decreased the luciferase activity, whereas co-transfection of miR-181d inhibitor and pGL-3-K-ras-wt increased the luciferase activity. Likewise, cells co-transfected with miR-181d mimic, miR-181d inhibitor and pGL-3-K-ras-mut showed no obvious change in luciferase activity. Then, we explore whether miR-181d can modulate the expression of K-ras. As shown in **Figure 3C**, miR-181d overexpression down-regulated K-rasexpression at the protein levels, whereas increased after inhibition of miR-181d. All these data indicate that K-rasis a direct target of miR-181d.

miR-181d functions as a tumor suppressor by targeting K-ras

Next, we measured miR-181d expression level and K-ras mRNA level in 10 pairs of OSCC tissues and the matched normal tissues. As shown in **Figure 4A**, an inverse correlation was observed between K-ras expression and the miR-181d expression level. To further investi-

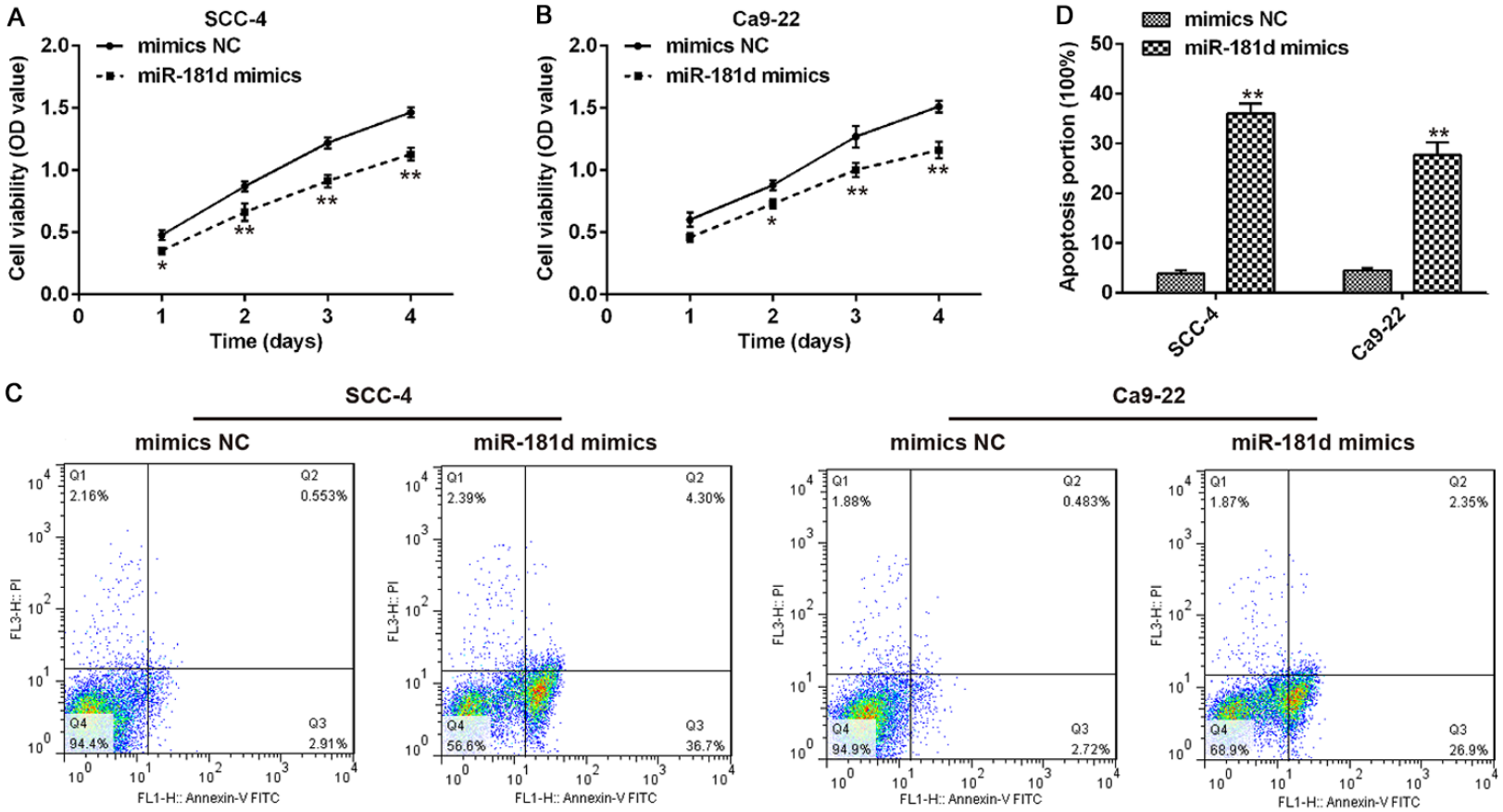

Figure 2. miR-181d inhibited cell proliferation and induced cell apoptosis. SCC-4 and Ca9-22 cells at 70% confluence were transfected with miR-181d mimics or mimics N (C) After transfection for 1 d, 2 d, 3 d and 4 d, the viability of SCC-4 cells (A) and Ca9-22 cells (B) were measured by CCK-8 assay. (C, D) After transfection for 48 h, Cell apoptosis was assessed by flow cytometry assay. Data are shown as means ± SD of three separate experiments. *P < 0.05, **P < 0.01 compared with the miR-NC group.

