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Original Article

miR-17-5p promotes proliferation and migration of CAL-27 human tongue squamous cell carcinoma cells involved in autophagy inhibition under hypoxia

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Abstract: Autophagy contributes to head and neck squamous cell carcinoma (HNSCC) development and progression. MiR-17-5p down-regulates Beclin-1 and thus plays an important role in autophagy, but little is known about the function and regulation of miR-17-5p in HNSCC autophagy. This study aimed to investigate the role of miR-17-5p on proliferation, migration, and autophagy under hypoxia in CAL-27 human tongue squamous cell carcinoma cells. CAL-27 cells were transfected with 50 nmol miR-17-5p mimics to overexpress miR-17-5p. Cell proliferation and migration were determined by CCK-8 and wound healing assays, respectively, under hypoxia. Autophagy induced by hypoxia was detected by transmission electron microscope and Beclin-1 mRNA and protein expressions. The miR-17-5p mimics successfully increased the expression of miR-17-5p in CAL-27 cells by almost 700 fold compared with the miRNA mimic negative control. After 3 days, cells transfected with the miR-17-5p mimics showed higher proliferation compared with controls ($P < 0.05$) under hypoxia. MiR-17-5p transfected CAL-27 cells had a higher migratory capacity compared with the control cells ($P < 0.05$) under hypoxia. Furthermore, transmission electron microscopy showed that miR-17-5p overexpression inhibited the formation of autophagosomes in hypoxic cells. Real-time quantitative polymerase chain reaction (RT-qPCR) and western blot showed that miR-17-5p overexpression inhibited the mRNA and protein expression of Beclin-1 in CAL-27 cells submitted to hypoxia. MiR-17-5p overexpression promoted the proliferation and migration of the CAL-27 cells, but inhibited autophagy under hypoxia.

Keywords: miR-17-5p, autophagy, Beclin-1, head and neck squamous cell carcinoma, proliferation, migration

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the 6th cancer in term of worldwide incidence [1]. About 90% of all head and neck cancers is HNSCC [2]. HNSCC may affect the lip, oral cavity, pharynx, and larynx. The risk factors for HNSCC include smoking, alcohol consumption, and human papilloma virus (HPV) infection [3]. Over the past decades, the diagnosis and treatment of HNSCC have made great progress, but mortality and prognosis have not been significantly improved [3]. A comprehensive multidisciplinary approach is needed to treat HNSCC, but the 5-year survival rate of patients with HNSCC is only about 50% [4]. HNSCC often shows factors of poor prognosis such as poor differentiation and lymph node invasion, but the molecular mechanisms are still poorly understood. The study of HNSCC development at the molecular level could be of great significance for its prevention, control, and treatment.

Autophagy is a survival process by which eukaryotic cells recycle proteins, nucleotides, and organelles in the normal development of organisms, and certain environmental stress res-

ponse is critical [5]. Autophagy takes part in many physiologic and pathologic processes such as development, differentiation, tumor, and neurodegenerative diseases [6]. Data about autophagy and its role in tumorigenesis are inconsistent among cancer types and organs [7]. In early cancer, autophagy can inhibit tumor formation; during cancer development, moderate autophagy will influence various death stimuli, thereby promoting tumor survival, and excessive autophagy can also induce autophagic cell death [8]. Cancer cells can enhance autophagy in order to survive to microenvironment stresses such as hypoxia and nutrient deprivation [9]. Indeed, hypoxia is commonly encountered in tumors since fast-growing tumors may lack the microcirculation necessary to provide oxygen to all cancer cells [10]. The hypoxia inducible factor (HIF) is central in the response to hypoxia and promotes autophagy, angiogenesis, metastasis, and resistance to chemotherapy and radiation therapy [10, 11]. In HNSCC, the autophagy induced by hypoxia is associated with an aggressive phenotype [12]. The complex network involved in the response to hypoxia and autophagy is still poorly understood. Nevertheless, Beclin-1 is recognized to play a critical role in autophagy and cell death by interacting with either BCL-2 or PI3K class III [13]. Zhao et al. [14] found that Beclin-1 and LC3B may contribute to the development of metastatic colorectal carcinoma. The study of autophagy in HNSCC and its regulation mechanism has wide clinical significance and good application prospects in the treatment of HNSCC [15].

