

Original Article

Decreased expression of miR-153-3p in oral squamous cell carcinoma contributes to cell migration and invasion

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Abstract: Emerging evidence suggests that dysregulation of miR-153-3p may contribute to tumor development and progression in several types of human cancers. However, its role in oral squamous cell carcinoma (OSCC) is still unknown. MicroRNA profiles were obtained via miRNA microarray in OSCC tissues and adjacent normal tissues, and quantitative RT-PCR was performed to further validate microarray data. Subsequently, associations of miR-153-3p with Nrf2 were assessed using Pearson correlation. The clinical significance of miR-153-3p in OSCC was analyzed using receiver operating characteristic (ROC) curve. *In vitro*, wound healing and Transwell assays, as well as western blot were conducted to determine the biological function of miR-153-3p in CAL-27 cells transfected with miRNA mimics, inhibitor, or the empty vector. The miR-153-3p target was validated through a luciferase reporter assay, RT-PCR, and western blot. miRNA microarray revealed 18 aberrant miRNAs in OSCC tissues, including miR-153-3p, which was significantly reduced ($P < 0.01$); miR-153-3p expression was lower in patients with aggressive tumors ($P < 0.01$). Additionally, Nrf2 mRNA amounts, which were higher ($P < 0.01$) in OSCC tissues, showed a negative correlation with miR-153-3p levels ($r = -0.74$, $P < 0.001$). Moreover, miR-153-3p inhibited cell migration and invasion abilities *in vitro* ($P < 0.01$) and Nrf2 was confirmed as a target of miR-153-3p. MiR-153-3p is downregulated in human OSCC tissues, and is associated with tumorigenesis of oral squamous cells; in agreement, miR-153-3p inhibits CAL-27 cell migration and invasion by downregulating Nrf2.

Keywords: Oral squamous cell carcinoma, miR-153-3p, invasion, Nrf2

Introduction

Oral cavity squamous cell carcinoma (OSCC) is the eighth most common cancer worldwide and poses a significant health threat [1]. In 2015, 45780 patients were newly diagnosed with oral cancer in the United States [2]. Surgery and radiation therapy (RT) represent the standards of care for early-stage and locally advanced resectable lesions within the oral cavity [3]. Although the functional outcome after primary surgical management is often quite good, diagnosed patients often face a low five-year survival rate of 58%, which has remained unchanged over the past three decades despite recent treatment advances [4]. Improvement in patient survival rate requires a better understanding of the initiation and progression of OSCC. The various mechanisms underlying oral carcinogenesis have been extensively studied in the past [5, 6], but remain incompletely understood.

MicroRNAs (miRNAs), as promising biomarkers, can bring insights into tumorigenesis of oral cancers. MiRNAs are a class of endogenous small non-coding RNAs that regulate target mRNAs predominantly by binding to the 3' untranslated region (UTR) at the post-transcriptional level [7, 8]. Increasing evidence indicates that microRNAs play a crucial role in regulating fundamental processes such as cell cycle, differentiation, metastasis, angiogenesis, and apoptosis in OSCC [9-12]. For instance, miR-320a plays an important role in the acquisition of an aggressive and/or metastatic phenotype in tumor budding cells of tongue squamous cell carcinoma, and decreased miR-320a promotes invasion and metastasis by targeting Suz12 expression [13]. MiR-146a levels were lower in OSCC tissues and cell lines. The loss of miR-146a expression is associated with aggressive oral cancer, and miR-146a suppresses oral cancer cell aggressiveness *in vitro* and *in vivo*.

through its binding to the 3'-UTR of SOX2 mRNA [14]. In addition, Jamali et al. found that miR-153 was markedly downregulated in cells that underwent epithelial-mesenchymal transition by targeting SNAIL1 and ZEB2 in human epithelial cancer [15]. However, the functional role of miR-153-3p in OSCC development and progression has not been experimentally established. Therefore, this study aimed to assess the role of miR-153-3p in OSCC, evaluating clinical data, and exploring its biological functions in OSCC.

Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) is a key transcription factor that regulates the expression of over a hundred cytoprotective and antioxidant genes that provide cellular protection from reactive oxygen species [16]. Dysregulation of the Nrf2 system is implicated in various disease states such as lung [17], ovarian [18], prostate [19], and breast cancer [20]. Nrf2 expression is also frequently higher in head and neck squamous cell carcinoma (HNSCC) and therefore, may be used as a useful biomarker for the early detection of HNSCC [21]. We searched for direct targets of miR-153-3p in TargetScan and miRBase, and established Nrf2 as a potential target, driving us to assess whether miR-153-3p could modulate Nrf2 expression in OSCC cells. Recent studies have demonstrated that Kelch-like ECH-associated protein 1 (Keap1) is highly effective in regulating Nrf2 expression through its binding with the latter. Therefore, we also studied its expression.

In the present study, we identified 18 aberrantly expressed miRNAs in OSCC tissues, and miR-153-3p showed the largest downregulation. Further assays demonstrated that decreased miR-153-3p levels were proportional to OSCC severity. Besides, we found that the loss of miR-153-3p promotes migration and invasion of CAL-27 by regulating the expression of Nrf2. This study established the tumor suppressing function of miR-153-3p, which may represent a new biomarker and therapeutic target for the treatment of OSCC.

Materials and methods

Patients and tissue samples

Surgical samples of 28 human primary OSCC tissues and corresponding non-tumor tissues were obtained during the surgical resection of

OSCC patients, immediately frozen in liquid nitrogen, and stored at -80°C. Patients with OSCC enrolled from May 2013 to January 2015 at the Center of Stomatology, and were studied with the approval of China-Japan Friendship Hospital. Inclusion criteria were: a) primary OSCC patients confirmed by pathology following surgery; b) patients that did not undergo any treatment prior to surgery; c) clinical and pathological data of these patients were complete. Exclusion criteria: a) family history of cancers; b) patients suffering from other chronic system diseases; c) patients unwilling to participate in this study, who refuse or are unable to receive definitive treatment. Histological grading was performed according to the World Health Organization classification. All patients were treated according to the clinical guideline for standardized diagnosis and treatment of oral and maxillofacial malignancy in China. Written informed consents were obtained from all patients.

