

Original Article

Expression of miR-214 and RASSF5 in oral cancer patients and their effects on apoptosis of oral cancer cells

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Abstract: Objective: To explore the expression and prognosis of miR-214 and RASSF5 in oral cancer patients and their effects on apoptosis of oral cancer cells. Methods: Oral squamous cell carcinoma tissues and adjacent normal tissues from 45 patients were collected. Normal oral squamous cells from 45 healthy volunteers were set as the control group. qRT-PCR was used to detect the expression of miR-214 and RASSF5 in oral squamous cell carcinoma tissues, adjacent normal tissues and control group. Then miR-214 inhibitors and RASSF5 mimic mRNA were transfected into oral cancer cell line KB cells. Untransfected cells were treated as the blank group and negative RNA control group (NC group). The effects of miR-214 and RASSF5 on apoptosis of oral cancer cells were observed by flow cytometry. Univariate and multivariate analysis of factors related to the formation of oral squamous cell carcinoma was performed. Results: The expression level of miR-214 in oral squamous cell carcinoma was significantly higher than that of adjuvant normal tissue and control group, while the expression level of RASSF5 in oral squamous cell carcinoma was significantly lower than that of adjuvant normal tissue and control group (all $P < 0.01$). The apoptosis ability of KB cells transfected with miR-214 mimics was significantly higher than that of the control group ($P < 0.05$). The apoptosis rate of KB cells transfected with miR-214 inhibitors and RASSF5 mimics was significantly higher than that of NC and blank groups ($P < 0.05$). Conclusion: Properly inhibiting the expression of miR-214 and up-regulating the expression of RASSF5 can significantly promote the apoptosis of oral cancer KB cells; and both are independent risk factors for prognosis of oral squamous cell carcinoma.

Keywords: miR-214, RASSF5, oral cancer, apoptosis

Introduction

Oral cancer is a malignant tumor found throughout the world [1]. Because the symptoms of early oral cancer are easily overlooked by patients, it is usually mistaken for conventional oral inflammation and develops to advanced stages in most patients [1-3]. The mortality of patients with advanced oral cancer is extremely high, and the number of deaths in patients with oral squamous cell carcinoma accounts for the majority of patients with advanced oral cancer [4-6]. At present, the clinical treatment of oral squamous cell carcinoma is mainly based on surgical treatment and radiation therapy [6, 7]. In recent years, with the in-depth discussion of the mechanism of oral squamous cell carcinoma, regulation of tumor suppressor genes or cancer-promoting genes may affect

the occurrence and development of cancer. This is expected to provide new ideas and targets for the treatment and prognosis of oral squamous cell carcinoma [8, 9].

MicroRNAs (miRNAs) are endogenous, non-coding RNAs involved in the regulation of cell proliferation, differentiation, and apoptosis [10, 11]. It is currently believed that miR-214 plays a role in promoting cancer or is a tumor suppressor miRNA in different solid tumors. Among them, studies have shown that inhibition of miR-214 expression in oral cancer can significantly inhibit cancer cell proliferation, cell cycle progression and migration ability [12, 13]. Some researchers have confirmed that RASSF5 is abnormally expressed in many malignant tumors such as oral squamous cell carcinoma, and the expression level is signifi-

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Table 1. Primer sequences of miR-214, RASSF and internal reference U6, β -actin

Group	Upstream primer sequence	Downstream primer sequence
miR-214	5'-GCACAGCAGGCACAGAC-3'	5'-GCA-CAGCAGGCACAGAC-3'
U6	5'-CTCGCTTCGGCAGCACA-3'	5'-AACGCTTCACGAATTTGCGT-3'
RASSF5	5'-GGCGTCGTGCGCAAAGGCC-3'	5'-GAACCTTGATGAAGCCTGTG-3'
β -actin	5'-GGCGGCACCAACCATGTACCCT-3'	5'-AGGGGCCGGACTCGTCATACT-3'

cantly down-regulated in tumor tissues [14]. However, the expression of miR-214 and RASSF5 and their effects on apoptosis of oral squamous cell carcinoma cells need to be further explored. Therefore, this paper studies the expression of miR-214 and RASSF5 in oral squamous cell carcinoma and the biological characteristics of oral squamous cell carcinoma.

