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Original Article

MiR-205-3p inhibits proliferation, invasion and promotes apoptosis of oral squamous cell carcinoma stem cells by targeting LAMC2 gene

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Abstract: Objective: To explore the regulatory mechanism of miR-205-3p targeting Laminin γ2-chain (LAMC2) gene on the biological characteristics of oral stem squamous cell carcinoma (OSCC) stem cells. Methods: The targeting relationship between miR-205-3p and LAMC2 gene was predicted by the bioinformatic database and verified by dual luciferase reporter assay system. qRT-PCR was used to detect the expression of miR-205-3p and LAMC2 in epithelial tissues from patients with OSCC or benign mass in oral mucosa. Normal oral mucosa epithelial cells were selected as the normal group. OSCC stem cells were divided into blank group, miR-205-3p mimic group, miR-205-3p inhibitor group, siRNA-LAMC2 group and miR-205-3p mimic + siRNA-LAMC2 group, respectively. qRT-PCR and western blot were performed to investigate the expression level of miR-205-3p, LAMC2, Bax, Bcl-2 and epithelial-mesenchymal transition (EMT)-related factors in cells of each group. Cell proliferation, invasion ability and apoptosis were detected by MTT assay, transwell assay and flow cytometry, respectively. Results: Compared with tissues from patients with benign mass of oral mucosa, reduced miR-205-3p expression level and enhanced LAMC2 expression level were detected in epithelial tissue from OSCC patients (both $P<0.05$). Compared with the blank group, up-regulation of miR-205-3p or down-regulation of LAMC2 could promote expression levels of E-cadherin and Bax, and suppress expression levels of N-cadherin, vimentin, and Bcl-2 in OSCC stem cells, which could also inhibit proliferation, invasion ability and facilitate apoptosis of OSCC stem cells (all $P<0.05$). In the miR-205-3p mimic + siRNA-LAMC2 group, the changes of above indicators were more notable. Inhibition of the miR-205-3p expression had the opposite effect (all $P<0.05$). Conclusion: Up-regulation of miR-205-3p can inhibit proliferation, invasion ability and promote apoptosis of OSCC stem cells by inhibiting LAMC2 gene expression.

Keywords: MiR-205-3p, LAMC2, OSCC, tumor stem cells

Introduction

Oral squamous cell carcinoma (OSCC) is a malignant tumor that typically occurs in the oral and maxillofacial region, with high recurrence rates and high metastasis rates [1]. In recent years, tumor stem cell theory has gradually become a hot spot. Tumor stem cells refer to cells that have the capabilities and attributes of stem cell in tumors, which have been confirmed to be associated with tumor growth, proliferation and metastasis [2-4]. Therefore, research on tumor stem cells is of great significance for clarifying the tumorigenesis, progression and drug resistance of tumors.

As a non-coding small molecule RNA, miRNA can regulate the expression of its target genes at the post-transcriptional level [5]. MiR-205 has been proven to play a pro- or anti-cancer role in a variety of cancers by acting on the target genes [6]. A previous research on the association between miR-205 and the development of oral cancer found that, compared with normal human oral keratinocytes, miR-205 expression in oral cancer cells was significantly elevated and overexpression of miR-205 could facilitate apoptosis of oral cancer cells [7]. Furthermore, Kim et al. discovered that miR-205 could act as an anti-oncogene in oral cancer through inhibiting the expression of Axis inhibition protein 2 (Axin-2) [8].

Laminin γ2-chain gene (LAMC2) is a subunit of the trimeric glycoprotein laminin 332. More and

more studies have shown that LAMC2 is an important risk factor for various cancers such as colorectal cancer and lung adenocarcinoma [9, 10]. Moreover, Ding et al. confirmed that high expression level of LAMC2 could promote the progression of tongue squamous cell carcinoma [11].

This study investigated the biological functions and potential regulatory mechanisms of miR-205-3p and LAMC2 in OSCC stem cells, hoping to boost the research on molecular mechanism of OSCC and inspire new insights for future treatment strategies for OSCC.

Methods

Tissue sample collection

Cancer tissue specimens of 68 patients who were diagnosed as OSCC in Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology from September 2017 to December 2018 were collected. The patients included 47 males and 21 females with an average age of 59.2±12.7 years. All patients did not receive chemotherapy before surgery, and were treated with orthotopic lesion resection and cervical lymphadenectomy. After surgery, they were routinely followed up. In addition, oral mucosa tissues around benign mass from 36 cases admitted in the hospital at the same time were collected as the normal group. These 36 cases included 19 males and 17 females with an average age of 56.3±11.4 years. All the tissue specimens were cleaned with saline to remove the surface blood, cut into small pieces (0.5 cm×0.5 cm), and stored in -80°C refrigerator after liquid nitrogen freezing.

This study was approved by the ethics committee of Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology and all patients signed the informed consent.

Cell culture

Human normal oral mucosal epithelial cell line (LM-H004, LMAI Bio, Shanghai, China) and human OSCC cell line SCC25 (CL-0569, Procell Life Science & Technology, Wuhan, China) were cultured in RPMI 1640 medium containing 10% fetal bovine serum at 37°C, 5% CO_2. When cells reached about 80% growth density, they were digested with 0.25% trypsin (9002-07-7, Yuanye Bio-Technology, Shanghai, China) for subculture.

Isolation of OSCC stem cells by immunomagnetic beads sorting

SCC25 cells were digested and the suspension was centrifugated at 1,000 rpm for 10 min. The supernatants were discarded, and then the cells were resuspended by 100 μl of buffer solution. Next, 20 μl of CD44 (130-110-293, Miltenyi Biotec, Germany) or CD133 (130-050-801, Miltenyi Biotec, Germany) immunomagnetic beads were added into the suspension. After incubation for 10 min at 4°C, suspension was centrifuged at 1,000 rpm for 10 min again and resuspended by 500 μl of buffer solution. After the magnetic field and the sorting rack being connected, the CD44 (or CD133) sorting column was placed in the magnetic field and rinsed with 3 ml of buffer solution. Then the cell suspension was added into the sorting column, and the column was washed by 3 ml of buffer solution three times. Subsequently, the sorting column was taken out from the magnetic field and washed by 5 ml of buffer solution to collect the target cells which were then seeded in culture flasks.