MicroRNA microarrays

Tumor tissues and matched normal tissues were randomly chosen for microarrays. Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, CA) and further purified with a Dynabeads mRNA DIRECT Kit (Invitrogen). Samples with RNA integrity number (RIN) > 8 were processed for hybridization. Total RNA was then amplified, labeled, and hybridized to the miRNA microarray, according to the instructions provided by CapitalBio Corporation. Scanned images were then imported into a confocal LuxScan scanner (CapitalBio Corp). The SpotData Pro software (CapitalBio Corp) was used for data analysis. The raw data were normalized using an Expression Console.

RNA extraction and quantitative real-time PCR

Total RNA was isolated from tumor and tumor-free tissues as well as cultured cells using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. First strand cDNA was synthesized according to the manufacturer's instructions using TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, USA). The reverse transcription condition was 25°C for 10 min, 37°C for 120 min, and 85°C for 5 min. Then qRT-PCR, using SYBR-Green detection chemistry (Sigma-Aldrich, USA), was performed on a Roche Lightcycler

Table 1. TaqMan stem-loop primers for reverse transcription PCR and forward and reverse primers for real-time PCR

Accession	ID		Sequence
MIMAT0000445	Has-miR-126	Sequence	UCGUACCGUGAGUAAUAAUGCG
		TaqMan primer	GTCGTATCCAGTGCCTGTCGTGGAGTCGGCAATTGCACTGGATACGACTCAGAC
		PCR-F	TCGTACCGTGAGTAAT
MIMAT0000439	Has-mir-153-3P	Sequence	UUGCAUAGUCACAAAAGUGAUC
		TaqMan primer	GTCGTATCCAGTGCCTGTCGTGGAGTCGGCAATTGCACTGGATACGACGATCAC
		PCR-F	GGGTTGCATAGTCACAAA
MIMAT0000256	Has-mir-181a	Sequence	AACAUUCAACGCUGUCGGUGAGU
		TaqMan primer	GTCGTATCCAGTGCCTGTCGTGGAGTCGGCAATTGCACTGGATACGACACTCAC
		PCR-F	AACATTCAACGCTGTCG
MIMAT0000430	Has-mir-138	Sequence	AGCUGGUGUUGUGAAUCAGGCCG
		TaqMan primer	GTCGTATCCAGTGCCTGTCGTGGAGTCGGCAATTGCACTGGATACGACCGGCCT
		PCR-F	AGCTGGTGTGTGAATC
M14486	U6	PCR-F	CTCGCTTCGGCAGCAC
		PCR-R	AACGCTTCACGAATTTGCGT
		PCR-F	CTGGAACGGTG AAGGTGACA
	β -actin	PCR-F	AAGGGACTTCTGTACAATGCA
		PCR-R	CCAGCACATCCAGTCAGAAA
		PCR-F	CGTAGCCGAAGAAACCTCAT
	Nrf2	PCR-F	GTACCTGTTGAGGCACTTTT
		PCR-R	GCTTTGGACTTCTTTTGAGA
		PCR-F	
	Keap1	PCR-F	
		PCR-R	
		PCR-F	

480 Real Time PCR System (Applied Biosystems, CA, USA). The reaction mixtures were incubated at 95°C for 2 min followed by 40 cycles of denaturation at 95°C (15 s), and annealing at 60°C (60 s). All PCR reactions were carried out in triplicate. Relative quantification of miRNA and mRNA expression levels was calculated using the $2^{-\Delta\Delta C_t}$ method. The raw data were presented as the relative quantity of target miRNA and mRNA, normalized with respect to U6 and β -actin, respectively. The primers used for real-time PCR are shown in **Table 1**.

Cell culture and transient transfection

CAL 27 was purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA) and grown in Dulbecco's MEM (Invitrogen, USA) supplied with 1% of 100 U/mL penicillin and streptomycin (Invitrogen, CA). Cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

miR-153-3p mimics (50 nM, GenePharma Co., Ltd), miR-153-3p inhibitor (100 nM, GenePharma Co., Ltd), and their negative controls were transfected into CAL-27 cells using Lipofectamine® 2000 Transfection Reagent (Thermo

Fisher) for downstream arrays. Transfected efficiency was determined through fluorescence detection. RT-qPCR showed that miR-153-3p increased more than 1000 fold after transfection with mimics, and was reduced by about 1 fold by the inhibitor.

Western blot

CAL-27 cells were washed with PBS and subsequently disrupted in lysis buffer (Beyotime, China) for 30 min. The lysates were then centrifuged at 10000× g for 5 min to remove insoluble material. The BCA Protein Assay kit (Beyotime, China) was used for protein quantitation. Equal amounts of protein were separated by 10% sodium dodecylsulfate-polyacrylamide gel electrophoresis and transferred onto PVDF membranes blocked with 5% non-fat milk. Then, the membranes were functionalized with relevant antibodies specific to Nrf2 (1:500, Abcam), Keap1 (1:200, Abcam), EZH2 (1:2000, Millipore), and MMP9 (1:2000, Abcam), followed by incubation with secondary antibodies (Merck, 1:5000) for 1.5 h at room temperature. Immunoreactive bands were detected using the ECL detection system; ImageJ software 6.0 was used to analyze the expression of target