MicroRNAs (miRNAs) are short non-coding RNA molecules that play a pivotal role in the regulation of the expression of a number of genes. The abnormal expression of miRNAs plays a key role in the course of many diseases including cancer [16]. Previous studies have shown that miR-17-5p is involved in the development of colorectal, prostate, ovarian, pancreatic, gastric, esophageal, lung, and nasopharyngeal cancers [17-20], but the role of miR-17-5p in the development of HNSCC remains elusive. The expression level of miR-17-5p is associated with cancer aggressiveness and therapy resistance [21]. In glioma cells, overexpression of miR-17-5p decreases Beclin-1-mediated autophagy and sensitizes the cells to radiation [22]. In non-small lung cancer, downregulation of miR-17-5p is associated with upregulation of Beclin-1 and resistance to paclitaxel [23]. Hence, miR-17-5p is involved in cancer development and progression by autophagy.

Despite vast research, the molecular mechanisms involved in hypoxia-induced autophagy of HNSCC cells are still poorly understood. Therefore, the aim of the present study was to transfect miR-17-5p mimics into CAL-27 human tongue squamous cell carcinoma cells to explore the role of miR-17-5p in CAL-27 cell proliferation, migration, and autophagy induced by hypoxia. The results could help unravel the mechanisms involved in hypoxia-induced autophagy and could provide hints for targeted treatments.

Materials and methods

Cells and treatment

The CAL-27 human tongue squamous cell carcinoma cell line was provided by the 9th People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine (Shanghai, China). Cells were cultured in Dulbecco's modified eagle medium (DMEM) (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher, Waltham, MA, USA) and 100 μM each of penicillin and streptomycin (Invitrogen, Carlsbad, CA, USA) in a humidified 5% CO_2 environment at 37°C.

The cells were treated under hypoxic conditions that were achieved with a gas-controlled chamber (Thermo Electron Corp., Marietta, OH, USA) maintained at 1% O_2, 94% N_2, and 5% CO_2 at 37°C [24].

MiRNA mimic transfection

We seeded CAL-27 cells on six-well plates at 1 × 10^5 cells/well, then transfected miR-17-5p mimics (and mimic negative control) at 50 nM using the riboFECT CP reagent (Ribobio, Guangzhou, China) after the cells had reached 50-60% confluence, according to the manufacturer's instructions.

Real-time quantitative polymerase chain reaction (RT-qPCR)

Forty-eight hours after transfection, the expressions of miR-17-5p and Beclin-1 were detected by RT-qPCR. Total small RNA was extracted from cells using the RNAiso for Small RNA re-

agent (Takara, Otsu, Japan). The concentration and purity of RNA were determined spectrophotometrically using the NanoDrop 2000 (NanoDrop Technologies, Wilmington, DE, USA). The purity of total small RNA was analyzed by the ratio of A260:A280. The Mir-X miRNA First-Strand Synthesis Kit (Takara, Otsu, Japan) was used for converting miRNAs into cDNA to enable specific RNAs to be RT-qPCR. The total RNA was reverse-transcribed using the PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) (Takara, Otsu, Japan) according to the manufacturer's instructions. RT-qPCR was performed using SYBR Premix Ex Taq II (Takara, Otsu, Japan) for miRNA and SYBR Premix Ex Taq (Takara, Otsu, Japan) for mRNA on a LightCycler (Roche Diagnostics, Basel, Switzerland). The relative quantification of miRNA or mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The raw data are presented as the relative quantity of target miRNA or mRNA, normalized with respect to U6 or GAPDH, and relative to a calibrator sample. The following primers were used: hsa-miR-17-5p forward 5'-CGG CGG CAA AGT GCT TAC AG-3' and reverse 5'-TGG TGT CGT GGA GTC G-3'; U6 forward 5'-CTC GCT TCG GCA GCA CA-3' and reverse 5'-AAC GCT TCA CGA ATT TGC GT-3'; BECN1 forward 5'-AAG GGT CTA AGA CGT CCA ACA A-3' and reverse 5'-GCC TGG GCT GTG GTA AGT AAT G-3'; and GAPDH forward 5'-AAG GTG AAG GTC GGA GTC AAC-3' and reverse 5'-CTT GAT TTT GGA GGG ATC TCG-3'.

Western blot

Forty-eight hours after transfection, Beclin-1 protein expression was detected by western blot. The cells were washed twice with cold PBS and lysed on ice in RIPA buffer (Beyotime, Shanghai, China) for 30 min. The cells were centrifuged at 12,000 × g for 15 min at 4°C. The supernatant was collected and the protein concentration was determined by the BCA Protein Assay Kit (Beyotime, Shanghai, China). Equal amounts of proteins (20 μg) were separated by SDS-PAGE using 10% polyacrylamide gel and transferred to polyvinylidenefluoride membrane (PVDF; Millipore corp., Billerica, MA, USA). After blocking with 5% nonfat milk in PBS, the membrane was immunoblotted overnight at 4°C with primary antibodies: anti-Beclin-1 monoclonal antibody (ab114071, Abcam, Cambridge, UK) and anti-β-actin monoclonal antibody (AA128, Beyotime, Shanghai, China). The secondary antibody, HRP-labeled Goat Anti-Mouse IgG (H+L) (Beyotime, A0216, Shanghai, China), was incubated with the membrane for 1 h after three washes with TBST. The signals were detected using an Immobilon Western Chemiluminescence HRP substrate (WBKLS0-500, Millipore corp., Billerica, MA, USA). The images were obtained and quantified using an Amersham Imager 600 system (GE Healthcare, Waukesha, WI, USA).