The sorted CD133, CD44 cells were digested by 0.25% of trypsin, centrifugated at 1,000 rpm for 5 min. The supernatants were discarded, then the cells were resuspended by 3 ml of PBS and divided into two groups after cell counting: cells in one group were incubated with 95 μl of PBS solution and 5 μl of CD44-FITC (or CD133-PE) antibodies for 30 min at 4°C, while cells in the other group were mixed with 95 μl of PBS solution and 5 μl of CD44-PE (or CD133-FITC) antibodies for 30 min at 4°C as isotype control. After incubation, the suspensions were centrifuged and the supernatants were discarded. The cells were resuspended by 400 μl of PBS solution and the purity of CD44+, CD133+ cells were detected by flow cytometry (CytoFLEX S, Beckman coulter, USA) [12].

Cell grouping and transfection

Normal oral mucosal epithelial cells were used as the normal group. The sorted OSCC stem cells were divided into the following 5 groups

Table 1. Primer sequences used in qRT-PCR

Gene	Primer sequences (5'-3')
miR-205-3p	
Forward	CTAAAATGATGGGGGAATCCTCC
Reverse	GCCTTCCTTGGGTAAAGCTAC
LAMC2	
Forward	GTTAGGGAGGAAGATAATCC
Reverse	CCCCTTGGTAAGCATTCGTA
Bax	
Forward	TGTGGGGCGGGTAAAACGTGTA
Reverse	GTGCAGAGGTTAGATTGGGTGGTA
Bcl-2	
Forward	CAGTCCACCGCCAAGCCTT
Reverse	CACACCCTAAGCTTCGCCG
E-cadherin	
Forward	CCTGTAACATGTGGATATCGTCC
Reverse	GGAAGAGGTTAGCTGTTCCC
N-cadherin	
Forward	CTTGACGCCGATGCGTTTCAG
Reverse	GACCTGCCTTGAGCGCTTAAT
Vimentin	
Forward	GTGGGTGTGCAGTGTTGGG
Reverse	CTCATAGAGTTGTCGTCGCACC
U6	
Forward	AGTGCTGGGTGTGGGTGTG
Reverse	GTATCTACGATGTCCGTCGA
GAPDH	
Forward	GGTGGTGTAATTCCAATTGGG
Reverse	ATGGCAGAATCCTATAGTCCCT

Note: LAMC2, Laminin γ2-chain.

and received corresponding treatments respectively: blank group (transfection with empty plasmid), miR-205-3p mimic group (transfected with miR-205-3p mimic), miR-205-3p inhibitor group (transfected with miR-205-3p inhibitor), siRNA-LAMC2 group (transfected with si-LAMC2 plasmid), miR-205-3p mimic + siRNA-LAMC2 group (co-transfected with miR-205-3p mimic and si-LAMC2 plasmid). Transfection procedures were performed strictly according to the Lipofectamin 2000 instructions (1166-8027, Invitrogen, USA). The plasmid was first diluted using 250 μl serum-free Opti-MEM medium (11058021, ThermoFisher, USA), and incubated at room temperature for 10 min after mixing. Then 5 μl lipofectamin 2000 was diluted with equal amount of serum-free Opti-MEM medium. The above two were mixed well, incubated at room temperature for 30 min, and then placed in an incubator containing 5% CO_2 at 37°C. The culture medium was replaced every 6-8 h and cultured continuously for 48 h. All plasmids used in this study were purchased from Genechem (Shanghai, China).

Dual luciferase reporter assay

The binding site of miR-205-3p and LAMC2 was predicted by Targetscan online prediction website (http://www.targetscan.org/mamm_31/). The LAMC2-3'UTR fragment was obtained by double digestion and cloned onto the upstream of the pmirGLO vector as LAMC2-3'UTR-WT plasmid (WT: wild type). The purified plasmid was used for site-directed mutagenesis at the binding site of miR-205-3p and LAMC2 to construct LAMC2-3'UTR-MUT plasmid (MUT: mutant type). MiR-205-3p mimic or NC mimic were co-transfected into 293T cells with LAMC2-3'UTR-WT and LAMC2-3'UTR-MUT plasmids respectively, according to the instructions of LipofectamineTM 2000 (11668-019, Invitrogen, USA). The culture medium was replaced after 4 h of incubation. Cells were incubated at 37°C, 5% CO_2 for 48 h. The luciferase activity was detected by chemiluminescence detector according to the instructions of the dual luciferase reporter gene kit (RG027, Beyotime Biotechnology, Shanghai, China).

QRT-PCR

MiR-205-3p expression and mRNA expressions of LAMC2, Bax, Bcl-2, E-cadherin, N-cadherin and vimentin in cells were examined by qRT-PCR. Total RNAs in cells was extracted according to Trizol kit instructions (Solarbio, Beijing, China). Subsequently, total RNAs were dissolved in diethyl pyrocarbonate (DEPC, 1609-47-8, Jizhi Biochemical Technology, Shanghai, China)-treated ultrapure water. Ultraviolet-visible spectrophotometer (DR6000, HACH, USA) was used to determine the total RNAs concentration at 260 nm and 280 nm. RT and PCR reactions were performed according to the instructions of qRT-PCR gene expression analysis detection kit (QPG-023, Genepharma, Suzhou, China). U6 was used as the internal control for miR-205-3p, while GAPDH was served as the internal control for LAMC2, Bax, Bcl-2, E-cadherin, N-cadherin and vimentin. All primer sequences were synthesized by Beijing Genomics Institute (Beijing, China; **Table 1**).

The relative expression of target genes was calculated by $2^{-\Delta\Delta Ct}$ method. $\Delta Ct = Ct_{target\ gene} - Ct_{control\ gene}$, $\Delta\Delta Ct = \Delta Ct_{experimental\ group} - \Delta Ct_{control\ group}$. This experiment was repeated 3 times independently to obtain average values. The expression of genes in tissue samples was detected in the same way.