Materials and methods

Data collection

Forty-five patients with oral squamous cell carcinoma who underwent surgical resection in our hospital were selected. There were 25 males and 20 females with an age range of 35-83 years and an average age of 50.03 ± 8.53 years. With the patient's consent, 45 oral squamous cell carcinoma tissues and adjacent tissues were excised and collected during the operation. At the same time, normal oral squamous cells of 45 healthy volunteers in the same period were taken and used as a control group. Inclusion criteria was that the tissue sections of the patients included were diagnosed as oral squamous cell carcinoma tissue or adjuvant normal tissue by our hospital pathology department. All specimens were placed in a liquid nitrogen tank immediately after excision. Patients who had undergone chemotherapy, immunotherapy, and radiation therapy before surgery were excluded. Patients and their families were informed in advance of the study and informed consent was signed. Ethics committee approval was obtained in our hospital.

Main reagents, instruments and testing methods

Main reagents and instruments: Human oral cancer KB cells were purchased from the Shanghai Cell Bank of the Chinese Academy of

Sciences. Trizol reagent was purchased from Applide Invitrogen, USA. qRT-PCR kit and minScript reverse transcription kit (Dalian TaKaRa), HBS-1096A enzyme analyzer (Nanjing Detie experiment Co., Ltd.); Real-time quantitative PCR instrument (BioRad, USA); DMEM medium (Gibco, USA); HyClone Standard Fetal Bovine Serum (FBS) and trypsin (Hyclone, USA); CyFlow Cube 8 flow cytometer (Partec, Germany). The primer sequences of miR-214, RASSF5 and internal reference materials U6 and β -actin were synthesized by Shanghai Jima Company (Table 1).

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Detection of miR-214 and RASSF: qRT-PCR was used to detect the expression of miR-214 and RASSF8 in oral squamous cell carcinoma tissues, adjacent normal tissue and control group. Total tissue RNA was extracted and dissolved in 20 μ L of DEPC water according to the Trizol reagent protocol; reverse transcription was performed with a reverse transcription kit for the total RNA. Reaction system: 1 μ L of M-MLV, 1 μ L of Olig (d T), 0.5 μ L of RNase inhibitor, 1 μ L of d NTPs, and 15 μ L of RNase free water. Incubate for 60 min at 38°C. One μ L of cDNA was taken at 85°C for 5 s; the synthesized DNA was used as a template for qRT-PCR amplification; a PCR reaction system was prepared: 2.5 μ L PCR buffer (10X), 1 μ L d NTPs, 1 μ L upstream and 1 μ L downstream primers, 0.25 μ L Taq DNA Polymerase, and added to 25 μ L with dd H₂O. Reactions were carried out at 94°C for 1 min; 94°C for 15 s, 60°C for 20 s, with a total of 39 cycles; experiments were performed in triplicate and repeated three times. U6 and β -actin were used as internal reference materials for miR-214 and RASSF, respectively. After the completion of the reaction, the amplification curve and the melting curve of the Real-Time PCR were confirmed, and the relative amount of the target gene was calculated based on the resulting parameters. The target gene is measured using $2^{-\Delta\Delta Ct}$ method.

