European Review for Medical and Pharmacological Sciences 2018; 22: 7735-7741

MiR-21 regulates proliferation and apoptosis of oral cancer cells through TNF-α

Y.-F. QIU[1], M.-X. WANG[2], L.-N. MENG[3], R. ZHANG[4], W. WANG[5]

[1]Department of Image Diagnoses, Stomatological College, the First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China
[2]Department of Stomatology, the Forth Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China
[3]Department of Stomatology, Nangang Branch of Heilongjiang Provincial Hospital, Harbin, Heilongjiang, China
[4]Department of Stomatology, Harbin Children's Hospital, Harbin, Heilongjiang, China
[5]Department of Oral and Maxillofacial Surgery, the First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China

Abstract. – OBJECTIVE: MicroRNA (miRNA) can widely regulate gene expression. More importantly, various miRNA molecules have been found with regulatory functions for tumor cell proliferation or apoptosis. The study showed that miR-21 inhibited apoptosis of cultured cancer cells, whilst tumor necrosis factor α (TNF-α) plays important roles in the proliferation of tumor cells. This study manipulated miR-21 expression in cultured oral cancer cells and aimed to investigate its effects on TNF-α expression, and on proliferation or apoptosis of cancer cells.

MATERIALS AND METHODS: Specific agonist and antagonist were synthesized based on miR-21 sequence. *In vitro* cultured oral cancer cell line, SCC-15 was transfected with agonist or antagonist, in parallel with normal cultured cells as negative control group. Quantitative Real-time PCR (qRT-PCR) was used to measure mRNA expression of miR-21 and TNF-α in transfected cells. Western blot was used for measuring TNF-α expression, and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) or Hoechst-33342 staining was used to measure proliferation and apoptosis of SCC-15 cells.

RESULTS: MiR-21 expression was potentiated or depressed with transfection of agonist or antagonist, respectively, illustrating the effectiveness of synthesized sequence in cultured SCC-15 cells. Moreover, TNF-α expression was positively correlated with miR-21, TNF-α up-regulation significantly potentiated the proliferation potency of SCC-15 cells, and TNF-α down-regulation remarkably weakened proliferation potency ($p<0.05$). The TNF-α expression did not affect apoptosis of SCC-15 cells ($p>0.05$ compared to the control group).

CONCLUSIONS: MiR-21 could participate in the proliferation of cultured SCC-15 cells via targeting TNF-α expression, but without any significant effects on cell apoptosis.

Key Words:
Oral cancer, miR-21, SCC-15, TNF-α, Cell proliferation, Cell apoptosis

Introduction

Oral cancer is a commonly occurred malignant tumor in human head-neck regions, and is one of the malignant tumors occurred within oral cavity. It occupies about 1.9-3.5% of total malignant tumors, and about 4-20% of head-neck cancers, with incidence only lower than nasopharyngeal carcinoma. Mainly consisting of lip carcinoma, gingival carcinoma, and tongue cancer, oral cancer has relatively higher incidence worldwide. In general males had higher incidence of oral cancer than females, probably due to relatively higher percentage of populations with smoking or alcohol abuse history. Currently treatment approaches targeting oral cancer are similar as those for other cancers, mainly including surgical resection, radiotherapy, chemotherapy or Chinese medicine, although no satisfactory efficiency has been reached[1, 2]. With the development of biological targeting treatment, novel treatment plans including gene therapy, immune therapy, and tumor stem cells have shown major potency. However, these