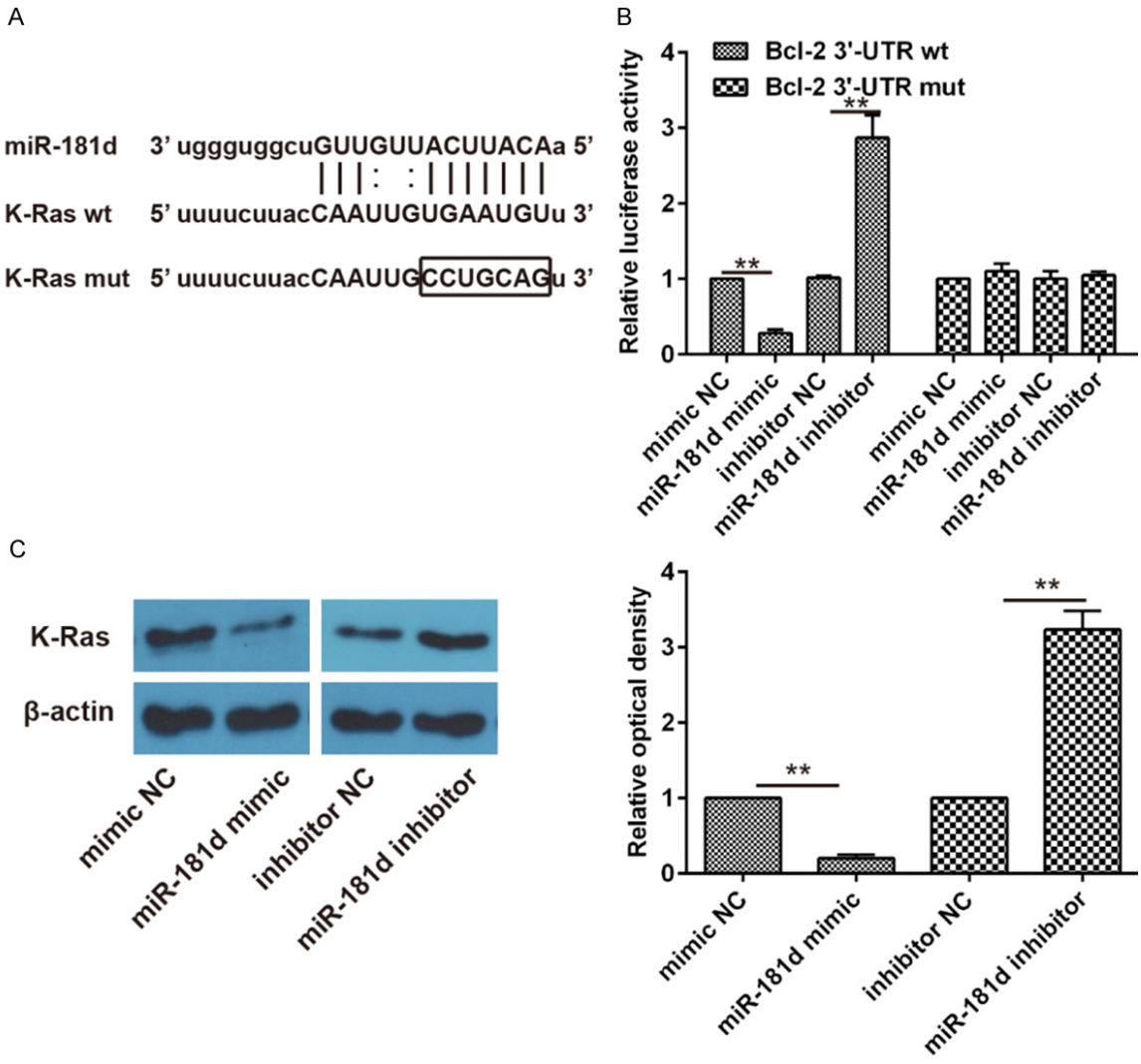

Figure 3. miR-181d directly targets K-ras. A. miR-181d binds to the predicted site of the 3'-UTR of K-ras. B. Dual-luciferase reporter gene analysis of K-ras 3'-UTR in HEK293T cells following co-transfection of miR-181d mimics, miR-181d inhibitors or miR-NC with pGL3 constructs containing WT- or MUT-3'-UTR of K-ras. Relative luciferase activities are the ratios of Renilla luciferase normalized to the control groups. C. Protein levels of K-ras in the indicated cells transfected with 20 nM miR-181d mimics, miR-181d inhibitors or miR-NC were measured by Western blot assays. β-actin was used as internal controls. Data are shown as means ± SD of three separate experiments. **P < 0.01 vs miR-NC.

gate whether miR-181d affected cell proliferation and apoptosis through its target K-ras, pcDNA-K-ras plasmids together with miR-181d mimics were transfected into SCC-4 and Ca9-22 cells. Then, cell viability and apoptosis was examined. As shown in **Figure 4B**, overexpression of K-ras attenuated the reduction of cell viability induced by miR-181d mimics. Furthermore, overexpression of K-ras reversed the promoting effect of miR-181d mimics on cell apoptosis (**Figure 4C**). These results indicate that miR-181d inhibits proliferation and induces apoptosis of OSCC cells by targeting K-ras.