Western blot

Appropriate amount of protein lysate (P0013B, Beyotime Biotechnology, Shanghai, China) was added into the transfected cells. Cells collected in EP tubes were lysed on ice and then centrifuged at 2,000 rpm for 30 min, at 4°C. The protein concentration in the supernatant was determined by using the BCA kit (P0009, Beyotime Biotechnology, Shanghai, China). SDS-PAGE gel electrophoresis was conducted to separate protein. After being transferred to a PVDF membrane by wet transfer method, protein was blocked for 1 h with 5% BSA. The PVDF membrane was washed and then incubated with rabbit anti-human primary antibodies overnight at 4°C. The primary antibodies used in this research were as follows: LAMC2 (ab96327, 1:1,000, Abcam, UK), Bax (ab182733, 1:2,000, Abcam, UK), Bcl-2 (ab182858, 1:2,000, Abcam, UK), E-cadherin (ab40772, 1:20,000, Abcam, UK), N-cadherin (ab18203, 1 μg/ml, Abcam, UK) and vimentin (ab24525, 1:10,000, Abcam, UK). The PVDF membrane was washed 3 times with TBST for 10 min each time. Horseradish peroxidase-labeled goat anti-rabbit IgG (ab200699, 1 μg/ml, Abcam, UK) was used to incubate the PVDF membrane for 4-6 h at 4°C. The PVDF membrane was then washed 3 times with TBST for 10 min each time. Equal amounts of chemiluminescence liquids A and B were mixed and added onto the PVDF membrane. The blots were photographed and the relative expression of protein was analyzed using Image J software.

MTT assay for cell proliferation

When the cell growth density reached about 80%, OSCC stem cells in each group were inoculated in 96-well plates (1×10^7 cells/ml, 20 μl). At 24 h, 48 h and 72 h of culture, 20 μl of MTT solution (5 mg/ml, SBJ-0190, SenBeiJia Biological Technology, Nanjing, China) was added into each well for 4-h incubation. The supernatant in each well was discarded and 150 μl of dimethyl sulfoxide (DMSO) solution (D2650, Lianshuo Biological Technology, Shanghai, China) was then added into each well. The plates were shaken and placed on a microplate reader (Thermo MK3, Thermo Scientific, Shanghai, China) to measure the absorbance of each well at 490 nm wavelength. The cell growth curve was plotted with the time point as the abscissa and the absorbance value as the ordinate.

Transwell experiment for cell invasion

Matrigel (356234, Surejbio, Shanghai, China) was diluted by serum-free RPMI 1640 medium (PM150110, Procell, Wuhan, China) contain 0.2% BSA (bull serum albumin) and 50 μl of the diluted Matrigel was spread onto the upper chamber of the transwell inserts. Next day, 500 μl of RPMI 1640 medium with 10% FBS and 100 μl of serum-free RPMI 1640 medium with 0.2% BSA were respectively added into the upper chamber and lower chamber. After digestion, cells in each group were washed by PBS and resuspended by RPMI 1640 medium to a density of 1×10^6 cells/ml. The cell suspension was then added into the lower chamber. The chamber was taken out after 24 h of culture. Cells were fixed with 4% paraformaldehyde for 30 min, followed by being stained with crystal violet solution (Solarbio, Beijing, China). After washed with PBS, cells passed through the membrane were observed with an inverted microscope (37XC, Optical Instrument Factory, Shanghai, China). The number of cells passed through the membrane was photographed and counted in 5 fields of vision to obtain an average value in each group.

Apoptosis detection by flow cytometry

Apoptosis was detected by Annexin V-FITC/PI kit (BB-4101, Bestbio, Shanghai, China). The transfected cells were digested and resuspended to 1×10^5 cells/ml. Pre-cooled 75% ethanol was used to fix cells overnight at 4°C. Then 1 ml of cells suspension was taken out and centrifuged at 1,500 rpm for 20 min. After being washed with PBS, cells were resuspended in 200 μl binding buffer. Then 5 μl of annexin V-FITC and 10 μl of PI solution were added into cells suspension for 15 min incubation at room temperature. Subsequently, 300 μl of HEPES buffer was added and apoptosis was measured by using a flow cytometer (FACSCalibur, BD, USA). The excitation wavelength was 488 nm

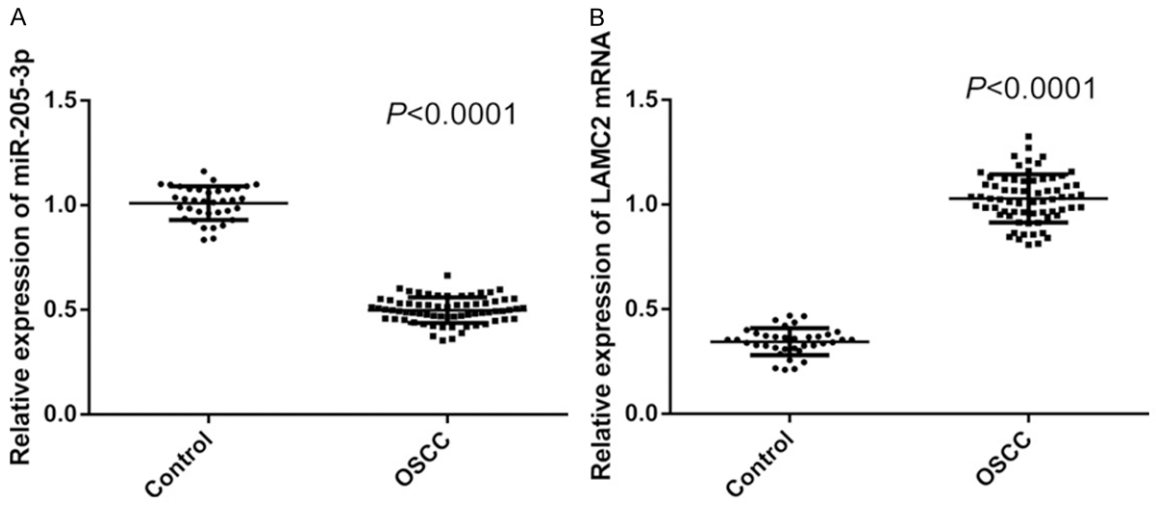

Figure 1. The expression of miR-205-3p and LAMC2 in tissues of OSCC patients and patients in Control group. A: MiR-205-3p expression level in OSCC tissues (OSCC) and normal oral mucosa around benign mass (Control). B: LAMC2 mRNA expression level in OSCC tissues (OSCC) and normal oral mucosa around benign mass (Control). LAMC2, Laminin γ2-chain; OSCC, oral stem squamous cell carcinoma.