Corresponding Author: Yanfen Qiu, MD; e-mail: xpnxgvhaim@sina.com

methods largely invade body injury taken by classical treatment methods and had better foresights in improving post-op survival and prognosis of patients. Therefore, the identification of valuable treatment target for oral cancer is of critical importance for development of specific molecular targeting medicine and prognostic evaluation of oral cancer[3]. Tumor necrosis factor α (TNF-α) has certain regulatory functions on cell proliferation, apoptosis and differentiation, and has pluripotent biological effects inside the body[4]. Hoeben et al[4] found prominent expression of TNF-α in various tumor tissues including prostate cancer, pancreatic carcinoma and kidney cancer. It has dual roles in regulating tumor cells, as it can inhibit tumor cell growth and induce tumor necrosis, and improves tumor cell growth, migration and infiltration potency, thus becoming one critical gene in tumor target gene research[5,6]. Previous work showed significantly higher TNF-α expression in serum of oral cancer patients compared to normal people[7]. MicroRNA (miRNA) is widely distributed in eukaryotes, and participates in the regulation of various genes. It exerts the regulatory function mainly via complementary binding with target gene, including direct degradation of target gene mRNA or inhibition of gene translation at post-transcriptional stage to suppress target gene expression level[8,9]. More importantly, one single protein may be under the regulation of multiple miRNA. Currently the analysis for miRNA has made major progression, and has identified its important regulatory role in various human diseases. Certain miRNA molecules have become early signs of tumorigenesis, whilst other miRNA molecules have become target for clinical drug treatment[10]. Previous researches showed certain relationship between miR expression and alternation of abilities in proliferation, apoptosis and migration of oral cancer cells. Multiple miRNA molecules have been found to have up-regulation in oral cancer cells such as miR-134, miR-24 and miR-21[11,12]. Therefore, the investigation of miR-21 in regulating proliferation and apoptosis of oral cancer, and downstream targeting TNF-α, have solid theoretical grounds and feasibility. Our work measured changing patterns of TNF-α after miR-21 manipulation, and its correlation with proliferation or apoptosis of oral cancer cells, in order to investigate the function of miR-21 and TNF-α in oral cancer pathogenesis.

Table I. Primers used in experiment.

Name	Primer sequence (5′-3′)
TNF-α forward	CCCGCATCCCAGGACCTCTCT
TNF-αreverse	CGGGGGACTGGCGA
mir-21forward	CTAAGACCTGTGGAATGGC
mir-21 reverse	CTCAAAGATGTCATTGCC
GAPDH forward	CGGAGTCAACGGATTTGGTCGTA
GAPDH reverse	AGCCTTCTCCATGGTGGTGAAG

Materials and Methods

Major Reagents and Equipment

Human oral cancer cell line SCC-15 was purchased from Beinuo Biotech. Co. Ltd. (Shanghai, China) and was preserved in liquid nitrogen. Roswell Park Memorial Institute-1640 (RPMI-1640) medium, option minimum essential media (opti-MEM), fetal bovine serum (FBS) and trypsin for digestion were purchased from HyClone (South Logan, UT, USA). Phosphate-buffered saline (PBS) powder was purchased from Beyotime Biotech. (Shanghai, China), and was diluted by ultrapure water for filtering in 0.22 μm pore membrane to sterilize. Dual antibiotics in cell culture were produced by Tiangen Biotech Co. Ltd. (Beijing, China). Liposome cell transfection reagent, human TNF-α protein assay antibody were produced by Invitrogen/Life Technologies (Carlsbad, CA, USA). RNA extraction kit (TRIzol) and complementary DNA (cNDA) synthesis kit were all purchased from Bio-Tek Inc. (Winooski, VT, USA). Quantitative Real-time PCR (qRT-PCR) reagent was purchased from Omega Bio-Tek. Inc. (Norcross, GA, USA). Microplate reader was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Hoechest-33342 was obtained from Suolaibao Tech. (Shanghai, China). Inverted fluorescent microscope was produced by Leica (Frankfurt, Germany). Wet membrane transfer apparatus was purchased from Bio-Rad Laboratories (Hercules, CA, USA). All primers used were synthesized by Sangon Biotech. Co. Ltd. (Shanghai, China). Other common reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