Discussion

In the present study, we found that miR-181d was downregulated in OSCC tissues and cell lines. Moreover, we demonstrated that miR-181d served as a tumor suppressor in OSCC by targeting K-ras. Taken together, these results suggest that miR-181d maybe as a potential therapeutic target for OSCC.

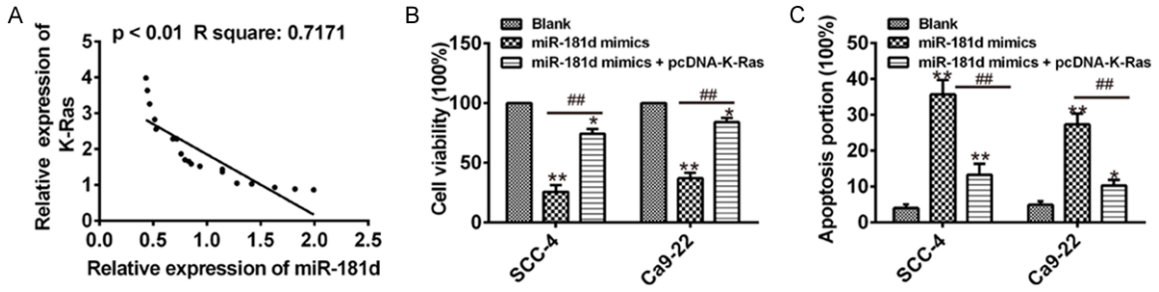

Figure 4. K-ras overexpression reversed the effects of miR-181d on the proliferation and apoptosis of OSCC cells. SCC-4 and Ca9-22 cells were transfected with miR-NC, miR-181d mimics, or miR-181d mimics plus K-ras-expressing plasmids. A. Pearson analysis for the correlation of K-ras and miR-181d expression levels in 20 pairs of OSCC tissues (n=20; r=0.7171; P < 0.01). B. After transfection for 24 h, the viability of SCC-4 cells and Ca9-22 cells were measured by CCK-8 assay. C. After transfection for 48 h, cell apoptosis was assessed by flow cytometry assay. Data are shown as means ± SD of three separate experiments. *P < 0.05, **P < 0.01 compared with the Blank group, ##P < 0.05 compared with the miR-181d mimic group.