MiR-153-3p inhibits cell migration and invasion in OSCC

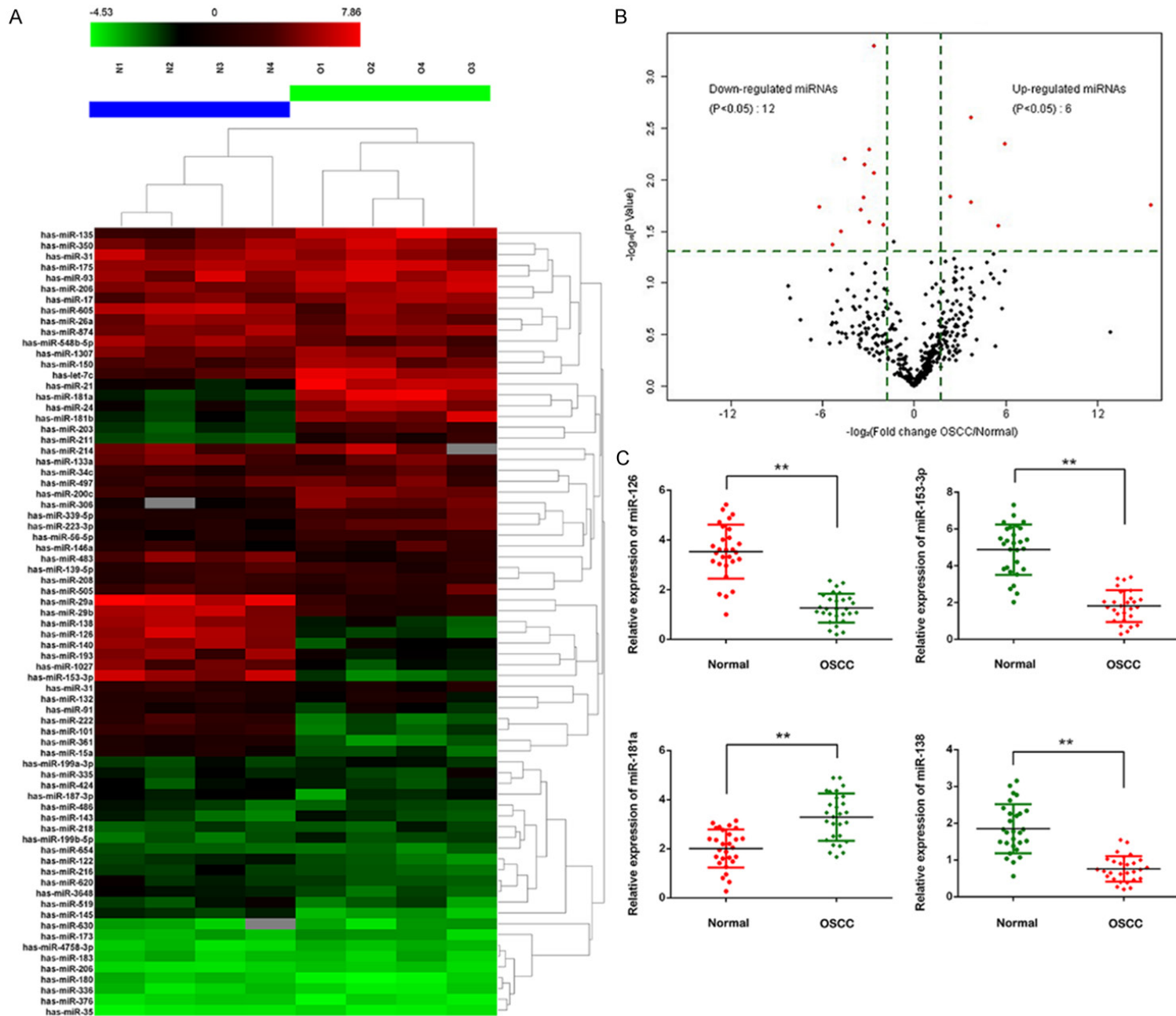


Figure 1. MiRNA signature of OSCC tissue samples. Four paired specimens were randomly chosen from 28 OSCC patients. A: Heat map of all miRNA expression differences between tumor tissues and corresponding normal tissues (The green bar represents downregulation and the red bars upregulation). B: Volcano plot showing the expression of miRNAs in OSCC tissues (red dot represents $P < 0.05$ and fold change > 1.5). C: RT-PCR of four miRNAs was used to validate the microarray data.

Table 2. Clinicopathologic factors and the expression of miR-153-3p in patients with oral squamous cell carcinoma

Characteristic	Cases	MiR-153-3p		P value
		Low expression	High expression	
Age(years)				0.573
< 60	17	10	7	
≥ 60	11	7	4	
Gender				0.684
Male	15	6	9	
Female	13	7	6	
Location				0.498
Tongue	10	6	4	
Lip	7	1	6	
Gingiva	6	3	3	
Buccal mucosa	2	2	0	
Mouth floor	2	0	2	
Alveolar ridge	1	1	0	
Differentiation				0.055
Well	13	8	5	
Moderate	9	6	3	
Poor	6	3	3	
Clinical stage				0.019
I,0	15	8	7	
III,50	13	10	3	
Lymphatic metastasis				< 0.001
Negative	19	9	10	
Positive	9	7	2	

proteins in comparison to β -actin (1:5000, Sigma).

Cell migration and invasion assay

Wound healing assays were performed when the cells reached 90% confluence in six-well plates. A wound was generated by dragging a pipette tip through the monolayer, and plates were washed using pre-warmed PBS to remove cellular debris. The plates were examined and photographed under a microscope at 0 h, and 48 h after wounding. Wound gaps were measured at each time point. Each cell condition was assayed in triplicate wells.

Transwell chambers coated with diluted matrix were used. After transfection, cells were

seeded into the upper chambers, with 5% fetal bovine serum (FBS) used as a chemoattractant in the lower wells. OSCC cells were allowed to migrate for 24 h before the medium was removed, and chambers were washed twice with PBS. Non-invading cells in the upper chamber were gently removed and those that migrated to the lower surface were stained with 0.1% crystal violet (Amresco, USA). The numbers of invaded cells were assessed using a light microscope (Axiovert 200 inverted microscope, Zeiss, Germany) and counted in three fields at $\times 200$ magnification.