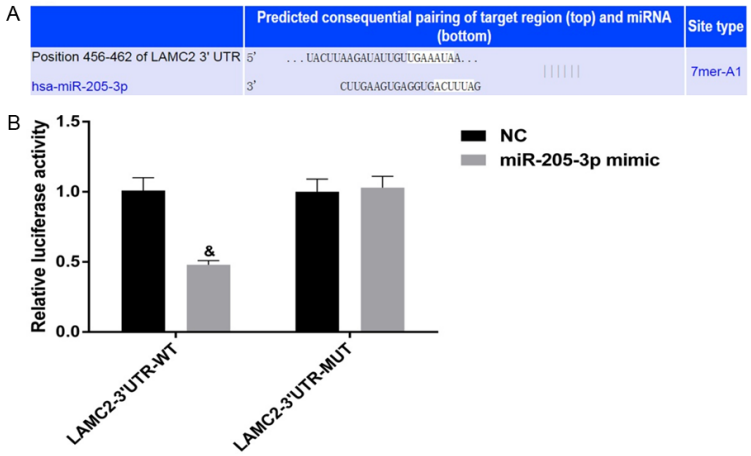

Figure 2. The targeting relationship between miR-205-3p and LAMC2. A: The prediction result of the binding site between miR-205-3p and LAMC2 according to Targetscan. B: Dual luciferase reporter system assay verified the prediction. &P<0.05 in comparison to 293T cells co-transfected by miR-205-3p mimic and LAMC2-3'UTR-MUT. LAMC2, Laminin γ2-chain.

and FITC was detected at 530 nm, while PI was detected at a wavelength of 575 nm.

Statistical analysis

The data was analyzed using the SPSS 21.0 software. All measurement data that fitted the normal distribution were expressed as mean ± standard deviation ($\bar{x}$ ± SD). The comparison between two groups was performed by independent sampled t test, while one-way analysis of variance was used for comparison among multiple groups. Count data was expressed as percentage and comparison between groups adopted Chi-square test. P<0.05 indicated that the difference was statistically significant.

Results

Reduced miR-205-3p expression and elevated LAMC2 expression was observed in OSCC tissues

The expression of miR-205-3p and LAMC2 in cancer tissues from OSCC patients (OSCC group) and normal tissues from patients (control group) was determined by qRT-PCR. As a result, in comparison with patients in control group, reduced miR-205-3p expression and elevated LAMC2 mRNA expression was detected in tissues from OSCC group (both P<0.05) (**Figure 1**).

MiR-205-3p directly inhibited the expression of LAMC2

Targetscan predicted that miR-205-3p possessed the binding site for LAMC2 (**Figure 2A**).

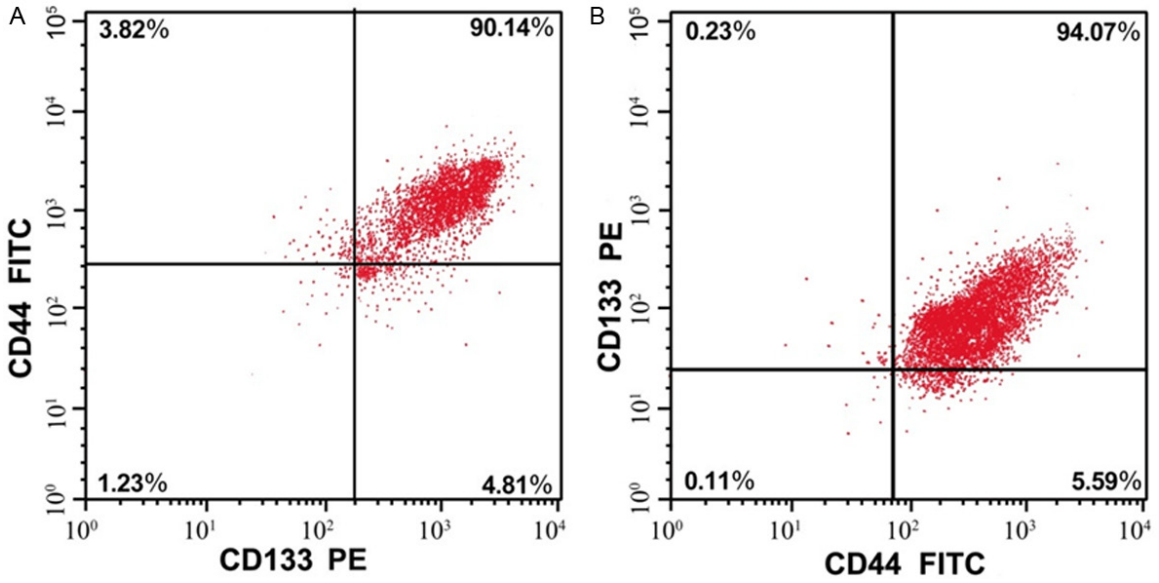

Figure 3. Purity of sorted OSCC stem cells. A: After immunomagnetic beads sorting, the expression of CD44 in OSCC cells. B: The expression of CD133 in OSCC cells after immunomagnetic beads sorting. OSCC, oral stem squamous cell carcinoma.

According to dual luciferase reporter system assay, compared with 293T cells co-transfected with mimic NC and LAMC2-3'UTR-MUT, those co-transfected with miR-205-3p mimic and LAMC2-3'UTR-WT had markedly lower luciferase activity (P<0.05) (**Figure 2B**). These results supported that miR-205-3p could directly inhibit the expression level of LAMC2.

Sorting and identification of OSCC stem cells

After sorting, the purity of CD44+, CD133+ OSCC stem cells was detected by flow cytometry. As shown in **Figure 3**, the purity of CD133+ and CD44+ cells were 90.14% and 94.07%, respectively. The results indicated that most of the sorted cells were OSCC stem cells, which could be used in the subsequent experiments.

MiR-205-3p over-expression or LAMC2 silencing suppressed the expression of epithelial-mesenchymal transition-related factors

Compared with normal group, cells in the other groups exhibited reduced miR-205-3p expression level, enhanced expression level of LAMC2 and epithelial-mesenchymal transition (EMT) related factors. In comparison with blank group, dramatically decreased LAMC2, N-cadherin and vimentin expression as well as increased E-cadherin expression were observed in miR-205-3p mimic group, siRNA-LAMC2 group, and miR-205-3p mimic + siRNA-LAMC2 group (all P<0.05). However, miR-205-3p inhibitor group showed opposite results (all P<0.05) (**Figure 4**).