Cell counting kit-8 (CCK-8) assay

Cell proliferation was determined using the CCK-8 method. Briefly, cells were seeded in a 96-well microplate with 100 μL of growth medium per well and at a density of 2×10^4/ml. After 24 h, the cells were transfected with miR-17-5p mimics and mimic negative control at 50 nM using the riboFECT CP reagent (Ribobio, Guangzhou, China). After transfection for 24, 48, and 72 h, the cells were treated using the cell proliferation CCK-8 Assay Kit (Beyotime, Shanghai, China). After adding the CCK-8 solution (10 μ/well) and incubating at 37°C for 1 h, the absorbance was measured using a microplate reader (Thermo Fisher Multiskan Go 1510-01981, Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm.

Wound healing assay

The wound healing assay was used to detect the ability of miR-17-5p transfected cells to repair scratches. We seeded CAL-27 cells on six-well plates at 1×10^5 cells/well, then transfected miR-17-5p mimics or mimic negative control at 50 nM using riboFECT CP reagent (Ribobio, Guangzhou, China) after the cells reached 50-60% confluence. When the cells reached 90% confluence, scratches were made with a 200-μl pipette tip across the centre of the wells. After washing with culture medium to remove cell debris, the cells were allowed to migrate for 24 h. The wounds were photographed at 0 and 24 h using an Olympus IX53 inverted phase contrast microscope (Olympus, Tokyo, Japan). Three random fields were marked and measured. The migration index was expressed as the ratio of the migrating distance of the treated cells to that of the control cells.

Transmission electron microscopy (TEM)

Forty-eight hours after transfection, the cells were fixed with 2.0% glutaraldehyde (Beijing

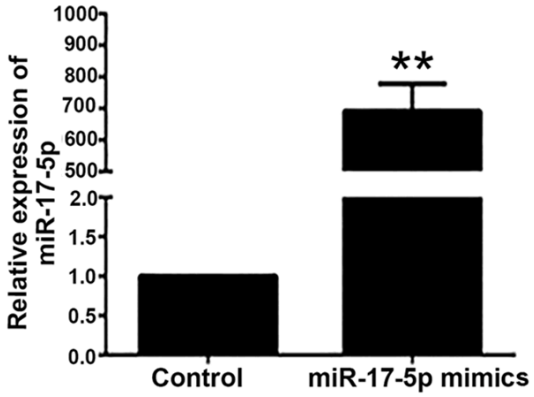

Figure 1. Effect of miR-17-5p mimics on the expression of miR-17-5p in CAL-27 tongue squamous cell carcinoma cells. CAL-27 cells were transfected with miR-17-5p mimics (50 nmol) or miRNA mimic negative control (50 nmol) for 48 h. The relative expression levels of miR-17-5p were analyzed by RT-qPCR. U6 was used as the inner control. Data are represented as mean ± standard error of the mean (SEM) of at least three independent experiments performed in duplicate. $^{**}P < 0.01$ *vs.* the control group.

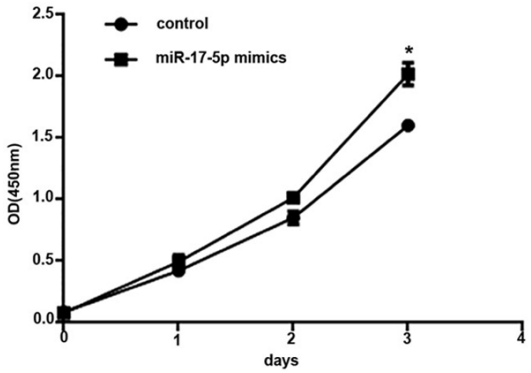

Figure 2. Overexpression of miR-17-5p promoted CAL-27 cell proliferation under hypoxia. CAL-27 cells were transfected with miR-17-5p mimics (50 nmol) or miRNA mimic negative control (50 nmol) for 1, 2, and 3 days under 1% O_2, 94% N_2, and 5% CO_2 at 37°C. Cell proliferation was determined by the CCK-8 assay. Data are presented as mean ± SEM of three independent experiments. $^{*}P < 0.05$ *vs.* the control group.