Luciferase reporter assay

To assess whether miR-153-3p targets the 3'-UTR of Nrf2, the sequence containing the 3'-UTR of FLOT1 was cloned downstream of the luciferase reporter in the pmirGLO dual-luciferase miRNA target expression vector (Promega, USA) between the SacI and SalI

sites, and verified by sequencing. Mutations in binding sites were introduced by site-directed mutagenesis, and a mutant (Mut) construct was obtained. For luciferase reporter assay, CAL-27 cells were cultured in 24-well plates and co-transfected with 50 nM miR-153-3p or NC and 100 ng of the luciferase vector (pmirGLO), using Lipofectamine 2000. Cells were harvested after 48 h of transfection and luciferase activity was assessed in each sample with a luciferase assay kit (Promega).

Statistical analysis

Data are mean \pm standard deviation from three independent experiments. Between-group differences were analyzed by the two-tailed

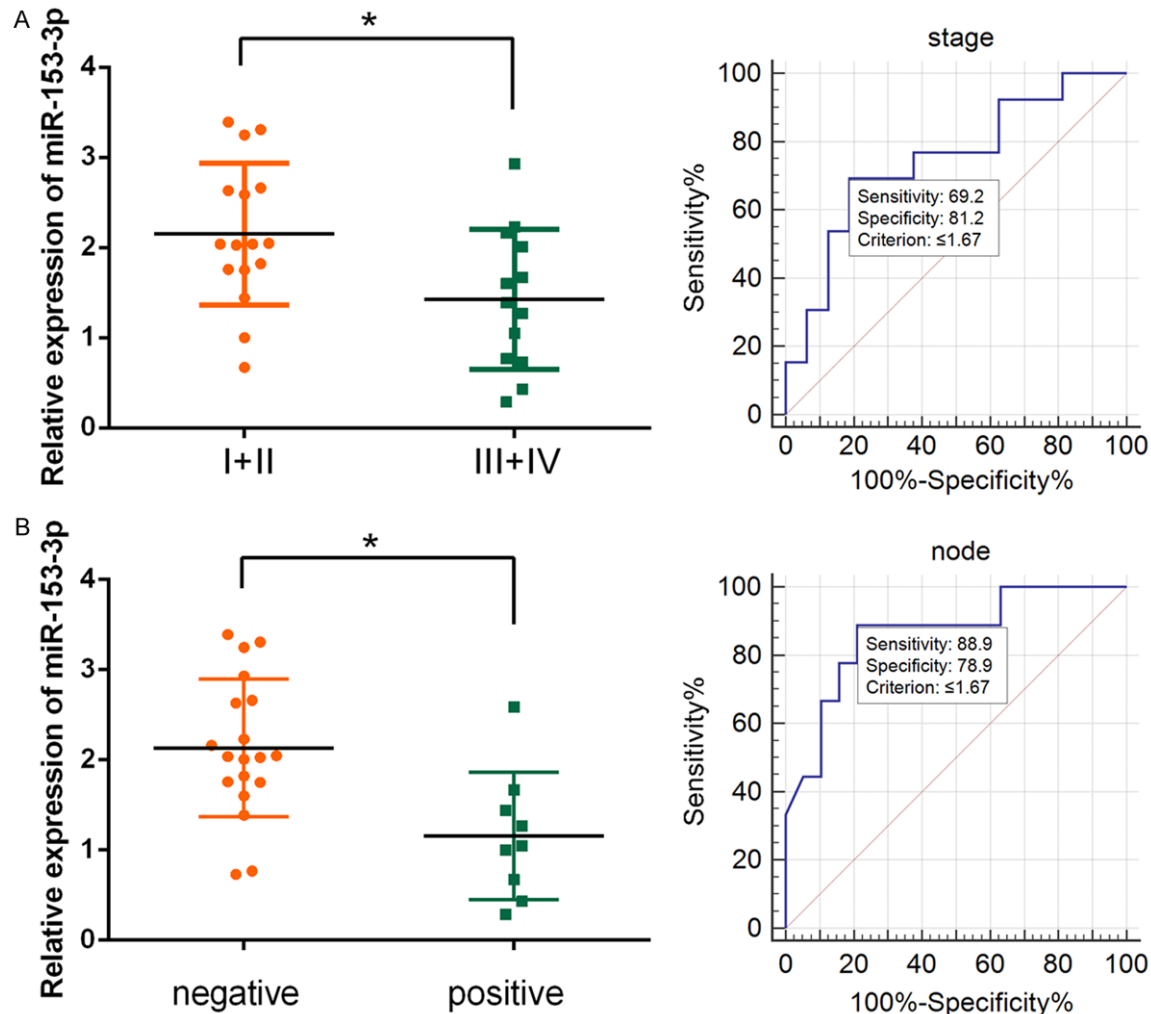


Figure 2. Expression of miR-153-3p and its ROC analysis for severity evaluation. A: Relative expression of miR-153-3p in groups of OSCC with different clinical stages and its ROC curve: AUC 0.75 (95% CI, 0.55-0.89), $P = 0.011$; Cut-off value is 1.67, sensitivity 69.2% and specificity 81.3%. B: Relative expression of miR-153-3p in groups of OSCC with or without lymphatic metastasis and ROC analysis of miR-153-3p for predicting lymph node metastasis; AUC 0.86 (95% CI, 0.68-0.96), $P < 0.001$; Cut-off value is 1.67, sensitivity 88.9% and specificity 79.0%.

Student t test. The associations between clinicopathological factors and miR-153-3p levels were analyzed using the chi-square test and Fisher's exact test, with the SPSS18.0 software. $P < 0.05$ was considered statistically significant. GraphPad Prism 5.0 was used for graphing.