Cell culture and transfection: A human oral squamous cell carcinoma KB (n = 45) cell line was placed in a medium containing 10% PBS DMEM and cultured at 37°C under 5% CO₂. When it was observed that the cell adherent

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Table 2. The expression of miR-214 and RASSF5 in oral squamous cell carcinoma, paracancerous normal tissues and control group

Group	Oral squamous cell carcinoma (n = 45)	Normal paracancerous tissue	Control group (n = 45)	F	P
miR-214	0.10 ± 0.05*	0.005 ± 0.002	0.005 ± 0.001	162.100	< 0.001
RASSF5	0.006 ± 0.002#	0.10 ± 0.05	0.10 ± 0.06	65.140	< 0.001

Note: * indicates that the expression level of miR-214 in this group is significantly lower than that in NC group and blank group; # indicates that the expression level of RASSF5 in this group is significantly higher than that in NC group and blank group (P < 0.001).

growth fusion reached 85%, then 25% trypsin was added for digestion. After the digestion is completed, the medium is changed, the culture is continued, and the passage is completed. The cells in the log phase of growth were selected for transfection and grouped prior to transfection. The cells that were not transfected were set as the blank group; as well as a negative RNA control (NC group), a miR-214 inhibitor group, and a RASSF5 mimic group. Lipofectamine 2000 and DNA were diluted and mixed according to the lipofectamine 2000 manufacturer's kit instructions. NC and miR-214p mimics, RASSFp mimics were transfected into oral squamous cell carcinoma cell lines using lipofectamine 2000, and incubated for 5 min at room temperature. Finally, the mixture was mixed with the cells and transfected at 37°C under 5% CO₂. After 48 hours of transfection, qRT-PCR was used to detect the expression of miR-214 and RASSF in KB cells transfected with miR-214, RASSF and NC.

Flow cytometry was used to detect apoptosis in each group: Cells treated with miR-214, RASSF8 and NC for 48 h were digested with trypsin, collected and fixed with 75% ethanol at a temperature of 20°C for 24 h. Then, all samples were centrifuged at 4°C and 3000 rpm for 5 min. Then the ethanol was discarded. After rinsing with PBS once, all samples were centrifuged at 4°C and 3000 rpm for 5 min. Then the supernatant was discarded. Five hundred ul of DNA Staining Solution was added to the sample and mixed well. Finally, transfer the prepared solution to the flow tube, incubate for 30 minutes in the dark. Then, detection was performed using a CyFlow Cube 8 flow cytometer.

Statistical methods

Statistical analysis was carried out using SPSS 17.0 (Beijing Strong-Vinda Information

Technology Co., Ltd.) software system. The count data was expressed by [n (%)], and the comparison between the two groups was performed by X² test. The measurement data were expressed by (X ± s), and the comparison between groups was performed with one-way ANOVA followed by post hoc Bonferroni test. Univariate and multivariate analysis of factors related to the formation of oral squamous cell carcinoma was performed as well. P < 0.05 indicated the difference was statistically significant.

Results

Expression of miR-214 and RASSF5 in oral squamous cell carcinoma, adjacent normal tissue and control group

The expression of miR-214 in oral squamous cell carcinoma was significantly higher than that of adjacent normal tissue and control group (P < 0.001). The expression level of RASSF58 in oral squamous cell carcinoma was significantly lower than that of adjuvant normal tissue and control group (P < 0.001) (Table 2 and Figure 1).

Relative expression of miR-214 and RASSF5 in each group of cells after transfection

The expression of miR-214 in the miR-214 inhibitor group was significantly lower than that in the NC group and the blank group (P < 0.001). The expression of RASSF5 in the RASSF5 mimic group was significantly higher than that in the NC group and the blank group (P < 0.001) (Figures 2 and 3).