SCC-15 Cell Culture and Liposome Transfection

SCC-15 cell line preserved in liquid nitrogen was resuscitated following routine protocols. Cells were firstly cultured in RPMI-1640 medium containing 13% fetal bovine serum (FBS), and were continuously cultured. After passage, the third generation of cells was used for further

assay. Before each passage, 10 μl cell re-suspensions were used for enumeration. About 10×10^4 cells were added into each well, and cell transfection was performed after 16 h normal culture. Before transfection, antibiotic-free culture medium was changed. MiR-21 agonist or antagonist was diluted to 100 nM using 50 μl opti-MEM medium. Meanwhile, 2 μl liposome solution were diluted within 50 μl opti-MEM followed by 5 min room temperature incubation. After mixture of two solutions, these were incubated at room temperature for 20-30 min. Within each well, 200 μl solution A+B mixture was added. Cells were cultured for 4 h and culture medium was changed. Three parallel replicates were performed in each group, using normal cultured cells as the control group. This study was approved by the Ethics Committee of the First Affiliated Hospital of Harbin Medical University (Harbin, China).

qRT-PCR for mRNA Expression in Transfected Cells

Following the manual instruction of test kit, total RNA was extracted from cells before transfection, 24 h and 48 h after transfection. cDNA strand was synthesized, and was placed on ice for 5-10 min. A total of 4 μl reverse transcriptase buffer, 2 μl dithiothreitol (DTT) and 1 μl deoxy-ribonucleoside triphosphate (dNTPs) were added into the mixture, which was incubated at 42°C for 2 min. A total of 1 μl reverse transcriptase was added for 42°C continuous incubation for 1 h. The reverse transcription reaction was quenched by 65°C. Using miR-21 gene sequence as the template, testing primers were designed and synthesized. Using GAPDH as the internal reference, primers sequences were shown in Table I. The qRT-PCR system consisted of 0.08 μM forward/reverse primer, 10 μl master mix, 2 μl cDNA, 0.4 μl PCR Taq polymerase, and ddH_2O up to 20 μl. The sample was briefly centrifuged at 1000 r/min. qRT-PCR conditions were: 94°C for 3 min, followed by 45 cycles each containing 95°C 13 s, and 63°C 40 s. Triplicated wells were set for each sample to reduce the error.

Western Blot for Serum TNF-α Expression in Supernatant from Transfected Cells

Total cellular proteins were collected at 24 h and 48 h after transfection. Culture medium was firstly removed from culture plate. The plate was then washed using pre-cold 1×PBS buffer. After removing all liquids, the plate was placed on ice, and protein lysis buffer was added (50 μl per well). Well bottom and wall were repeated washed using PBS buffer. Cell suspensions were collected into 1.5 ml enzyme-free Eppendorf (EP) tubes for mixture repeated in 3-4 times until complete lysis of cells. All operations were performed on ice. After 4°C centrifugation at 12000 r/min for 15 min, the supernatant was collected into pre-cold 1.5 ml EP tube. After mixture, 1 μl sample was used for bicinchoninic acid (BCA) protein quantification, and the remaining samples were kept at -80°C fridge. A total of 20 μg protein samples were used for sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separation using 4% condensing gel and 12% separating gel. Gel electrophoresis was performed under 80 V for 20 min, and 120 V for 35 min. After electrophoresis, the protein was transferred to polyvinylidene difluoride (PVDF) membrane in a wet transferring apparatus at 385 mA current for 30-60 min. Using β-actin as the internal reference, primary antibody was diluted at 1:100 ratio and was used for 4°C incubation for 8-10 h. After Tris-buffered saline and Tween-20 (TBST) rinsing, 500-fold diluted secondary antibody was added for 2 h room temperature incubation, followed by 3-5 times of rinsing. Developing and chromogenic substrates were added, and Bio-Rad automatic illuminator was used for analysis of gray values from each group. Using β-actin as the internal reference, expression of TNF-α protein was quantified.