MiR-205-3p over-expression or LAMC2 silencing inhibited OSCC stem cells proliferation

Compared with the normal group, reduced Bax expression level and enhanced Bcl-2 expression level and cell proliferation was found in the other groups. In comparison with blank group, OSCC stem cells in miR-205-3p mimic group and siRNA-LAMC2 group exhibited significantly elevated Bax expression level and reduced Bcl-2 expression level and cell proliferation (all P<0.05). Notably, OSCC stem cells of miR-205-3p mimic + siRNA-LAMC2 group showed more obvious changes in the above indicators. In addition, compared with blank group, OSCC stem cells in miR-205-3p inhibitor group had significantly decreased Bax expression level and increased Bcl-2 expression level and cell proliferation (all P<0.05) (**Figure 5**).

MiR-205-3p over-expression or LAMC2 silencing suppressed OSCC stem cells invasion

Transwell assay was used for the detection of OSCC stem cells invasion ability (**Figure 6**). Compared with normal group, more invasive cells were observed in the other groups. The invasive cell numbers of miR-205-3p mimic

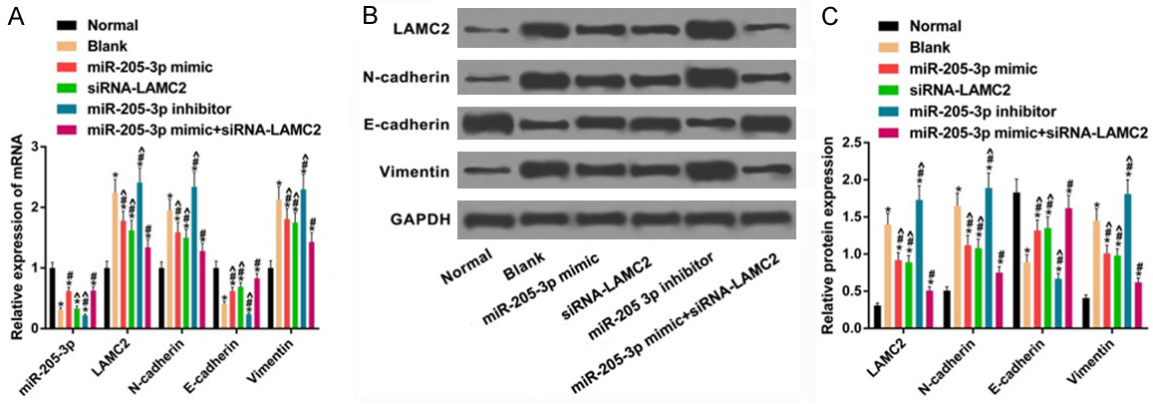

Figure 4. Expression level of miR-205-3p, LAMC2, N-cadherin, E-cadherin and Vimentin in each group of OSCC stem cells after transfection. A: mRNA expression level. B, C: Protein expression level. Compared with Normal group, *P<0.05. Compared with Blank group, #P<0.05. Compared miR-205-3p mimic + siRNA-LAMC2 group, ^P<0.05. LAMC2, Laminin γ2-chain; OSCC, oral stem squamous cell carcinoma.

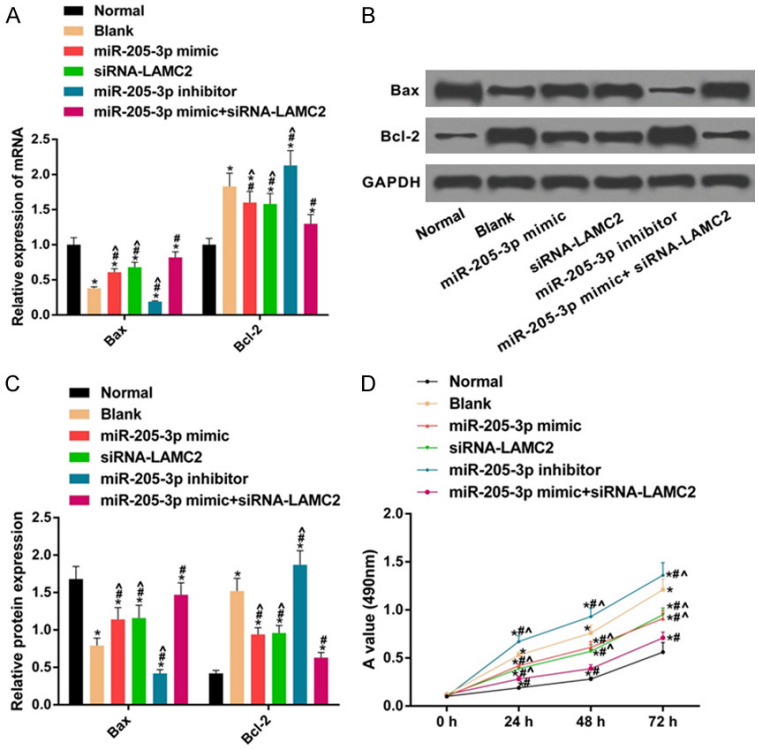

Figure 5. Effect of miR-205-3p overexpression or LAMC2 silencing on Bax and Bcl-2 expression level and cell proliferation of OSCC stem cells. A: mRNA expression level of Bax and Bcl-2 in each group of OSCC stem cells. B, C: Protein expression level of Bax and Bcl-2 proteins in each group of OSCC stem cells. D: Cell proliferation of OSCC stem cells in each group. Compared with normal group, *P<0.05. Compared with blank group, #P<0.05. Compared miR-205-3p mimic + siRNA-LAMC2 group, ^P<0.05. LAMC2, Laminin γ2-chain; OSCC, oral stem squamous cell carcinoma.

group and siRNA-LAMC2 group were significantly less than that of blank group (all P<0.05).

OSCC stem cells in miR-205-3p mimic + siRNA-LAMC2 exhibited more pronounced inhibitory effects on invasion. Moreover, remarkably increased invasive cell number occurred in OSCC stem cells of miR-205-3p inhibitor group when compared with blank group (P<0.05).

MiR-205-3p over-expression or LAMC2 silencing promoted OSCC cells apoptosis

Flow cytometry was carried out to detect apoptosis in OSCC stem cells in each group. Compared with normal group, much lower apoptosis rate was observed in the other groups. In comparison with blank group, miR-205-3p overexpression or LAMC2 silencing could markedly facilitate OSCC cells apoptosis. Meanwhile, combination of miR-205-3p overexpression and LAMC2 silencing could more seriously promote OSCC cells apoptosis (all P< 0.05). Furthermore, the apoptosis rate of OSCC stem cells in miR-205-3p inhibitor group was much lower than that in blank group (P<0.05) (**Figure 7**).