Results

MiR-153-3p is downregulated in OSCC

To obtain the miRNA signature of OSCC, miRNA expression levels were evaluated by microRNA microarrays among 4 paired specimens randomly chosen from 28 tissue samples. Interestingly,

18 miRNAs exhibited significantly different expression levels ($P < 0.05$ and fold change ≥ 1.5), including 12 upregulated miRNAs and 6 suppressed ones (**Figure 1A**). The volcano plot shows the changes in these expressions in **Figure 1B**. MiR-153-3p showed the largest downregulation in OSCC tissues compared with adjacent non-tumor tissues. To confirm the expression pattern of miRNAs in OSCC provided by microarray, four of the miRNAs showing the most aberrant expressions, including miR-126, miR-153-3p, miR-181a, and miR-138, were assessed by qRT-PCR. As expected, although fold changes differed, the expression trends were consistent with the microarray findings. To

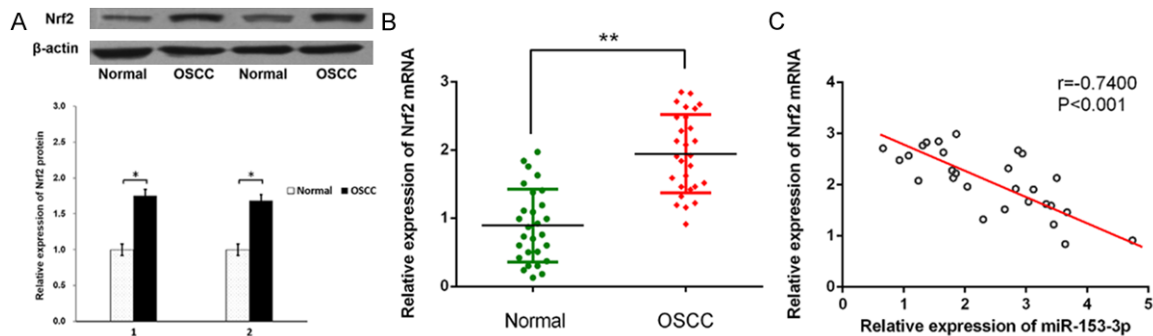


Figure 3. An inverse correlation between Nrf2 and miR-153-3p expressions in OSCC tissues. A: The expression of Nrf2 protein was significantly increased in OSCC tissues compared with normal tissues (* $P < 0.01$). B: Nrf2 mRNA expression levels were significantly higher in OSCC tissues (* $P < 0.01$). C: Pearson correlation was used to analyze the relationship between Nrf2 mRNA and miR-153-3p.

our knowledge, among these four miRNAs, the biological function of miR-153-3p has not yet been investigated in OSCC, making it a valuable subject with in the scope of this study.

Decreased expression of miR-153-3p in OSCC is correlated with disease severity

To further investigate the role of miR-153-3p expression in OSCC progression, clinicopathological factors were analyzed in relation to miR-153-3p levels in OSCC samples. Patients were subdivided into two groups according to clinicopathological features, and ROC curve analysis was used to determine the associations between miR-153-3p and clinical indexes. Details are showed in **Table 2**. The results indicate that there was no significant difference between low expression of miR-153-3p and age ($P = 0.573$), gender ($P = 0.684$), or tumor location ($P = 0.498$). However, the P value resulting from the comparison between miR-153-3p and histology/differentiation was 0.055 and an increase in sample size may give rise to a different conclusion. Interestingly, the expression of miR-153-3p was significantly lower in patients with lymphatic metastasis and higher clinical stage. The AUC value for miR-153-3p was 0.86 (95% CI, 0.68-0.96) ($P < 0.001$; **Figure 2A**) in predicting lymph node metastasis, with a cut-off value of 1.67, and its sensitivity and specificity were 88.9% and 79.0%, respectively. Furthermore, for predicting tumor stage, the AUC value was 0.75 (95% CI, 0.55-0.89; $P = 0.011$) (**Figure 2B**), with 69.2% sensitivity and 81.3% specificity. Taken together, these results demonstrate that miR-153-3p represents a

good candidate for a future application toward evaluating OSCC severity.

Inverse expression patterns of Nrf2 and miR-153-3p in OSCC tissues

It is essential to elucidate the potential molecular mechanisms involving miR-153-3p in oral squamous cell carcinoma due to its important role. We predicted the putative target genes of miR-153-3p in human cells using the tools TargetScan, miRBase, miRDB, and miRWalk. Among the predicted candidates, Nrf2 was of interest in this study, as it is known to be overexpressed in many cancers, including OSCC, with an involvement in tumor progression [22-24]. Therefore, the expression of Nrf2 was assessed in OSCC tissue samples using western blot and qRT-PCR. As shown in **Figure 3A**, **3B**, Nrf2 levels in OSCC tissues were significantly higher than those detected in the corresponding normal tissues; meanwhile, the results obtained from the Pearson correlation analysis indicated that Nrf2 mRNA expression was negatively correlated with miR-153-3p levels ($r = -0.74$, $P < 0.001$) (**Figure 3C**). These results supported our hypotheses and motivated *invitro* experiments to further validate Nrf2 as a direct target of miR-153-3p.