3-(4,5-Dimethyl-2-Thiazolyl)-2,5-Diphenyl-2-H-Tetrazolium Bromide (MTT) Assay for Effect of TNF-α Expression on Proliferation of SCC-15 Cells

Cells after transfection were enumerated and transferred into 96-well plate (1×10^5 cells per well). At 36 h or 48 h after transfection, cells were rinsed in serum-free medium. Each well was added with 20 μl MTT solution for 4 h incubation. Culture medium was then removed, and 150 μl dimethylsulfoxide (DMSO) were added for 10 min room temperature incubation. Optical density (OD) values of each well were measured in triplicates from each group. Averaged values were taken for calculating cell proliferation rate, which was equal to OD of experimental group divided by OD of control group.

Hoechest-33342 Staining for the Effect of TNF-α Expression on Cell Apoptosis

Transfected cells were inoculated into 6-well plate, and were continuously cultured till 24 h or

48 h. After removing culture medium, the culture plate was rinsed in PBS buffer for 3 to 5 times. Each well was fixed by 200 µl paraformaldehyde at 4°C for 20 min, followed by PBS rinsing. About 1 ml staining buffer was then added into each well for 20 min incubation. With repeated PBS rinsing, the plate was observed under an inverted fluorescent microscope for statistical analysis.

Statistical Analysis

SPSS 11.3 software (SPSS Inc., Chicago, IL, USA) was used for data analysis and statistics. The Student's *t*-test was used to compare the differences between two groups. Tukey's post-hoc test was used to validate the analysis of variance (ANOVA) for comparing measurement data among groups. A statistical significance was defined when $p<0.05$, and extremely significant difference.

Results

qRT-PCR for miR-21 mRNA Expression

After extracting total RNA from transfected cells, qRT-PCR was employed to measure miR-21 expression level using GAPDH as the internal reference. As shown in Figure 1, at 24 h and 48 h after transfection of agonist, miR-21 level was significantly elevated compared to control group ($p<0.05$). The transfection of inhibitor significantly depressed miR-21 expression at 24 h and 48 h ($p<0.01$ compared to control group). These data suggested that using agonist and inhibitor to alter cellular miR-21 expression was feasible, paving grounds for further assays.

qRT-PCR Assay for Cellular TNF-α Expression

Using cDNA extracted from transfected cells, qRT-PCR was employed to test TNF-α expression. As shown in Figure 2, TNF-α expression was positively correlated with miR-21, and TNF-α expression showed significant increase or decrease at 24 h and 48 h after transfecting agonist or inhibitor, respectively. Such patterns of TNF-α were consistent with that of miR-21, showing significant difference with control group ($p<0.05$).

Western Blot for TNF-α Expression in Cell Supernatant after Transfection

Test kit was used to extract total cell proteins at 24 h and 48 h after transfection. Western blot was used to describe TNF-α expression in all groups

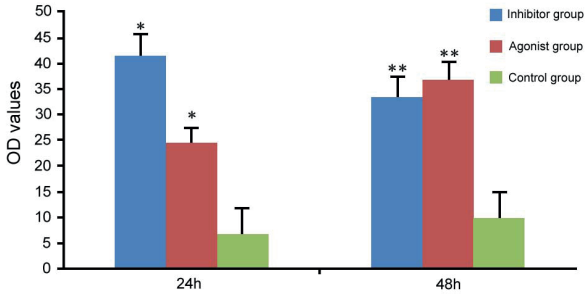

Figure 1. MiR-21 expression at 24 h and 48 h after transfection. $^{*}p<0.05$ comparing between groups, $^{**}p<0.01$ comparing to control group.

of cells. As shown in Figure 3, TNF-α expression was gradually increased in agonist group with elongated transfection time, and was gradually decreased with usage of inhibitor ($p<0.05$ compared to control group). These data showed certain relationship between TNF-α expression and miR-21 levels.