Comparison of apoptosis of each group of cells after transfection

There was no significant difference in apoptosis rate between the miR-214 inhibitor group

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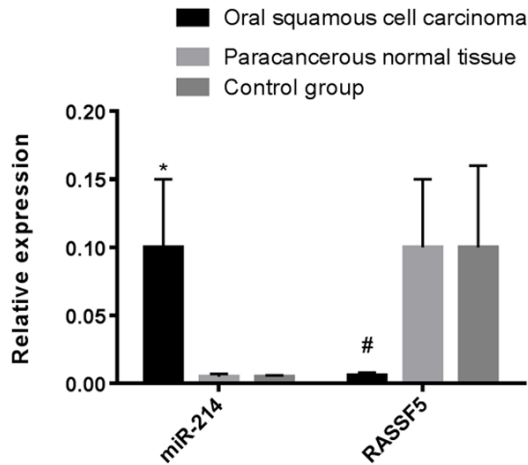


Figure 1. Expression of miR-214 and RASSF5 in oral squamous cell carcinoma, adjacent normal tissues and control group. The expression of miR-214 in oral squamous cell carcinoma was significantly higher than that of adjacent normal tissue and control group ($P < 0.001$). There was no significant difference between adjacent normal tissue and control group ($P > 0.05$). The expression of RASSF58 in oral squamous cell carcinoma was significantly lower than that of adjacent normal tissue and control group ($P < 0.001$). There was no significant difference between adjacent normal tissue and control group ($P > 0.05$). Note: * indicated that the expression of this group was significantly higher than that of the adjacent normal tissue and the control group, and the difference was statistically significant ($P < 0.001$); # indicated that the expression of this group was significantly lower than that of the adjacent normal tissue and the control group, and the difference was statistically significant ($P < 0.001$).

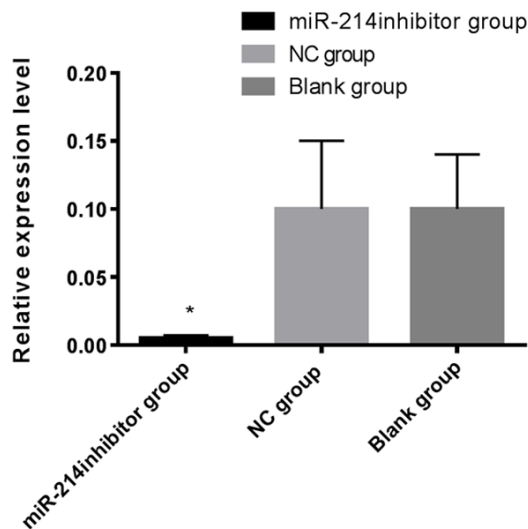


Figure 2. Relative expression of miR-214 in each group of cells after transfection. The miR-214 expression level of miR-214 inhibitor was significantly lower

than that of miR-214 in the NC group and the blank group. The difference was statistically significant ($P < 0.001$). There was no significant difference in the expression of miR-214 between the NC group and the blank group ($P > 0.05$). Note: * indicated that the expression of miR-214 in this group was significantly lower than that in the NC group and the blank group, and the difference in expression was statistically significant ($P < 0.001$).

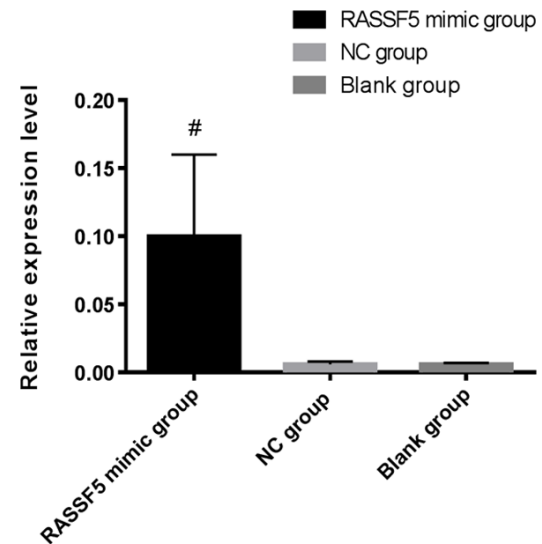


Figure 3. Relative expression of RASSF5 in each group of cells after transfection. The expression of RASSF5 in the RASSF5 mimic group was significantly higher than that in the NC group and the blank group, and the difference was statistically significant ($P < 0.001$). There was no significant difference in the expression of RASSF5 between NC group and blank group ($P > 0.05$). Note: # indicated that the expression level of RASSF5 in this group was significantly higher than that in NC group and blank group, and the difference was statistically significant ($P < 0.001$).

and the RASSF5 mimic group ($P > 0.05$), but they were significantly higher than that of the NC group and the blank group ($P < 0.05$). There was no significant difference in apoptosis rate between the NC group and the blank group (> 0.05) (Table 3).