Chemical Industry Group, Co., Ltd., Beijing, China) in 0.1 M Sorensen buffer (pH 7.3; Beijing Chemical Industry Group, Co., Ltd., Beijing, China) for 1 h at 4°C, and post-fixed in 1% osmium tetroxide (Beijing Chemical Industry Group, Co., Ltd., Beijing, China) in 0.1 M cacodylate buffer (Beijing Chemical Industry Group, Co., Ltd., Beijing, China) for 1 h at room temperature. The cells were dehydrated in solutions of ethanol (30-90%), then embedded in Epon resin (Beijing Chemical Industry Group, Co., Ltd., Beijing, China) and cut with an UC7 microtome (Leica, Wetzlar, Germany) to obtain 70-nm sections. The sections were placed on uncoated copper grids. Sections were subsequently counterstained with 4% uranyl acetate (Beijing Chemical Industry Group, Co., Ltd., Beijing, China) and examined using transmission electron microscopy (TEM) (Model JEM-1200EX; JEOL, Tokyo, Japan) at 100 kV.

Statistical analysis

The Prism 5.0 software (GraphPad Software Inc., San Diego, CA, USA) was used for data analysis. All experiments were performed in duplicate with data averaged from at least three independent experiments. The data are presented as mean ± standard error of the mean (SEM). Statistical significance was evaluated by independent sample t-test. Two-sided *P*-values < 0.05 were considered significant.

Results

Effect of miR-17-5p mimics on the expression of miR-17-5p in CAL-27 cells

Figure 1 shows that the miR-17-5p mimics successfully increased the expression of miR-17-5p in CAL-27 cells by almost 700 fold compared with the miRNA mimic negative control.

MiR-17-5p promoted CAL-27 cell proliferation and migration

Figure 2 shows the effect of miR-17-5p overexpression on CAL-27 cells proliferation under hypoxia. After 3 days, cells transfected with the miR-17-5p mimics showed higher proliferation compared with controls ($P < 0.05$).

Figure 3 shows that miR-17-5p transfected CAL-27 cells had a higher migratory capacity compared with that of the control cells ($P < 0.05$) under hypoxia.

MiR-17-5p inhibits hypoxia-induced autophagy in CAL-27 cells

CAL-27 cells were submitted to hypoxic conditions after transfection with miR-17-5p mimics or mimic negative control. TEM showed that miR-17-5p overexpression inhibited the formation of autophagosomes in hypoxic cells (**Figure 4**). The role of Beclin-1 was explored in this observation. RT-qPCR and western blot showed