MTT Assay for the Effect of TNF-α Expression on SCC-15 Cell Proliferation

Transfected cells were enumerated and transferred into 96-well palate, in which MTT assay was performed at 36 h and 48 h post-transfection, in order to examine the effect of TNF-α expression on proliferation of SCC-15 cells. As shown in Figure 4, those SCC-15 cells with TNF-α up-regulation showed positive relationship with proliferation, in a temporal dependent manner.

Hoechest-33342 Staining for the Effect of TNF-Ð on Cell Apoptosis

Transfected cells were transferred into 6-well plate, in which they were inoculated until 24h and 48h. Hoechest-33412 staining was then employed

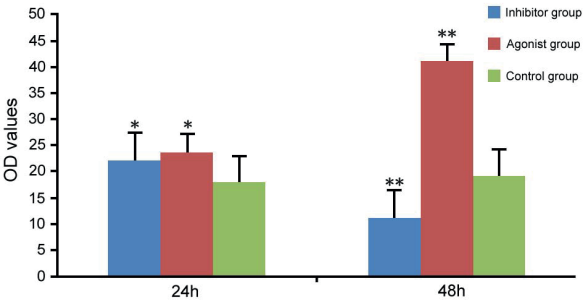

Figure 2. TNF-α expression assay at 36 h and 48 h after transfection. $^{*}p<0.05$ comparing between groups, $^{**}p<0.01$ comparing to control group.

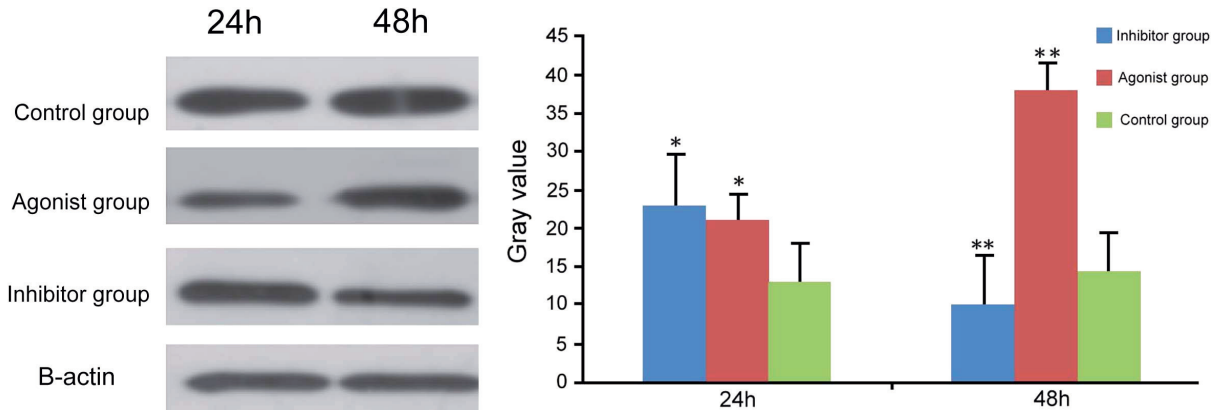

Figure 3. Western blot for TNF-α expression in agonist or inhibitor transfection groups. $^{*}p<0.05$ comparing between groups, $^{**}p<0.01$ comparing to control group.

to detect cell apoptosis after TNF-α expression. Apoptotic cells showed dense staining and bright view, whilst normal cells showed only light blue staining. Under inverted fluorescent microscope, the number of apoptotic cells was measured from three different fields. No significant difference was found in apoptotic rate among three groups (Figure 5, $p>0.05$).