Single factor and multi-factor analysis of oral squamous cell carcinoma formation

Single factor analysis of risk factors for oral squamous cell carcinoma: Logistic single factor analysis was performed on the risk factors associated with oral squamous cell carcinoma. It was found that the patient's gender, body

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Table 3. Comparison of the apoptosis ability of cells in each group after transfection

Group	miR-214inhibitor group	RASSF5 mimic group	NC group	Blank group	F	P
Apoptosis rate (%)	20.06 ± 3.47*	19.98 ± 4.41*	3.78 ± 0.71	3.69 ± 0.83	486.900	< 0.001

Note: * indicates that the apoptotic rate of the two groups is significantly higher than that of the NC group and the blank group (P < 0.05).

Table 4. Univariate analysis of risk factors for oral squamous cell carcinoma

Group	Oral squamous cell carcinoma (n= 45)	Control group (n = 45)	t/X ²	P
Gender			0.000	1.000
Male	25 (55.56)	25 (55.56)		
Female	20 (44.44)	20 (44.44)		
Age (years)			0.047	0.829
< 50	18 (40.00)	17 (37.78)		
≥ 50	27 (60.00)	28 (62.22)		
Body mass index (kg/m ²)				
< 19.50	10 (22.22)	10 (22.22)	0.000	1.000
19.50~25.00	18 (40.00)	17 (37.78)	0.047	0.829
≥ 25.00	17 (37.78)	18 (40.00)	0.047	0.829
History of smoking			60.960	< 0.001
Have	40 (88.89)*	3 (6.67)		
NO	5 (11.11)*	42 (93.33)		
Drinking history			78.750	< 0.001
Have	45 (100.00)*	3 (6.67)		
NO	0 (0.00)*	42 (93.33)		
Brushing times			68.140	< 0.001
< 3	40 (88.89)*	1 (2.22)		
≥ 3	5 (11.11)*	44 (97.78)		
Oral disease visits			90.000	< 0.001
< 3	0 (0.00)*	45 (100.00)		
≥ 3	45 (100.00)*	0 (0.00)		
Dental cleanliness			86.090	< 0.001
Clean	1 (2.22)*	45 (100.00)		
Not clean	44 (97.78)*	0 (0.00)		
miR-214	0.10 ± 0.05*	0.005 ± 0.001		
RASSF5	0.006 ± 0.002*	0.10 ± 0.06		

Note: * indicates that the group is compared with the control group (P < 0.001).

mass index, smoking history, drinking history, brushing condition, tooth cleaning at the time of treatment, miR-214, RASSF5, and oral visits were related to the formation of oral squamous cell carcinoma, which was a prognostic risk factor for oral squamous cell carcinoma (**Table 4**).

Multi-factor analysis of the formation of oral squamous cell carcinoma: Multivariate logistic regression analysis was performed on risk factors related to the formation of oral squamous cell carcinoma. The results showed that the history of smoking, drinking history, brushing,

dental hygiene at the time of treatment, high expression of miR-214, low expression of RASSF5, and the number of oral visits were independent prognostic risk factors for oral squamous cell carcinoma (**Table 5**).