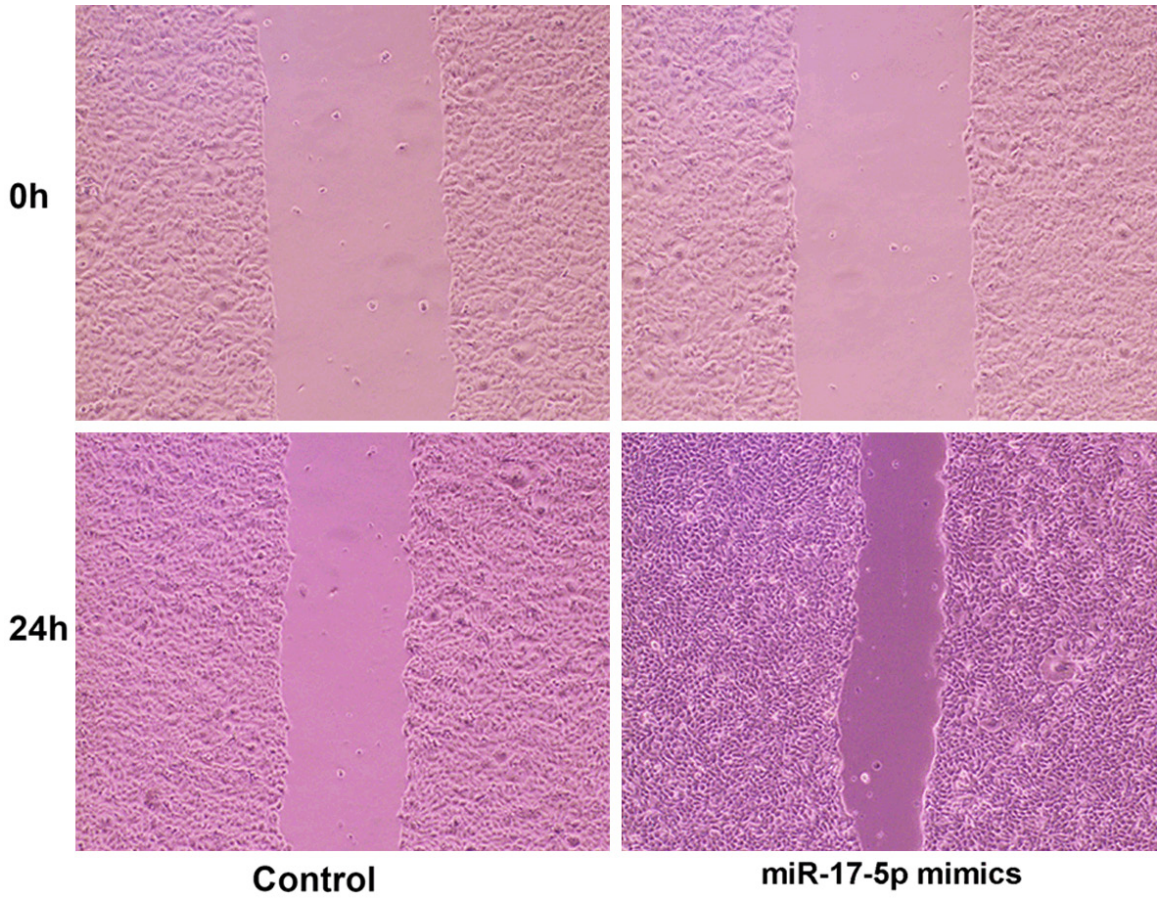

Figure 3. Overexpression of miR-17-5p promoted CAL-27 cell migration under hypoxia. CAL-27 cells were transfected with miR-17-5p mimics (50 nmol) or miRNA mimic negative control (50 nmol) under 1% O_2, 94% N_2, and 5% CO_2 at 37°C for 24 h. Cell migration was determined by the wound healing assay. Magnification: × 100.

that miR-17-5p overexpression inhibited the mRNA and protein expressions of Beclin-1 in CAL-27 cells subjected to hypoxia (**Figure 5**).

Discussion

Autophagy contributes to HNSCC development and progression [11]. MiR-17-5p downregulated Beclin-1 and thus plays an important role in autophagy [18, 19, 21-23], but little is known about the function and regulation of miR-17-5p in HNSCC autophagy. Therefore, this study aimed to investigate the role of miR-17-5p on the proliferation, migration, and autophagy under hypoxia of CAL-27 human tongue squamous cell carcinoma cells. The results suggest that miR-17-5p overexpression promoted the proliferation and migration of the CAL-27 cells, but inhibited autophagy under hypoxia.

Data about autophagy and its role in tumorigenesis are inconsistent among cancer types and organs [7]. In addition, the degree of autophagy varies during cancer progression and may play differential roles [8]. Nevertheless, in HNSCC, the autophagy induced by hypoxia is associated with an aggressive phenotype [12]. In addition, autophagy in HNSCC has good application prospects in targeted treatment of HNSCC [15].

Many miRNAs are associated with the occurrence, invasion, and metastasis of oral cancer [25, 26]. Recent studies have reported that miRNAs can be used as biomarkers for predicting the prognosis and sensitivity of cancer to radiotherapy and chemotherapy [27]. MiR-17-5p imbalance has been reported to be associated with the metastasis and invasion of hepatocellular carcinoma, ovarian cancer, breast cancer, and prostate cancer [17-20], but its role in HNSCC is still not clear. The expression levels of miR-17-5p is associated with cancer aggressiveness and therapy resistance [21]. Furth-