A large number of studies have indicated that miRNAs may play an important role in OSCC initiation and development. For example, Jun Hu et al reported that miR-497 enhanced OSCC metastases through SMAD7 inhibition [16]. A study performed by Wang X et al showed that miR-204-5p regulated cell proliferation and metastasis through inhibiting CXCR4 expression in OSCC [17]. Therefore, understanding the roles of other miRNAs in OSCC is necessary. In the present study, we found that miR-181d was downregulated in OSCC tissues by miRNA microarray. It is well known that miR-181d acts as a tumor suppressor in glioma and human esophageal squamous cell carcinoma [10, 11], but the role of it in OSCC is not known. In agreement with these previous reports, our study found that miR-181d significantly inhibited cell growth and induced apoptosis in Ca9-22 and SCC-4 cells. Although the evidence highlighted the important roles of miR-181d in OSCC, the underlying mechanism through which miR-181d inhibits OSCC remains unclear.

K-ras is a member of the RAS oncogene family. The known function of K-ras was involved in various biological processes, including cell proliferation, differentiation, survival, and death [18]. Increasing evidence suggests that K-ras was able to promote tumorigenesis and progression in several human malignancies [19-22], suggesting that K-ras functioned as an oncogene. Recently, miRNAs were reported to be involved in the posttranscriptional regulation of K-ras and miR-induced K-ras dysregulation is frequently observed in human cancers [21, 23-26]. For example, Keklikoglou et al found that miR-206 reduced cell proliferation by depressing K-Ras in pancreatic adenocarcinoma [27]. A recent study demonstrated that miR-181d is frequently down-regulated in glioma and may be a potential tumor suppressor in glioma by targeting K-ras [10]. However, it is unknown whether K-ras mediates the functional effects of miR-181d on OSCC cells. In this study, we conformed that K-ras was a target of miR-181d by luciferase activity assay and western blot in OSCC. In addition, our data reveal a negative correlation between miR-181d and K-ras in OSCC tissues. More importantly, overexpression of K-ras effectively reversed the tumor suppressive functions of miR-181d on OSCC proliferation and apoptosis. These results might suggest that miR-181d exerted its suppressive role in OSCC by targeting K-ras.

In conclusion, we found that miR-181d was downregulated in OSCC and may be a potential tumor suppressor in OSCC by targeting K-ras. Thus, miR-181d may serve as a new target for OSCC therapy.

Acknowledgements

This study was supported by the Foundation from Zhejiang Province Natural Science (491020+N21394).

Disclosure of conflict of interest

None.

Address correspondence to: Ying Li, Department of Oral Medicine, The People's Hospital of Fuyang City, 429 North Ring Road, Fuyang District, Hangzhou

311400, China. Tel: +86-571-63322551; E-mail: liying_liy@126.com

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Ferlito A, Rinaldo A, Devaney KO, MacLennan K, Myers JN, Petruzzelli GJ, Shaha AR, Genden EM, Johnson JT, de Carvalho MB and Myers EN. Prognostic significance of microscopic and macroscopic extracapsular spread from metastatic tumor in the cervical lymph nodes. Oral Oncol 2002; 38: 747-751.

[3] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-297.

[4] Calin GA and Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.

[5] Wu BH, Xiong XP, Jia J and Zhang WF. MicroRNAs: new actors in the oral cancer scene. Oral Oncol 2011; 47: 314-319.

[6] Manikandan M, Deva Magendhra Rao AK, Arunkumar G, Manickavasagam M, Rajkumar KS, Rajaraman R and Munirajan AK. Oral squamous cell carcinoma: microRNA expression profiling and integrative analyses for elucidation of tumourigenesis mechanism. Mol Cancer 2016; 15: 28.

[7] Hui Y, Li Y, Jing Y, Feng JQ and Ding Y. miRNA-101 acts as a tumor suppressor in oral squamous cell carcinoma by targeting CX chemokine receptor 7. Am J Transl Res 2016; 8: 4902-4911.

[8] Sun X and Zhang L. MicroRNA-143 suppresses oral squamous cell carcinoma (OSCC) cell growth, invasion and glucose metabolism through targeting hexokinase 2. Biosci Rep. 2017; 37.

[9] Kozaki K, Imoto I, Mogi S, Omura K and Inazawa J. Exploration of tumor-suppressive microRNAs silenced by DNA hypermethylation in oral cancer. Cancer Res 2008; 68: 2094-2105.

[10] Wang XF, Shi ZM, Wang XR, Cao L, Wang YY, Zhang JX, Yin Y, Luo H, Kang CS, Liu N, Jiang T and You YP. MiR-181d acts as a tumor suppressor in glioma by targeting K-ras and Bcl-2. J Cancer Res Clin Oncol 2012; 138: 573-584.