Discussion

MiRNA plays an important role in carcinogenesis and tumor progression, and miRNA expression affects tumor cell apoptosis [15]. Studies have shown that changes in expression miR-214 have been confirmed to be associated with

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Table 5. Multivariate analysis of the formation of oral squamous cell carcinoma

Influencing factor	β	SE	Wald	P	Exp (β)	95% CI
History of smoking	1.106	0.459	5.208	0.010	2.530	1.231~5.914
Drinking history	1.097	0.902	1.604	< 0.001	3.146	1.294~9.783
Brushing times	1.505	0.924	2.658	0.005	4.775	1.584~11.730
Dental hygiene	1.625	0.977	2.505	0.002	4.813	1.752~13.531
Oral disease visits	0.828	0.689	1.484	0.042	2.056	1.018~6.284
miR-214	2.538	2.197	1.425	< 0.001	11.069	6.241~20.364
RASSF5	2.770	1.945	1.877	< 0.001	12.005	8.001~22.299

the occurrence and development of malignant tumors [16]. RASSF5 is a novel candidate tumor suppressor gene [17]. Studies have confirmed that abnormal methylation of RASSF5 gene promoter leads to silencing of gene transcription and expression, which is related to the occurrence and development of various tumors [18]. However, at present, there are few reports on miR-214 and RASSF5 genes in oral mucosal carcinogenesis. The specific mechanism of miR-214 and RASSF5 in oral squamous cell carcinoma cells is unclear. Therefore, this study explored the expression of miR-214 and RASSF5 in oral squamous cell carcinoma and the biological characteristics of oral squamous cell carcinoma.

We found that the expression level of miR-214 in oral squamous cell carcinoma was significantly higher than that of adjuvant normal tissue and control group, the expression level of RASSF5 in oral squamous cell carcinoma was significantly lower than that of adjuvant normal tissue and control group. Similar results also showed that the expression of miR-214 in oral squamous cell carcinoma was significantly higher than that of adjacent normal tissue. The RASSF5 gene is a tumor suppressor gene that is often inactivated in human tumors [13]. A large number of studies have shown that the RASSF5 gene is widely expressed in normal tissues, while the expression in tumor tissues is down-regulated [19]. Thus, we believe that miR-214 is overexpressed in oral squamous cell carcinoma, and the RASSF5 gene is under-expressed in oral squamous cell carcinoma.

Then, through transfecting the relative expression levels of miR-214 and RASSF5 in each group of cells, the survival of oral cancer KB cells was observed. We found that there was no significant difference in apoptosis rate between

the two groups in the miR-214 inhibitor group and the RASSF5 mimic group, but they were significantly higher than those in the NC group and the blank group. There was no significant difference in the apoptotic rate between the NC group and the blank group. Rapid apoptosis of cancer cells can alleviate the growth of

tumors and facilitate the recovery of the immune mechanism of the body [20, 21]. Some studies on the biological function of oral cancer KB cells have shown that regulating the expression of miR-214 or RASSF5 can significantly affect the biological functions of proliferation, invasion and apoptosis of oral cancer KB cells [22, 23]. Thus, we hypothesized that inhibition of miR-214 and up-regulation of RASSF5 expression would have a greater effect on accelerating the apoptosis rate of oral cancer KB cells.

Finally, we found that the patient's gender, body mass index, smoking history, drinking history, tooth brushing conditions, dental hygiene during treatment, miR-214, RASSF5, and number of oral visits were related to the formation of oral squamous cell carcinoma. Multi-variate Logistic regression analysis showed that the history of smoking, drinking history, brushing condition, dental hygiene during treatment, miR-214, RASSF5, and oral diseases were all independent risk factors for the prognosis of oral squamous cell carcinoma. Therefore, we believe that the regulation of miR-214 and RASSF5 expression show great reference value for gene-targeted therapy of clinical oral squamous cell carcinoma. At present, relevant clinical studies have shown that the development of oral cancer is inhibited by regulating changes in miR-214 and RASSF5 expression [24, 25]. This is a good complement to this study.