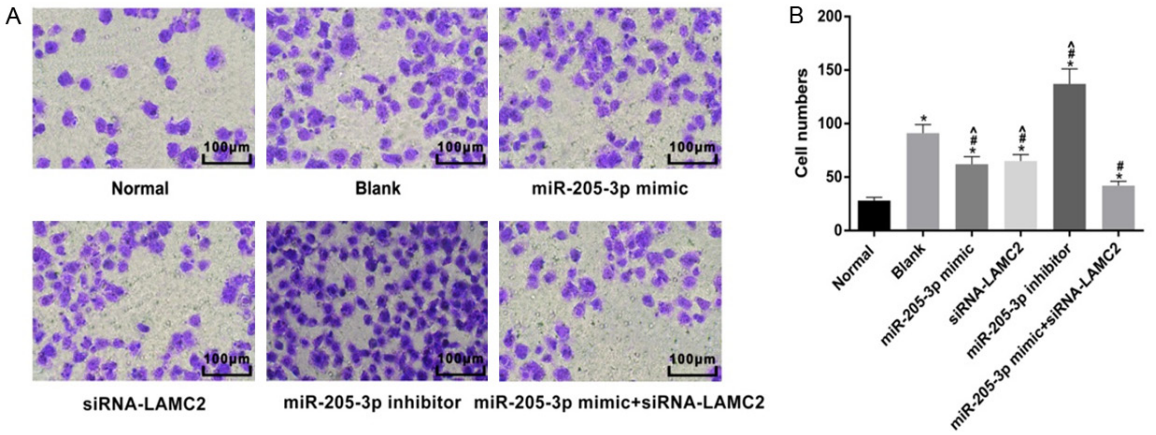

Figure 6. Effect of miR-205-3p overexpression or LAMC2 silencing on invasion ability in OSCC stem cells. A: OSCC stem cells invasion ability was detected by transwell assay. B: The number of invasive OSCC stem cells was counted. Compared with normal group, *P<0.05. Compared with blank group, #P<0.05. Compared miR-205-3p mimic + siRNA-LAMC2 group, ^P<0.05. LAMC2, Laminin γ2-chain; OSCC, oral stem squamous cell carcinoma.

Discussion

Previous studies have reported that miR-205 plays a tumor-suppressing role in a variety of cancers. For instance, Salajegheh et al. demonstrated that miR-205 could suppress the apoptosis and angiogenesis of thyroid cancer cells [13]. A research also indicated that miR-205 could attenuate TGF-β-induced EMT in lung cancer [14]. Furthermore, the role of miR-205 in oral cancer has also been gradually revealed. A study revealed that miR-205 expression was elevated in oral cancer cells [8]. Nagai et al. discovered that miR-205-5p could suppress the invasion of OSCC cells by inhibiting the expression of TIMP2 [15]. Moreover, the role of LAMC2 in oral cancer has also started to be noticed recently [11]. In this article, the expression of miR-205-3p and LAMC2 in OSCC tissues or oral mucosa around benign mass was examined. As a result, we found that the expression level of miR-205-3p was significantly lower and LAMC2 was significantly higher in OSCC tissues than those in the oral mucosa. This result indicated that miR-205-3p and LAMC2 might be involved in the pathogenesis of OSCC.

The expression level of CD44+ and CD133+ has been confirmed to be of great significance in predicting the prognosis of OSCC [16]. In addition, the CD44+ and CD133+ cell populations in OSCC cells have the characteristics of stem cells, which possessed high survival, invasion and migration abilities, and resistance to chemoradiation. Therefore, CD44+ and CD133+ are used as markers of OSCC tumor stem cells [17]. In this study, OSCC stem cells were successfully purified by immunomagnetic beads sorting and their gene expression of miR-205-3p and LAMC2 was regulated. Finally, we found that miR-205-3p overexpression or LAMC2 silencing could promote apoptosis and reduce proliferation and invasion of OSCC stem cells, while miR-205-3p inhibition had opposite effects. In addition, we also found that miR-205-3p could directly inhibit the expression level of LAMC2. All these findings indicated that upregulation of miR-205-3p could promote apoptosis and reduce proliferation and invasion of OSCC stem cells by inhibiting LAMC2 gene expression.

EMT is the process by which epithelial cells transform into mesenchymal cells. It is also a key process in the early stages of cancer metastasis [18-21]. More and more studies showed that, EMT occurred in a variety of cancers, such as lung cancer, pancreatic cancer, breast cancer and oral cancer. The degree of EMT directly affects the malignancy grade of tumors [22-27]. Down-regulation of cell surface epithelial marker E-cadherin and up-regulation of interstitial markers N-cadherin and Vimentin are important markers of EMT [28-31]. In this study, EMT-related factors expression in OSCC stem cells was assessed after transfection. It could be noted that miR-205-3p overexpres-