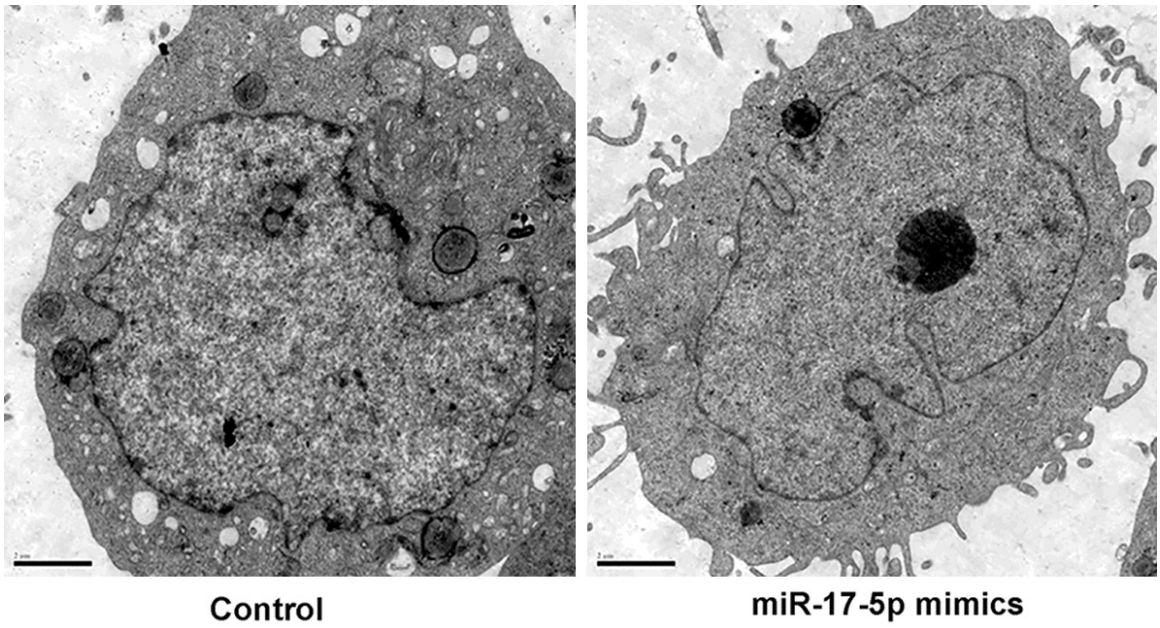

Figure 4. Overexpression of miR-17-5p inhibited the formation of autophagosomes induced by hypoxia in CAL-27 cells. CAL-27 cells were transfected with miRNA mimic negative control (50 nmol) or miR-17-5p mimics (50 nmol) under 1% O_2, 94% N_2, and 5% CO_2 at 37°C for 48 h. Transmission electron microscope (TEM) was used to observe the formation of autophagosomes (magnification: × 6000).

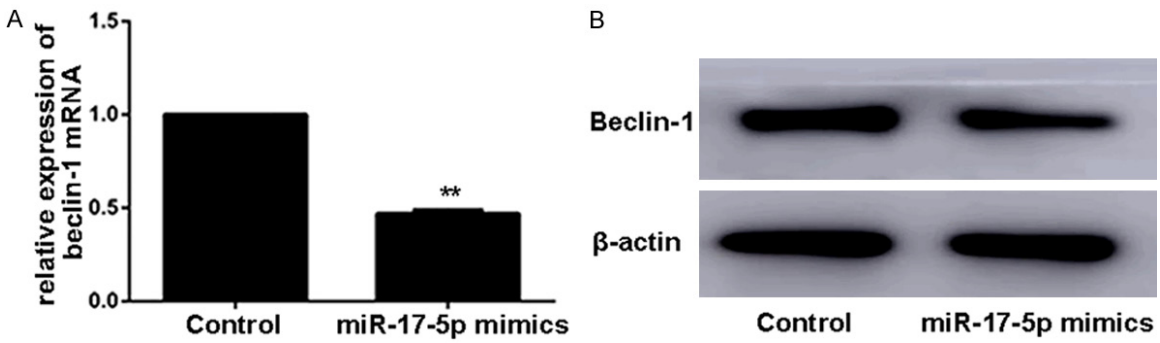

Figure 5. Overexpression of miR-17-5p inhibited the mRNA and protein expression of Beclin-1 in CAL-27 cells under hypoxia. CAL-27 cells were transfected with miRNA mimic negative control (50 nmol) or miR-17-5p mimics (50 nmol) under 1% O_2, 94% N_2, and 5% CO_2 at 37°C for 48 h. The relative mRNA (A) and protein (B) expression levels of Beclin-1 were detected by RT-qPCR and western blotting, respectively. Data are presented as mean ± SEM of at least three independent experiments performed in duplicate. $^{**}P < 0.01$ *vs.* the control group.

ermore, hypoxia is an important factor associated with HNSCC aggressiveness and resistance to treatments [11, 12]. MiR-17-5p has been shown to be involved in autophagy and cell death in response to hypoxia [28].

In the present study, miR-17-5p appears to have a dual role in HNSCC. Indeed, the overexpression of miR-17-5p increased cancer cell proliferation and migration, but decreased autophagy under hypoxia. A previous study in gastric cancer cells showed that miR-17-5p binds to the TGFBR2 mRNA and that decreased expression of TGFBR2 leads to uncontrolled cell growth and invasion [29], supporting the present study. Such a relationship between miR-17-5p has been shown in different cell types [30-32]. On the other hand, miR-17-5p has been shown to be a tumor suppressor in triple-negative breast cancer [33], highlighting the differential roles of miR-17-5p in different tumor types.

Regarding autophagy, miR-17-5p is important in regulating many genes involved in autophagy. Overexpression of miR-17-5p in glioma cells decreases Beclin-1-mediated autophagy and radioresistance [22], supporting the present study. In non-small lung cancer, downregulation of miR-17-5p is associated with upregulation of Beclin-1 and resistance to paclitaxel [23].