[11] Li D, Shi M, Ji H, Chen G, Jiang H and Wang Z. MicroRNA-181d is a tumor suppressor in human esophageal squamous cell carcinoma inversely regulating Derlin-1. Oncol Rep 2016; 36: 2041-2048.

[12] Castoldi M, Schmidt S, Benes V, Hentze MW and Muckenthaler MU. miChip: an array-based method for microRNA expression profiling using locked nucleic acid capture probes. Nat Protoc 2008; 3: 321-329.

[13] Wang J, Xu W, Zhong T, Song Z, Zou Y, Ding Z, Guo Q, Dong X and Zou W. miR-365 targets beta-arrestin 2 to reverse morphine tolerance in rats. Sci Rep 2016; 6: 38285.

[14] Jackson EL, Willis N, Mercer K, Bronson RT, Crowley D, Montoya R, Jacks T and Tuveson DA. Analysis of lung tumor initiation and progression using conditional expression of oncogenic K-ras. Genes Dev 2001; 15: 3243-3248.

[15] Shin KH, Bae SD, Hong HS, Kim RH, Kang MK and Park NH. miR-181a shows tumor suppressive effect against oral squamous cell carcinoma cells by downregulating K-ras. Biochem Biophys Res Commun 2011; 404: 896-902.

[16] Hu J, Xu JF and Ge WL. MiR-497 enhances metastasis of oral squamous cell carcinoma through SMAD7 suppression. Am J Transl Res 2016; 8: 3023-3031.

[17] Wang X, Li F and Zhou X. miR-204-5p regulates cell proliferation and metastasis through inhibiting CXCR4 expression in OSCC. Biomed Pharmacother 2016; 82: 202-207.

[18] Rajalingam K, Schreck R, Rapp UR and Albert S. Ras oncogenes and their downstream targets. Biochim Biophys Acta 2007; 1773: 1177-1195.

[19] Funato T, Ishii T, Kambe M, Scanlon KJ and Sasaki T. Anti-K-ras ribozyme induces growth inhibition and increased chemosensitivity in human colon cancer cells. Cancer Gene Ther 2000; 7: 495-500.

[20] Fleming JB, Shen GL, Holloway SE, Davis M and Brekken RA. Molecular consequences of silencing mutant K-ras in pancreatic cancer cells: justification for K-ras-directed therapy. Mol Cancer Res 2005; 3: 413-423.

[21] He XY, Chen JX, Zhang Z, Li CL, Peng QL and Peng HM. The let-7a microRNA protects from growth of lung carcinoma by suppression of k-Ras and c-Myc in nude mice. J Cancer Res Clin Oncol 2010; 136: 1023-1028.

[22] Caulin C, Nguyen T, Longley MA, Zhou Z, Wang XJ and Roop DR. Inducible activation of oncogenic K-ras results in tumor formation in the oral cavity. Cancer Res 2004; 64: 5054-5058.

[23] Tsang WP and Kwok TT. The miR-18a* microRNA functions as a potential tumor suppressor by targeting on K-Ras. Carcinogenesis 2009; 30: 953-959.

[24] Chen X, Guo X, Zhang H, Xiang Y, Chen J, Yin Y, Cai X, Wang K, Wang G, Ba Y, Zhu L, Wang J, Yang R, Zhang Y, Ren Z, Zen K, Zhang J and Zhang CY. Role of miR-143 targeting KRAS in colorectal tumorigenesis. Oncogene 2009; 28: 1385-1392.

[25] Yu S, Lu Z, Liu C, Meng Y, Ma Y, Zhao W, Liu J, Yu J and Chen J. miRNA-96 suppresses KRAS and functions as a tumor suppressor gene in pancreatic cancer. Cancer Res 2010; 70: 6015-6025.

[26] Zhao WG, Yu SN, Lu ZH, Ma YH, Gu YM and Chen J. The miR-217 microRNA functions as a potential tumor suppressor in pancreatic ductal adenocarcinoma by targeting KRAS. Carcinogenesis 2010; 31: 1726-1733.

[27] Keklikoglou I, Hosaka K, Bender C, Bott A, Koerner C, Mitra D, Will R, Woerner A, Muenstermann E, Wilhelm H, Cao Y and Wiemann S. MicroRNA-206 functions as a pleiotropic modulator of cell proliferation, invasion and lymphangiogenesis in pancreatic adenocarcinoma by targeting ANXA2 and KRAS genes. Oncogene 2015; 34: 4867-4878.