MiR-153-3p suppresses CAL-27 cell migration and invasion in vitro

To investigate the functional role of miR-153-3p in migration and invasion of OSCC, we used wound healing and Transwell assays. As shown in **Figure 4A**, miR-153-3p expression was remarkably higher when transfecting CAL-27 cells

MiR-153-3p inhibits cell migration and invasion in OSCC

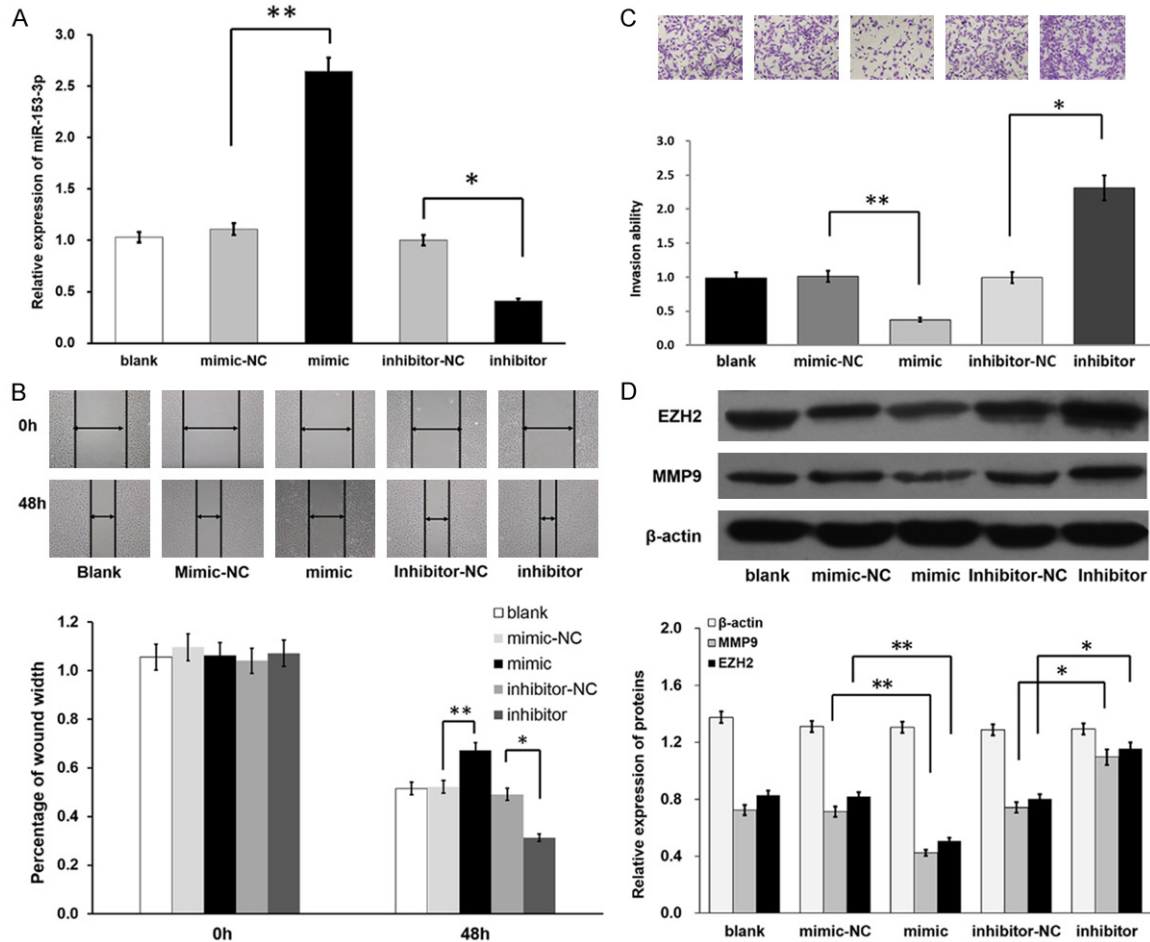


Figure 4. MiR-153-3p inhibited CAL-27 cell migration and invasion *in vitro*. A: The relative expression of miR-153-3p in CAL-27 cells transfected with miR-153-3p mimics, mimic-NC, miR-153-3p inhibitor, and inhibitor-NC. B: Wound healing assay on CAL-27 cells from different groups. C: Transwell invasion assay was used to determined invasion ability of CAL-27 cells within groups. D: The different expressions of migration and invasion-related proteins are shown using western blot. * $P < 0.01$ vs. inhibitor-NC, ** $P < 0.01$ vs. mimic-NC.

with mimics, as assessed by qPCR. Contrastingly, the expression was statistically down-regulated with miRNA inhibitor. Interestingly, the results obtained from the wound healing assay showed that miR-153-3p mimics impaired the migration of CAL-27 cells, but that the miR-153-3p inhibitor enhanced lateral cell migration (**Figure 4B**, $P < 0.01$). Similarly, the cell invasion ability was suppressed with over-expression of miR-153-3p and promoted when miR-153-3p expression was downregulated, as assessed by Transwell assay (**Figure 4C**, $P < 0.01$). Furthermore, the western blot results showed increased expressions of migration and invasion-related proteins when CAL-27 cells were treated with miR-153-3p mimics, whereas the opposite phenomenon occurred

with the inhibitor (**Figure 4D**, $P < 0.01$). Taken together, these findings suggest that miR-153-3p suppresses CAL-27 cell migration and invasion abilities *in vitro*.

MiR-153-3p directly downregulates Nrf2 in OSCC cells

We found that Nrf2 was negatively correlated with miR-153-3p in OSCC tissue samples in this study. Therefore, a bioinformatics analysis was further conducted. The putative complementary sequence of miR-153-3p was identified in the 3'-UTR of Nrf2 mRNA, as illustrated in **Figure 5A**. It is established that Nrf2 expression can be regulated by Keap1-dependent and Keap1-independent mechanisms. Therefore, to explore whether miR-153-5p regulates the