Discussion

Pathogenesis of oral cancer is a complicated pathological process, among which proliferation and apoptosis of cancer cells play critical modulatory functions. The proliferation and apoptosis of oral cancer cells are under the modulation of various signaling pathways such as p53, phosphate and tension homology deleted on chromosome ten (PTEN) and TNF-α. Among those, TNF-α has dual roles in tumor cells, as it can suppress tumor cell growth, induce sensitive cell death and tumor

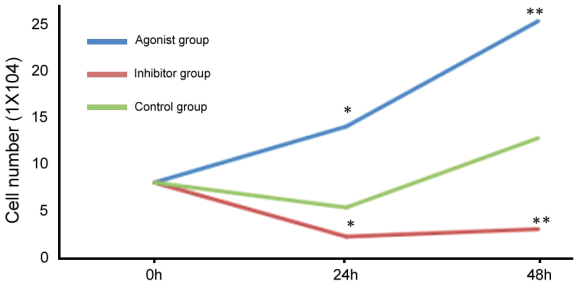

Figure 4. Effect of TNF-α expression on SCC-15 cell proliferation. $^{*}p<0.05$ comparing between groups, $^{**}p<0.01$ comparing to control group.

tissue necrosis via regulating downstream expressions of proteins such as nuclear factor kB (NF-κB), receptor-interacting protein 3 (RIP3) and epidermal growth factor receptor (EGFR), and can improve tumor growth, migration and infiltration. Therefore, the study of TNF-α has certain values in understanding proliferation and apoptosis of oral cancer cells[13-16]. Although miRNA plays critical roles in mediating cancer cell proliferation and apoptosis, currently little studies have been raised targeting TNF-α by miRNA. Previous studies showed that miR-29a over-expression could mediate atherosclerosis occurrence, indicating regulatory relationship between TNF-α and miRNA[17, 18]. MiR-21 is one prominent regulatory for cancer cell proliferation and apoptosis, and has received lots of researches regarding its regulatory patterns in tumor cells. For example,, suppression of miR-21 in oral cancer cells facilitate cancer cell migration via inhibiting RASA1 expression. Therefore, miR-21 might work as one potential biomarker for diagnosis of migrated oral cancer[19,20]. This work further investigated if miR-21 expression in oral cancer could participate in proliferation and apoptosis of cancer cells via mediating TNF-α expression. Our findings showed that TNF-α expression within oral cancer cells was positively correlated with miR-21. TNF-α up-regulation or down-regulation significantly facilitates or inhibits proliferation of oral cancer cells. However, the effect of differentiated TNF-α expression on apoptosis of oral cancer cells is not significant, indicating that TNF-α might be one of targeting sites of miR-21. Such miR-21/TNF-α pathway plays certain role in proliferation of oral cancer cells. However, this study only investigat-