In this study, there are some shortcomings. For example, we only monitored the gene expression levels of miR-214 and RASSF5 during the study, and did not monitor the RASSF5 protein expression levels. Second, there are some other miRNAs that may be involved in the development of oral squamous cell carcinoma, which needs to be considered in future studies. The

inclusion of factors related to the formation of oral squamous cell carcinoma may not be sufficient. We will continue to pay attention to studies related to oral cancer, and detect the protein expression levels of miR-214 and RASSF5 under appropriate experimental conditions to continuously enrich the study.

In summary, inhibition of miR-214 expression and up-regulation of RASSF5 expression can significantly promote the apoptosis of oral cancer KB cells. While smoking history, drinking history, tooth brushing conditions, dental hygiene during treatment, high expression of miR-214, low expression of RASSF5, and oral diseases were all independent risk factors for prognosis of oral squamous cell carcinoma.

Disclosure of conflict of interest

None.

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References

- [1] Mislav AR, Wildes TM, Kanesvaran R, Baldini C, Holmes HM, Nightingale G, Coolbrandt A and Biganzoli L. Adherence to oral cancer therapy in older adults: the international society of geriatric oncology (SIOG) taskforce recommendations. *Cancer Treat Rev* 2017; 57: 58-66.
- [2] Vaish R, Gupta S and D'Cruz AK. Elective versus therapeutic neck dissection in oral cancer. *N Engl J Med* 2015; 373: 2477.
- [3] Chen L, Zhang S, Wu J, Cui J, Zhong L, Zeng L and Ge S. circRNA_100290 plays a role in oral cancer by functioning as a sponge of the miR-29 family. *Oncogene* 2017; 36: 4551-4561.
- [4] 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 tumorigenesis mechanism. *Mol Cancer* 2016; 15: 28.
- [5] Wu Y, Zhang L, Zhang L, Wang Y, Li H, Ren X, Wei F, Yu W, Liu T, Wang X, Zhou X, Yu J and Hao X. Long non-coding RNA HOTAIR promotes tumor cell invasion and metastasis by recruiting EZH2 and repressing E-cadherin in oral squamous cell carcinoma. *Int J Oncol* 2015; 46: 2586-2594.
- [6] Lee AY, Fan CC, Chen YA, Cheng CW, Sung YJ, Hsu CP and Kao TY. Curcumin inhibits invasiveness and epithelial-mesenchymal transition in oral squamous cell carcinoma through reducing matrix metalloproteinase 2, 9 and modulating p53-E-cadherin pathway. *Integr Cancer Ther* 2015; 14: 484-490.
- [7] Zhou X, Liu S, Cai G, Kong L, Zhang T, Ren Y, Wu Y, Mei M, Zhang L and Wang X. Long non coding RNA MALAT1 promotes tumor growth and metastasis by inducing epithelial-mesenchymal transition in oral squamous cell carcinoma. *Sci Rep* 2015; 5: 15972.
- [8] Cao M, Ouyang J, Liang H, Guo J, Lin S, Yang S, Xie T and Chen S. Regional gene expression profile comparison reveals the unique transcriptome of the optic fissure. *Invest Ophthalmol Vis Sci* 2018; 59: 5773-5784.
- [9] Sritippho T, Chotjumlong P and Iamaroon A. Roles of human papillomaviruses and p16 in oral cancer. *Asian Pac J Cancer Prev* 2015; 16: 6193-6200.
- [10] Giraldez MD, Chevillet JR and Tewari M. Droplet digital PCR for absolute quantification of extracellular microRNAs in plasma and serum: quantification of the cancer biomarker hsa-miR-141. *Methods Mol Biol* 2018; 1768: 459-474.
- [11] Penna E, Orso F and Taverna D. miR-214 as a key hub that controls cancer networks: small player, multiple functions. *J Invest Dermatol* 2015; 135: 960-969.
- [12] Hayes J, Peruzzi PP and Lawler S. MicroRNAs in cancer: biomarkers, functions and therapy. *Trends Mol Med* 2014; 20: 460-469.
- [13] Yoon AJ, Wang S, Shen J, Robine N, Philipone E, Oster MW, Nam A and Santella RM. Prognostic value of miR-375 and miR-214-3p in early stage oral squamous cell carcinoma. *Am J Transl Res* 2014; 6: 580-592.
- [14] Feng X, Jiang J, Shi S, Xie H, Zhou L and Zheng S. Knockdown of miR-25 increases the sensitivity of liver cancer stem cells to TRAIL-induced apoptosis via PTEN/PI3K/Akt/Bad signaling pathway. *Int J Oncol* 2016; 49: 2600-2610.
- [15] Wen Z, Zhang Y, Wang X, Zeng X, Hu Z, Liu Y, Xie Y, Liang G, Zhu J, Luo H and Xu B. Novel 3',5'-diprenylated chalcones inhibited the proliferation of cancer cells in vitro by inducing cell apoptosis and arresting cell cycle phase. *Eur J Med Chem* 2017; 133: 227-239.
- [16] Phatak P, Byrnes KA, Mansour D, Liu L, Cao S, Li R, Rao JN, Turner DJ, Wang JY and Donahue JM. Overexpression of miR-214-3p in esophageal squamous cancer cells enhances sensitivity to cisplatin by targeting survivin directly and indirectly through CUG-BP1. *Oncogene* 2016; 35: 2087-2097.
- [17] Liao TJ, Tsai CJ, Jang H, Fushman D and Nussinov R. RASSF5: an MST activator and tumor