MiR-153-3p inhibits cell migration and invasion in OSCC

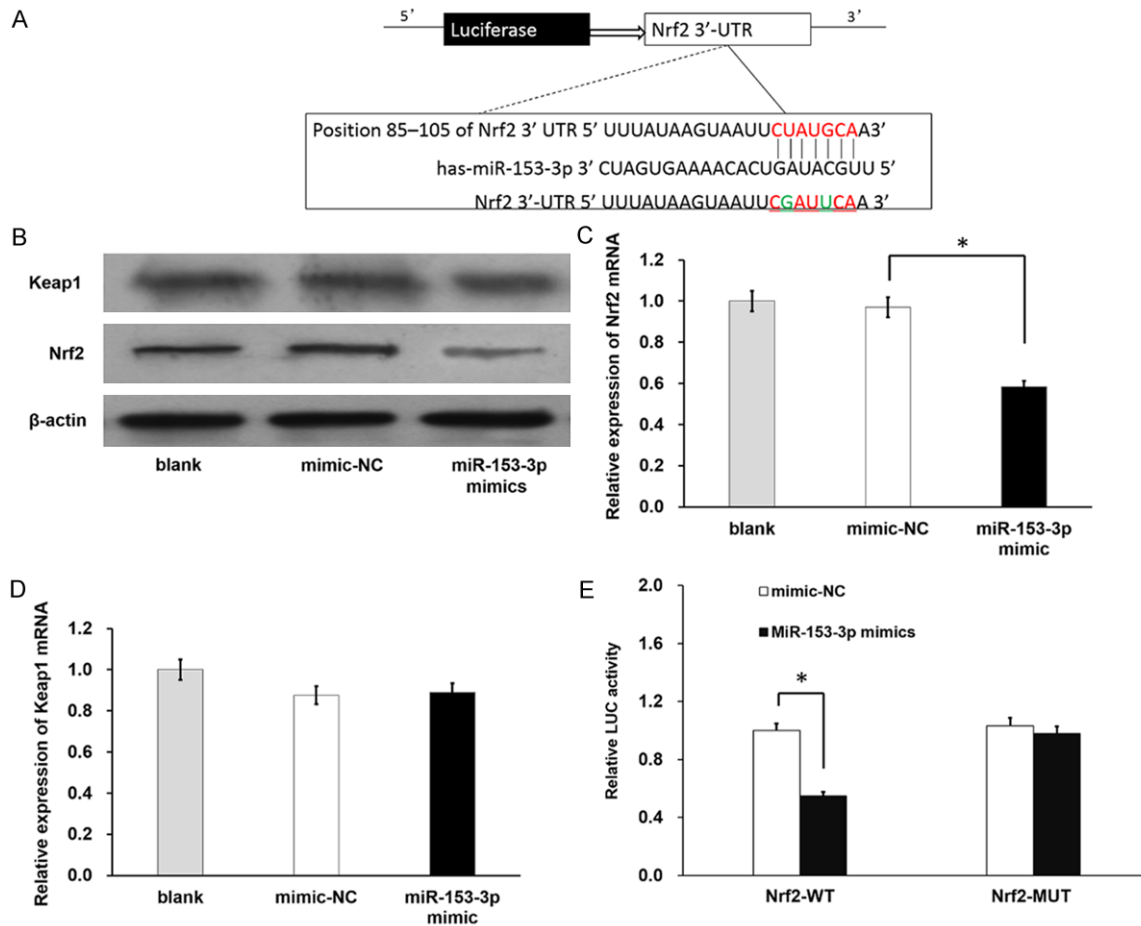


Figure 5. MiR-153-3p directly targeted Nrf2 in CAL-27 cells. A: Putative sites on which miR-153-3p binds the 3'-UTR of Nrf2 are shown. B: Western blot showing the altered expression of Nrf2 and Keap1 proteins in the different groups. β -actin was used as a loading control. C-D: RT-PCR was performed to detect the relative expressions of Nrf2 and Keap1. E: CAL-27 cells transfected with wild type (WT) or mutant (MUT) Nrf2 mRNA 3'-UTR reporter plasmids with vehicle control or miR-153-3p mimics. The luciferase activities were measured 48 h after transfection. MiR-153-3p inhibited luciferase activity in the Nrf2 containing 3'-UTR of WT binding sites, in contrast to 3'-UTR of MUT binding sites in Nrf2 mRNA.

expression of Nrf2 only or Nrf2 and Keap1, their expressions were assessed in miR-153-3p mimics, mimic-NC, and blank groups. Interestingly, lower Nrf2 protein levels were observed in the miR-153-3p mimics group compared with the other two (Figure 5B, $P < 0.01$), whereas there were no statistically significant changes in Keap1 protein expressions. Subsequently, Nrf2 and Keap1 mRNA amounts were evaluated by qRT-PCR in CAL-27 cells. In agreement with the western blot results, Nrf2 mRNA levels were lower in the miR-153-3p overexpression group ($P < 0.01$) and Keap1 mRNA levels showed no obvious alteration, as shown in Figure 5C, 5D. A luciferase reporter assay was then performed to further validate the interaction between miR-153-3p and Nrf2.

As shown in Figure 5E, the activity of the luciferase reporter gene containing the miR-153-3p combined site was significantly reduced following transfection with mimics ($P < 0.01$), while the activity of the luciferase reporter gene containing the mutant Nrf2 showed no significant change. Collectively, these findings suggested that Nrf2 is subject to regulation by miR-153-3p through its binding to 3'-UTR and that miR-153-3p mediated changes in Nrf2 expression were not due to the regulation of Keap1/Nrf2 interaction.

Discussion

Since microRNAs have shown significant potential in diagnosis and therapy, it is worth study-