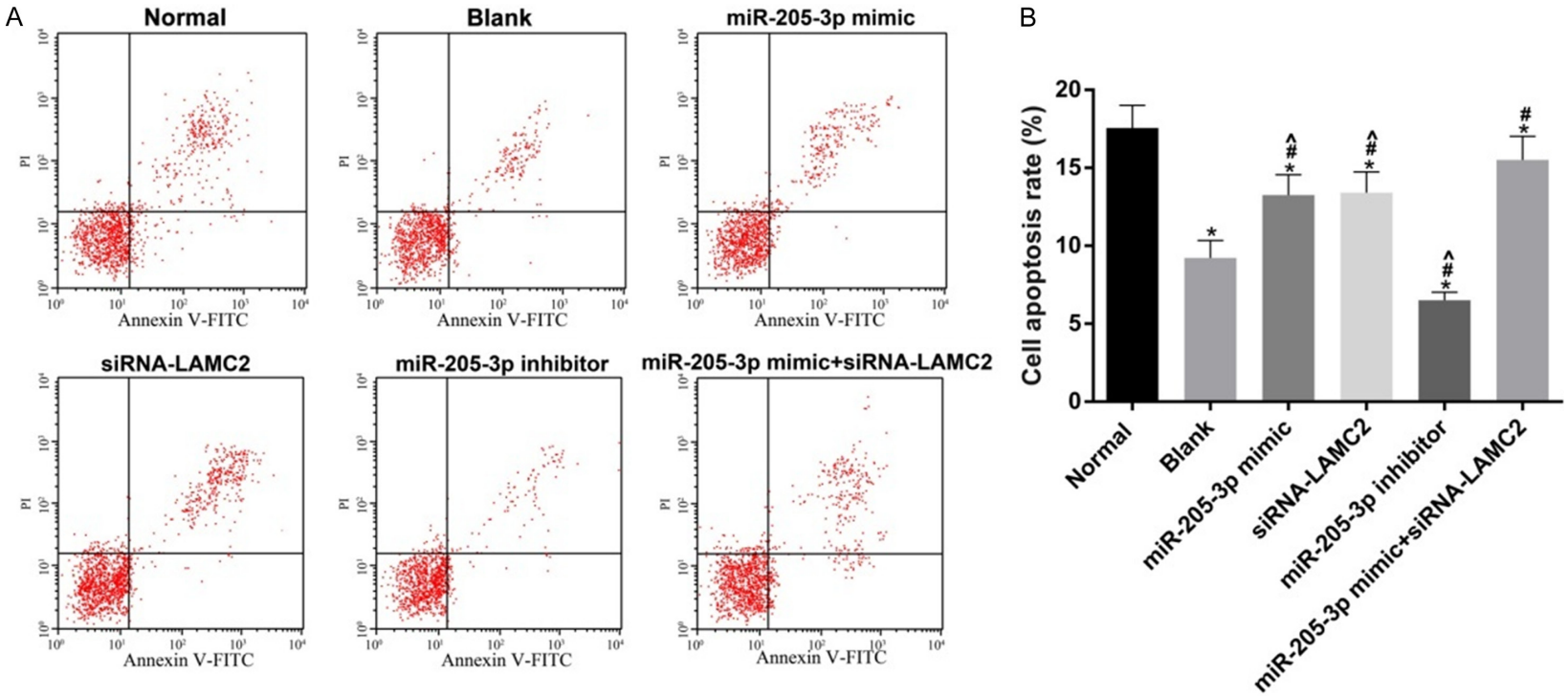

Figure 7. Effect of miR-205-3p overexpression or LAMC2 silencing on apoptosis in OSCC stem cells. A: The apoptosis map of OSCC stem cells in each group after transfection. B: The apoptosis rate of OSCC stem cells in each group after transfection. Compared with normal group, $^{*}P<0.05$. Compared with blank group, $^{\#}P<0.05$. Compared miR-205-3p mimic + siRNA-LAMC2 group, $^{\wedge}P<0.05$. LAMC2, Laminin γ2-chain; OSCC, oral stem squamous cell carcinoma.

sion or LAMC2 silencing could promoted E-cadherin expression and inhibited expression of N-cadherin and Vimentin. These results indicated that miR-205-3p might regulate OSCC development by weakening EMT via targeting and inhibiting LAMC2 gene expression.

However, there are limitations in this research, such as a lack of exploration of the cell death patterns and in vivo experiments. Therefore, future studies are needed to verify our results and investigate the mechanism, so as to provide a new direction for the treatment of OSCC.

In summary, miR-205-3p could inhibit proliferation and invasion but promote apoptosis of OSCC stem cells via inhibition of LAMC2 expression.

Disclosure of conflict of interest

None.

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References

[1] Fang CY, Wu CZ, Chen PN, Chang YC, Chuang CY, Lai CT, Yang SF and Tsai LL. Antimetastatic potentials of salvianolic acid A on oral squamous cell carcinoma by targeting MMP-2 and the c-Raf/MEK/ERK pathway. Environ Toxicol 2018; 33: 545-554.

[2] Relation T, Dominici M and Horwitz EM. Concise review: an (Im) penetrable shield: how the tumor microenvironment protects cancer stem cells. Stem Cells 2017; 35: 1123-1130.

[3] Wang L, Li Y, Xu J, Zhang A, Wang X, Tang R, Zhang X, Yin H, Liu M, Wang DD, Lin PP, Shen L and Dong J. Quantified postsurgical small cell size CTCs and EpCAM+ circulating tumor stem cells with cytogenetic abnormalities in hepatocellular carcinoma patients determine cancer relapse. Cancer Lett 2018; 412: 99-107.

[4] Guler G, Guven U and Oktem G. Characterization of CD133+/CD44+ human prostate cancer stem cells with ATR-FTIR spectroscopy. Analyst 2019; 144: 2138-2149.

[5] Tseng HH, Tseng YK, You JJ, Kang BH, Wang TH, Yang CM, Chen HC, Liou HH, Liu PF, Ger LP and Tsai KW. Next-generation sequencing for microRNA profiling: microRNA-21-3p promotes oral cancer metastasis. Anticancer Res 2017; 37: 1059-1066.

[6] Qin AY, Zhang XW, Liu L, Yu JP, Li H, Wang SZ, Ren XB and Cao S. MiR-205 in cancer: an angel or a devil? Eur J Cell Biol 2013; 92: 54-60.

[7] Kim JS, Yu SK, Lee MH, Park MG, Park E, Kim SG, Lee SY, Kim CS, Kim HJ, Chun HS, Chun SW and Kim DK. MicroRNA-205 directly regulates the tumor suppressor, interleukin-24, in human KB oral cancer cells. Mol Cells 2013; 35: 17-24.

[8] Kim JS, Park SY, Lee SA, Park MG, Yu SK, Lee MH, Park MR, Kim SG, Oh JS, Lee SY, Kim CS, Kim HJ, Chun HS, Kim JS, Moon SM and Kim DK. MicroRNA-205 suppresses the oral carcinoma oncogenic activity via down-regulation of Axin-2 in KB human oral cancer cell. Mol Cell Biochem 2014; 387: 71-79.

[9] Moon YW, Rao G, Kim JJ, Shim HS, Park KS, An SS, Kim B, Steeg PS, Sarfaraz S, Changwoo Lee L, Voeller D, Choi EY, Luo J, Palmieri D, Chung HC, Kim JH, Wang Y and Giaccone G. LAMC2 enhances the metastatic potential of lung adenocarcinoma. Cell Death Differ 2015; 22: 1341-1352.

[10] Gong J, Tian J, Lou J, Ke J, Li L, Li J, Yang Y, Gong Y, Zhu Y, Zhang Y, Zhong R, Chang J and Miao X. A functional polymorphism in lncLAMC2-1:1 confers risk of colorectal cancer by affecting miRNA binding. Carcinogenesis 2016; 37: 443-451.