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- suppressor in vivo but opposite in vitro. *Curr Opin Struct Biol* 2016; 41: 217-224.
- [18] Sun Z, Liu G and Xu N. Does hypermethylation of CpG island in the promoter region of the E-cadherin gene increase the risk of lung cancer? A meta-analysis. *Thorac Cancer* 2019; 10: 54-59.
- [19] Liao TJ, Jang H, Tsai CJ, Fushman D and Nussinov R. The dynamic mechanism of RASSF5 and MST kinase activation by ras. *Phys Chem Chem Phys* 2017; 19: 6470-6480.
- [20] de la Torre C, Dominguez-Berrocal L, Murguia JR, Marcos MD, Martinez-Manez R, Bravo J and Sancenon F. Polylysine-capped mesoporous silica nanoparticles as carrier of the C9h peptide to induce apoptosis in cancer cells. *Chemistry* 2018; 24: 1890-1897.
- [21] Wong CH, Iskandar KB, Yadav SK, Hirpara JL, Loh T and Pervaiz S. Correction: simultaneous induction of non-canonical autophagy and apoptosis in cancer cells by ROS-dependent ERK and JNK activation. *PLoS One* 2016; 11: e0159352.
- [22] Li TK, Yin K, Chen Z, Bao Y and Zhang SX. MiR-214 regulates oral cancer KB cell apoptosis through targeting RASSF5. *Genet Mol Res* 2017; 16.
- [23] Cao W, Zheng W and Chen T. Ruthenium polypyridyl complex inhibits growth and metastasis of breast cancer cells by suppressing FAK signaling with enhancement of TRAIL-induced apoptosis. *Sci Rep* 2015; 5: 9157.
- [24] Liu X, Yue P, Chen S, Hu L, Lonial S, Khuri FR and Sun SY. The proteasome inhibitor PS-341 (bortezomib) up-regulates DR5 expression leading to induction of apoptosis and enhancement of TRAIL-induced apoptosis despite up-regulation of c-FLIP and survivin expression in human NSCLC cells. *Cancer Res* 2007; 67: 4981-4988.
- [25] Tian C, Wu H, Li C, Tian X, Sun Y, Liu E, Liao X and Song W. Downregulation of FoxM1 by miR-214 inhibits proliferation and migration in hepatocellular carcinoma. *Gene Ther* 2018; 25: 312-319.