Nevertheless, this dual role of miR-17-5p could be the key to its eventual successful role in cancer treatment. Indeed, autophagy is a process by which cancer cells acquire resistance to adverse environmental conditions and cancer treatments [5, 10, 12, 23]. In addition, traditional chemotherapy and radiation therapy targets cells that are in active proliferation [34]. Hence, the increased cancer proliferation and invasion following miR-17-5p overexpression could be irrelevant in the context where the cells are more sensitive to therapy. In addition, the levels of miR-17-5p observed in the present study after mimic transfection are supraphysiological. Additional studies should be performed in cells naturally overexpressing miR-17-5p. Nevertheless, these results strongly suggest that miR-17-5p could be used for HNSCC treatment.

In conclusion, miR-17-5p promoted the proliferation and migration of CAL-27 cells, but inhibited autophagy under hypoxia.

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Disclosure of conflict of interest

None.

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References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Brocic M, Kozomara R, Cerovic S, Jovic N, Vukelic-Markovic S and Stosic S. Clinical significance of vascular endothelial growth factor expression in patients with carcinoma of the mouth floor and tongue. Vojnosanit Pregl 2009; 66: 440-448.

[3] Faraji F, Zaidi M, Fakhry C and Gaykalova DA. Molecular mechanisms of human papillomavirus-related carcinogenesis in head and neck cancer. Microbes Infect 2017; 19: 464-475.

[4] Guidi A, Codeca C and Ferrari D. Chemotherapy and immunotherapy for recurrent and metastatic head and neck cancer: a systematic review. Med Oncol 2018; 35: 37.

[5] Klionsky DJ. The molecular machinery of autophagy: unanswered questions. J Cell Sci 2005; 118: 7-18.

[6] Wu D, Wang X and Sun H. The role of mitochondria in cellular toxicity as a potential drug target. Cell Biol Toxicol 2018; 34: 87-91.

[7] Lei Y, Zhang D, Yu J, Dong H, Zhang J and Yang S. Targeting autophagy in cancer stem cells as an anticancer therapy. Cancer Lett 2017; 393: 33-39.

[8] Tsuchihara K, Fujii S and Esumi H. Autophagy and cancer: dynamism of the metabolism of tumor cells and tissues. Cancer Lett 2009; 278: 130-138.

[9] White E. The role for autophagy in cancer. J Clin Invest 2015; 125: 42-46.

[10] Muz B, de la Puente P, Azab F and Azab AK. The role of hypoxia in cancer progression, angiogenesis, metastasis, and resistance to therapy. Hypoxia (Auckl) 2015; 3: 83-92.

[11] Wu H, Huang S and Zhang D. Autophagic responses to hypoxia and anticancer therapy in head and neck cancer. Pathol Res Pract 2015; 211: 101-108.

[12] Vigneswaran N, Wu J, Song A, Annapragada A and Zacharias W. Hypoxia-induced autophagic response is associated with aggressive phenotype and elevated incidence of metastasis in orthotopic immunocompetent murine models of head and neck squamous cell carcinomas (HNSCC). Exp Mol Pathol 2011; 90: 215-225.

[13] Kang R, Zeh HJ, Lotze MT and Tang D. The Beclin 1 network regulates autophagy and apoptosis. Cell Death Differ 2011; 18: 571-580.

[14] Zhao H, Yang M and Zhao B. Beclin 1 and LC3 as predictive biomarkers for metastatic colorectal carcinoma. Oncotarget 2017; 8: 59058-59067.

[15] Zhang L, Zhang W, Wang YF, Liu B, Zhang WF, Zhao YF, Kulkarni AB and Sun ZJ. Dual induction of apoptotic and autophagic cell death by targeting survivin in head neck squamous cell carcinoma. Cell Death Dis 2015; 6: e1771.

[16] Lan J, Huang Z, Han J, Shao J and Huang C. Redox regulation of microRNAs in cancer. Cancer Lett 2018; 418: 250-259.

[17] Li Y, Lauriola M, Kim D, Francesconi M, D'Uva G, Shibata D, Malafa MP, Yeatman TJ, Coppola