[11] Ding J, Yang C and Yang S. LINC00511 interacts with miR-765 and modulates tongue squamous cell carcinoma progression by targeting LAMC2. J Oral Pathol Med 2018; 47: 468-476.

[12] Wang J, Wu Y, Gao W, Li F, Bo Y, Zhu M, Fu R, Liu Q, Wen S and Wang B. Identification and characterization of CD133+/CD44+ cancer stem cells from human laryngeal squamous cell carcinoma cell lines. J Cancer 2017; 8: 497-506.

[13] Salajegheh A, Vosgha H, Md Rahman A, Amin M, Smith RA and Lam AK. Modulatory role of miR-205 in angiogenesis and progression of thyroid cancer. J Mol Endocrinol 2015; 55: 183-196.

[14] Zeng Y, Zhu J, Shen D, Qin H, Lei Z, Li W, Huang JA and Liu Z. Repression of Smad4 by miR205 moderates TGF-β-induced epithelial-mesenchymal transition in A549 cell lines. Int J Oncol 2016; 49: 700-708.

[15] Nagai H, Hasegawa S, Uchida F, Terabe T, Ishibashi Kanno N, Kato K, Yamagata K, Sakai S, Kawashiri S, Sato H, Yanagawa T and Bukawa H. MicroRNA-205-5p suppresses the invasiveness of oral squamous cell carcinoma by inhibiting TIMP2 expression. Int J Oncol 2018; 52: 841-850.

[16] Oliveira LR, Castilho-Fernandes A, Oliveira-Costa JP, Soares FA, Zucoloto S and Ribeiro-Silva A. CD44+/CD133+ immunophenotype and matrix metalloproteinase-9: Influence on prognosis in early-stage oral squamous cell carcinoma. Head Neck 2014; 36: 1718-1726.
[17] Wang J, Wu Y, Gao W, Li F, Bo Y, Zhu M, Fu R, Liu Q, Wen S and Wang B. Identification and characterization of CD133+/CD44+ cancer stem cells from human laryngeal squamous cell carcinoma cell lines. J Cancer 2017; 8: 497-506.
[18] Sharma S, Goswami R and Rahaman SO. The TRPV4-TAZ mechanotransduction signaling axis in matrix stiffness- and TGFβ1-induced epithelial-mesenchymal transition. Cell Mol Bioeng 2019; 12: 139-152.
[19] Yokomichi N, Nishida N, Umeda Y, Taniguchi F, Yasui K, Toshima T, Mori Y, Nyuya A, Tanaka T, Yamada T, Yagi T, Fujiwara T, Yamaguchi Y, Goel A, Kudo M and Nagasaka T. Heterogeneity of epigenetic and epithelial mesenchymal transition marks in hepatocellular carcinoma with keratin 19 proficiency. Liver Cancer 2019; 8: 239-254.
[20] Gloushankova NA, Zhitnyak IY and Rubtsova SN. Role of epithelial-mesenchymal transition in tumor progression. Biochemistry (Mosc) 2018; 83: 1469-1476.
[21] Yang Z, Xie Q, Chen Z, Ni H, Xia L, Zhao Q, Chen Z and Chen P. Resveratrol suppresses the invasion and migration of human gastric cancer cells via inhibition of MALAT1-mediated epithelial-to-mesenchymal transition. Exp Ther Med 2019; 17: 1569-1578.
[22] Wang Y, Jia L, Wang B, Diao S, Jia R and Shang J. MiR-495/IGF-1/AKT signaling as a novel axis is involved in the epithelial-to-mesenchymal transition of oral squamous cell carcinoma. J Oral Maxillofac Surg 2019; 77: 1009-1021.
[23] Kurashige J, Yokobori T, Mima K, Sawada G, Takahashi Y, Ueo H, Takano Y, Matsumura T, Uchi R, Eguchi H, Sudo T, Sugimachi K, Mori M, Baba H and Mimori K. Plastin3 is associated with epithelial-mesenchymal transition and poor prognosis in gastric cancer. Oncol Lett 2019; 17: 2393-2399.
[24] Kim SD, Yi JM and Park MT. Irradiated endothelial cells modulate the malignancy of liver cancer cells. Oncol Lett 2019; 17: 2187-2196.
[25] Cao ZQ, Wang XX, Lu L, Xu JW, Li XB, Zhang GR, Ma ZJ, Shi AC, Wang Y and Song YJ. β-sitosterol and gemcitabine exhibit synergistic anti-pancreatic cancer activity by modulating apoptosis and inhibiting epithelial-mesenchymal transition by deactivating Akt/GSK-3β signaling. Front Pharmacol 2018; 9: 1525.
[26] Dai G, Sun B, Gong T, Pan Z, Meng Q and Ju W. Ginsenoside Rb2 inhibits epithelial-mesenchymal transition of colorectal cancer cells by suppressing TGF-β/Smad signaling. Phytomedicine 2019; 56: 126-135.
[27] Guo D, Song X, Guo T, Gu S, Chang X, Su T, Yang X, Liang B and Huang D. Vimentin acetylation is involved in SIRT5-mediated hepatocellular carcinoma migration. Am J Cancer Res 2018; 8: 2453-2466.
[28] Zhao L, Li J, Liu Y, Zhou W, Shan Y, Fan X, Zhou X, Shan B, Song Y and Zhan Q. Flotillin1 promotes EMT of human small cell lung cancer via TGF-β signaling pathway. Cancer Biol Med 2018; 15: 400-414.
[29] Xu Q, Fang L, Chen B, Zhang H, Wu Q, Zhang H, Wang A, Tong J, Tao S and Tian H. Radon induced mitochondrial dysfunction in human bronchial epithelial cells and epithelial-mesenchymal transition with long-term exposure. Toxicol Res (Camb) 2019; 8: 90-100.
[30] Ji S, Su X, Zhang H, Han Z, Zhao Y and Liu Q. MicroRNA-372 functions as a tumor suppressor in cell invasion, migration and epithelial-mesenchymal transition by targeting ATAD2 in renal cell carcinoma. Oncol Lett 2019; 17: 2400-2408.
[31] Wang Y, Liang S, Yu Y, Shi Y and Zheng H. Knockdown of SNHG12 suppresses tumor metastasis and epithelial-mesenchymal transition via the Slug/ZEB2 signaling pathway by targeting miR-218 in NSCLC. Oncol Lett 2019; 17: 2356-2364.