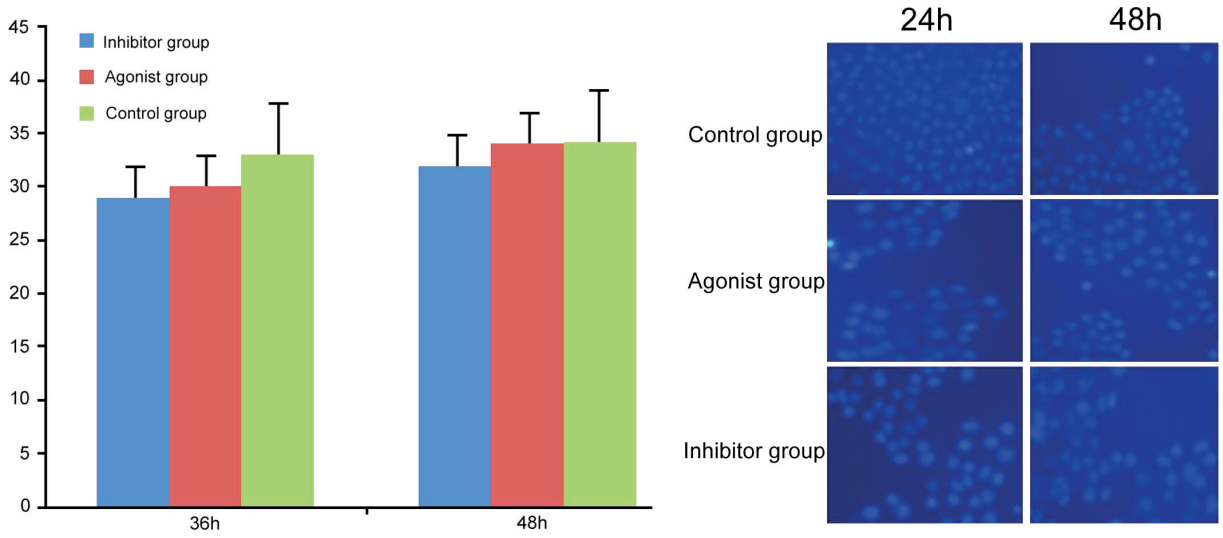

Figure 5. No significant difference of apoptosis among difference groups (p>0.05). Hoechst-33342 staining for the effect of TNF-α on cell apoptosis. Transfected cells were transferred into 6-well plate, in which they were inoculated until 24 h and 48 h. Hoechest-33412 staining was then employed to detect cell apoptosis after TNF-α expression. Apoptotic cells showed dense staining, bright view, whilst normal cells showed only light blue staining. Under inverted fluorescent microscope, number of apoptotic cells was measured from three different fields. No significant difference was found in apoptotic rate among three groups (p>0.05).

ed *in vitro* cultured SCC-15 cell line, which may not completely reflect true pathogenesis process of oral cancer. Therefore, whether miR-21 mediated TNF-α to regulate oral cancer cell proliferation is also influenced by other cytokines, genes or even environmental factors is not definitive and requires further substantiation.

Conclusions

Using miR-21 interference to treat *in vitro* cultured oral cancer cells, we found miR-21 expression in SCC-15 cells was negatively correlated with TNF-α expression, which was positively related with proliferation but not apoptosis of oral cancer cells. We thus speculate that miR-21 might participate in the regulation of oral cancer cell proliferation via negative control on TNF-α expression.

Acknowledgments

This work was supported by the Natural Science Foundation of Heilongjiang Province (Grant No. H2016044).

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Shen S, Tang JX. Mechanism of OGT2115 inhibition of invasion and migration in KB oral cancer cells. Eur Rev Med Pharmacol Sci 2017; 21: 3744.
2) Bundela S, Sharma A, Bisen PS. Potential compounds for oral cancer treatment: resveratrol, nimbolide, lovastatin, bortezomib, vorinostat, berberine, pterostilbene, deguelin, andrographolide, and colchicine. PLoS One 2015; 10: e0141719.
3) Shepperd JA, Emanuel AS, Howell JL, Logan HL. Predicting scheduling and attending for an oral cancer examination. Ann Behav Med 2015; 49: 828-838.
4) Hoeben A, Polak J, Van De Voorde L, Hoebers F, Grabsch HI, de Vos-Geelen J. Cervical esophageal cancer: a gap in cancer knowledge. Ann Oncol 2016; 27: 1664-1674.
5) Buda A, Elisei F, Palazzi S, De Ponti E, Arosio M, Vecchione F, Dell'Anna T, Cuzzocrea M, Bussi B, Giuliani D, Di Martino G, Crivellaro C. Quality of care for cervical and endometrial cancer patients: the impact of different techniques of sentinel lymph node mapping on patient satisfaction. Ann Surg Oncol 2016; 23: 2975-2981.
6) Seyyednejad F, Rezaee A, Haghi S, Goldust M. Survey of pre-inflammation cytokines levels in radiotherapy-induced-mucositis. Pak J Biol Sci 2012; 15: 1098-1101.
7) Brailo V, Vucicevic-Boras V, Lukac J, Biocina-Lukenda D, Zilic-Alajbeg I, Milenovic A, Balija M. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leu-

koplakia and oral cancer. Med Oral Patol Oral Cir Bucal 2012; 17: e10-e15.