ing microRNAs in the context of a specific disease or biological process. Profiling microRNA expression in OSCC tissues has been reported using microarray or quantitative PCR-based methods [25, 26]. In this work, we start to shed light on the importance of miR-153-3p as we revealed its extensive level modulation in OSCC tissues using a microarray assay. A number of studies have reported that miR-153 dysregulates cancer cell growth, invasion, metastasis, and apoptosis. Downregulated expression of miR-153 is observed in hepatocellular carcinoma (HCC) cell lines and tissues, and miR-153 has shown a tumor suppressor function via the regulation of the expression of Snail [27]. In addition, Wu et al. found that the expression of an homologous to the E6-associated protein carboxyl terminus domain containing 3 (HECTD3) was downregulated through the binding of miR-153 to its 3'-UTR and that miR-153 promotes apoptosis of breast cancer cells in the presence of TRAIL and cisplatin by targeting HECTD3 [28]. Meanwhile, Wang et al. detected significantly lower levels of miR-153 in 50 gastric cancer specimens and found that the overexpression of miR-153 inhibited Snail-mediated cell invasiveness, while a depletion of miR-153 increased Snail-mediated cell invasiveness [29]. Another study established that miR-153 was expressed at significantly lower levels in pancreatic ductal adenocarcinoma patients with lymph node metastasis, tumor recurrence, poor tumor differentiation, and advanced tumor stage, and showed, through an *in vitro* assay, that miR-153 inhibits tumor cell migration and invasion by targeting SNAIL [30]. Similarly, Zuo et al. showed that the expression of miR-153 was also noticeably downregulated in esophageal squamous cell carcinoma (ESCC) cell lines and tissues and that the suppression of miR-153 could dictate SNAIL upregulation during epithelial to mesenchymal transition and metastatic progression of ESCC [31]. Additionally, Yuan et al. determined that the level of miR-153 was reduced by 80% in lung cancer tissues compared with adjacent controls and could regulate the expression of AKT in terms of both protein and mRNA levels, significantly inhibiting proliferation and migration, and promoting apoptosis of cultured lung cancer cells [32]. Importantly, a recent study compared and analyzed the miRNA expression profiles of epithelial phenotype cancer cells, mesenchymal phenotype cancer cells, and TGF- β -induced EMT

cell models, and identified miR-153 as a key EMT suppressor, capable of inhibiting cancer cell invasion and metastasis. Low miR-153 expression levels in human oral epithelial cancer have also been shown to be correlated with clinical metastasis and poor survival [15]. However, miR-153 expression has also been shown to be upregulated in other cancers. For instance, upregulation of miR-153 increases colorectal cancer invasiveness and resistance to oxaliplatin and cisplatin [33]. Besides, miR-153 was shown to be overexpressed in prostate cancers and promote cell proliferation by targeting the PTEN tumor suppressor gene, activating AKT signaling, and downregulating FOXO1 transcriptional activity [34]. Hitherto, cancer type-specific feature of miR-153 have been showed in cancer development and progression. It would be of interest to define the role of miR-153 in OSCC. In this study, miR-153-3p was significantly downregulated in OSCC tissues compared with adjacent non-tumor ones. Furthermore, we examined the clinical significance of miR-153-3p in cancer progression, and found that low expression of miR-153-3p was associated with lymph node metastasis and advanced tumor stage in OSCC patients. Overexpression of miR-153-3p significantly suppresses the migration and invasion of cultured oral cancer cells *in vitro*. On the contrary, its downregulation yields opposite results.

In the last decades, increasing evidence has shown that Nrf2 activation can provide beneficial effects against numerous human diseases including cancer [35]. Therefore, Nrf2 has been viewed as a "good" protein that protects humans from genotoxic damage caused by carcinogens [36]. Loss of Nrf2 expression increases the sensitivity of cells to carcinogenesis and promotes tumor formation [37]. DeNicola et al. found that Nrf2 controls the expression of the key serine/glycine biosynthesis enzyme phosphoglycerate dehydrogenase (PHGDH) in non-small cell lung cancer [17]. There is abundant evidence that the activation of Nrf2 can suppress carcinogenesis, especially in its earliest stages [38, 39]. The important property of chemopreventive compounds, such as curcumin, sulforaphane, isothiocyanates, green and black tea, resides in their ability to activate Nrf2 [40]. A study suggested that plumbagin suppresses the translocation of Nrf2 from cytosol to nucleus, and may act as a promising anticancer com-

pound *via* inhibiting the Nrf2-mediated oxidative stress signaling pathway in tongue squamous cell carcinoma cells [41].

Keap1, a chemical and oxidative stress sensor protein in mammalian cells, is an E3 ligase that targets Nrf2 and IKK β for degradation [42]. Many studies indicated that, in contrast to normal cells, Nrf2 protein is constitutively upregulated in many tumors due to somatic mutations in the Keap1 or Nrf2 genes [42, 43]. Decreased Keap1 activity in cancer cells induced greater nuclear accumulation of Nrf2 in non-small-cell lung cancer cells [44]. Studies found that Nrf2 is also transcriptionally regulated by the aryl hydrocarbon receptor (AhR) [45]. Even though the dysregulation of the Nrf2 system is implicated, a recent study has also elucidated the potential influence of miRNA activity over modulations of the Nrf2 signaling network [46]. Eades et al. demonstrated that miR-200a regulates the Keap1/Nrf2 pathway in mammary epithelium, thereby inhibiting the anchorage-independent growth of breast cancer cells [20]. MiR-93 was shown to regulate oncogenic processes in mammary tissues through the regulation of its target gene *Nrf2*; however there was no indication on whether this interaction was associated with Keap1 [47]. Interestingly, Yang et al. investigated the function of miR-28 in breast cancer and found inverse expression patterns of Nrf2 mRNA and miR-28 in mammary epithelial cells. Further experiments indicated that miR-28 decreases Nrf2 mRNA and protein through a Keap1-independent process [48]. Similarly, in the present study, Nrf2 mRNA and protein showed high expressions in OSCC tissues. Additionally, we found that miR-153-3p could regulate the expression of Nrf2, but not Keap1, suggesting that Nrf2 downregulation is mediated by miR-153-3p directly through its binding to the 3'-UTR of Nrf2 mRNA, rather than through the Keap1/Nrf2 interaction.

In summary, this study demonstrated that miR-153-3p was downregulated in OSCC and that its low expression is directly correlated with lymph node metastasis and advanced tumor stage. Moreover, miR-153-3p inhibits oral cancer cell migration and invasion by targeting Nrf2, but not Keap1. These findings suggest that the miR-153-3p/Nrf2 interaction could be regarded as a potential therapeutic target in oral squamous cell carcinoma.

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Disclosure of conflict of interest

None.

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