D, Solmi R and Cheng JQ. Adenomatous polyposis coli (APC) regulates miR17-92 cluster through beta-catenin pathway in colorectal cancer. Oncogene 2016; 35: 4558-4568.
[18] Gu J, Wang D, Zhang J, Zhu Y, Li Y, Chen H, Shi M, Wang X, Shen B, Deng X, Zhan Q, Wei G and Peng C. GFRalpha2 prompts cell growth and chemoresistance through down-regulating tumor suppressor gene PTEN via Mir-17-5p in pancreatic cancer. Cancer Lett 2016; 380: 434-441.
[19] Chen P, Zhao H, Huang J, Yan X, Zhang Y and Gao Y. MicroRNA-17-5p promotes gastric cancer proliferation, migration and invasion by directly targeting early growth response 2. Am J Cancer Res 2016; 6: 2010-2020.
[20] Guo J, Mei Y, Li K, Huang X and Yang H. Downregulation of miR-17-92a cluster promotes autophagy induction in response to celastrol treatment in prostate cancer cells. Biochem Biophys Res Commun 2016; 478: 804-810.
[21] Bobbili MR, Mader RM, Grillari J and Dellago H. OncomiR-17-5p: alarm signal in cancer? Oncotarget 2017; 8: 71206-71222.
[22] Hou W, Song L, Zhao Y, Liu Q and Zhang S. Inhibition of Beclin-1-mediated autophagy by MicroRNA-17-5p enhanced the radiosensitivity of glioma cells. Oncol Res 2017; 25: 43-53.
[23] Chatterjee A, Chattopadhyay D and Chakrabarti G. miR-17-5p downregulation contributes to paclitaxel resistance of lung cancer cells through altering beclin1 expression. PLoS One 2014; 9: e95716.
[24] Dulloo I, Phang BH, Othman R, Tan SY, Vijayaraghavan A, Goh LK, Martin-Lopez M, Marques MM, Li CW, Wang de Y, Marin MC, Xian W, McKeon F and Sabapathy K. Hypoxia-inducible TAp73 supports tumorigenesis by regulating the angiogenic transcriptome. Nat Cell Biol 2015; 17: 511-523.
[25] Suh YE, Raulf N, Gaken J, Lawler K, Urbano TG, Bullenkamp J, Gobeil S, Huot J, Odell E and Tavassoli M. MicroRNA-196a promotes an oncogenic effect in head and neck cancer cells by suppressing annexin A1 and enhancing radioresistance. Int J Cancer 2015; 137: 1021-1034.
[26] Kinoshita T, Nohata N, Fuse M, Hanazawa T, Kikkawa N, Fujimura L, Watanabe-Takano H, Yamada Y, Yoshino H, Enokida H, Nakagawa M, Okamoto Y and Seki N. Tumor suppressive microRNA-133a regulates novel targets: moesin contributes to cancer cell proliferation and invasion in head and neck squamous cell carcinoma. Biochem Biophys Res Commun 2012; 418: 378-383.
[27] Huang Y, Tan D, Xiao J, Li Q, Zhang X and Luo Z. miR-150 contributes to the radioresistance in nasopharyngeal carcinoma cells by targeting glycogen synthase kinase-3beta. J Cancer Res Ther 2018; 14: 111-118.
[28] Hao MX, Wang X and Jiao KL. MicroRNA-17-5p mediates hypoxia-induced autophagy and inhibits apoptosis by targeting signal transducer and activator of transcription 3 in vascular smooth muscle cells. Exp Ther Med 2017; 13: 935-941.
[29] Qu Y, Zhang H, Duan J, Liu R, Deng T, Bai M, Huang D, Li H, Ning T, Zhang L, Wang X, Ge S, Zhou L, Zhong B, Ying G and Ba Y. MiR-17-5p regulates cell proliferation and migration by targeting transforming growth factor-beta receptor 2 in gastric cancer. Oncotarget 2016; 7: 33286-33296.
[30] Shan SW, Fang L, Shatseva T, Rutnam ZJ, Yang X, Du W, Lu WY, Xuan JW, Deng Z and Yang BB. Mature miR-17-5p and passenger miR-17-3p induce hepatocellular carcinoma by targeting PTEN, GalNT7 and vimentin in different signal pathways. J Cell Sci 2013; 126: 1517-1530.
[31] Wu Q, Luo G, Yang Z, Zhu F, An Y, Shi Y and Fan D. miR-17-5p promotes proliferation by targeting SOCS6 in gastric cancer cells. FEBS Lett 2014; 588: 2055-2062.
[32] Chen C, Lu Z, Yang J, Hao W, Qin Y, Wang H, Xie C and Xie R. MiR-17-5p promotes cancer cell proliferation and tumorigenesis in nasopharyngeal carcinoma by targeting p21. Cancer Med 2016; 5: 3489-3499.
[33] Li J, Lai Y, Ma J, Liu Y, Bi J, Zhang L, Chen L, Yao C, Lv W, Chang G, Wang S, Ouyang M and Wang W. miR-17-5p suppresses cell proliferation and invasion by targeting ETV1 in triple-negative breast cancer. BMC Cancer 2017; 17: 745.
[34] Liu H, Lv L and Yang K. Chemotherapy targeting cancer stem cells. Am J Cancer Res 2015; 5: 880-893.