8) Kong Q, Wang W, Li P. Regulator role of HPV E7 protein on miR-21 expression in cervical carcinoma cells and its functional implication. Int J Clin Exp Pathol 2015; 8: 15808-15813.

9) Han Y, Xu GX, Lu H, Yu DH, Ren Y, Wang L, Huang XH, Hou WJ, Wei ZH, Chen YP, Cao YG, Zhang R. Dysregulation of miRNA-21 and their potential as biomarkers for the diagnosis of cervical cancer. Int J Clin Exp Pathol 2015; 8: 7131-7139.

10) Yan LX, Liu YH, Xiang JW, Wu QN, Xu LB, Luo XL, Zhu XL, Liu C, Xu FP, Luo DL, Mei P, Xu J, Zhang KP, Chen J. PIK3R1 targeting by miR-21 suppresses tumor cell migration and invasion by reducing PI3K/AKT signaling and reversing EMT, and predicts clinical outcome of breast cancer. Int J Oncol 2016; 48: 471-484.

11) Ge Y, Zhang L, Nikolova M, Reva B, Fuchs E. Strand-specific in vivo screen of cancer-associated miRNAs unveils a role for miR-21(*) in SCC progression. Nat Cell Biol 2016; 18: 111-121.

12) Bumrungthai S, Ekalaksananan T, Evans MF, Chopjitt P, Tangsiriwatthana T, Patarapadungkit N, Kleebkaow P, Luanratanakorn S, Kongyingyoes B, Worawichawong S, Pientong C. Up-regulation of miR-21 is associated with cervicitis and human papillomavirus infection in cervical tissues. PLoS One 2015; 10: e0127109.

13) Niaz K, Maobool F, Khan F, Bahadar H, Ismail Hassan F, Abdollahi M. Smokeless tobacco (paan and gutkha) consumption, prevalence, and contribution to oral cancer. Epidemiol Health 2017; 39: e2017009.

14) Yang CY, Lin CK, Tsao CH, Hsieh CC, Lin GJ, Ma KH, Shieh YS, Sytwu HK, Chen YW. Melatonin exerts anti-oral cancer effect via suppressing LSD1 in patient-derived tumor xenograft models. Oncotarget 2017; 8: 33756-33769.

15) Lien MY, Lin CW, Tsai HC, Chen YT, Tsai MH, Hua CH, Yang SF, Tang CH. Impact of CCL4 gene polymorphisms and environmental factors on oral cancer development and clinical characteristics. Oncotarget 2017; 8: 31424-31434.

16) Tsai HT, Hsieh MJ, Lin CW, Su SC, Miao NF, Yang SF, Huang HC, Lai FC, Liu YF. Combinations of SERPINB5 gene polymorphisms and environmental factors are associated with oral cancer risks. PLoS One 2017; 12: e0163369.

17) Wang D, Fan Z, Liu F, Zuo J. Hsa-miR-21 and Hsa-miR-29 in tissue as potential diagnostic and prognostic biomarkers for gastric cancer. Cell Physiol Biochem 2015; 37: 1454-1462.

18) Wu X, Xu W, Feng X, He Y, Liu X, Gao Y, Yang S, Shao Z, Yang C, Ye Z. TNF-a mediated inflammatory macrophage polarization contributes to the pathogenesis of steroid-induced osteonecrosis in mice. Int J Immunopathol Pharmacol 2015; 28: 351-361.

19) Zhang L, Zhan X, Yan D, Wang Z. Circulating MicroRNA-21 is involved in lymph node metastasis in cervical cancer by targeting RASA1. Int J Gynecol Cancer 2016; 26: 810-816.

20) Wang GB, Liu JH, Hu J, Xue K. MiR-21 enhanced glioma cells resistance to carmustine via decreasing Spry2 expression. Eur Rev Med Pharmacol Sci 2017; 21: 5065-5071.