

Research Paper

# Hsa-let-7e-5p Inhibits the Proliferation and Metastasis of Head and Neck Squamous Cell Carcinoma Cells by Targeting Chemokine Receptor 7

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## Abstract

This study aimed at determining the role of hsa-let-7e-5p in the progression of head and neck squamous cell carcinoma (HNSCC). The relative levels of hsa-let-7e-5p transcripts in 15 paired of HNSCC and adjacent non-tumor tissues and cells were examined by quantitative real-time PCR (qRT-PCR). The potential targets of hsa-let-7e-5p were predicted and validated by luciferase assay. The impact of altered hsa-let-7e-5p expression on HNSCC cell proliferation and metastasis was determined by CCK-8, wound healing, transwell migration and invasion assays. The effect of hsa-let-7e-5p over-expression on the growth of HNSCC was examined *in vivo*. Hsa-let-7e-5p expression was significantly down-regulated in HNSCC tissues and highly metastatic PCI-37B cells. Bioinformatic analysis predicted that hsa-let-7e-5p bound to the 3'untranslated region (3'UTR) of chemokine receptor 7(CCR7), which was validated by luciferase assay. While transfection with hsa-let-7e-5p mimic significantly decreased CCR7 protein expression, transfection with hsa-let-7e-5p inhibitor increased CCR7 protein expression in HNSCC cells. Similarly, hsa-let-7e-5p over-expression inhibited PCI-37B cell proliferation, wound healing, migration and invasion, while inhibition of endogenous hsa-let-7e-5p had opposite effects in PCI-37A cells. Hsa-let-7e-5p over-expression inhibited PCI-37B tumor growth *in vivo*. Therefore, hsa-let-7e-5p acts as a tumor suppressor to inhibit the progression of HNSCC by targeting CCR7 expression. Hsa-let-7e-5p and CCR7 may be therapeutic targets of HNSCC.

Key words: head and neck squamous cell carcinoma (HNSCC), chemokine receptor 7(CCR7), hsa-let-7e-5p, proliferation, metastasis

## Introduction

Head and neck squamous cell carcinoma (HNSCC) is a commonly malignant tumor in the region [1]. Despite intensive researches and improvements in therapies, including surgery, radiation and chemotherapy, the five-year survival rate of HNSCC patients remains low due to its high local and distant metastasis [2]. Therefore, understanding the molecular mechanisms underlying the metastasis of HNSCC will be important for identifying the potential

targets to develop therapies for HNSCC patients.

MicroRNAs can bind to the 3'untranslational region (3'UTR) of their target mRNAs to promote their degradation and inhibit their translation [3, 4]. Currently, the role of hsa-let-7e-5p remains controversial. While down-regulated hsa-let-7e-5p expression is related to aggressiveness and poor prognosis of breast cancer [5], non-small-cell lung cancer (NSCLC) [6] and epithelial ovarian cancer [7],

other studies reported that hsa-let-7e-5p expression is up-regulated in hepatocellular carcinoma [8], colon cancer [9] and esophageal cancer [10]. Recent studies have revealed that hsa-let-7e-5p expression is up-regulated in oral tongue squamous cell carcinoma, particularly in some young cancer patients [11] and up-regulated hsa-let-7e-5p expression promotes the invasion and metastasis of esophageal squamous cell carcinoma [10]. Hence, what is the hsa-let-7e-5p expression pattern in HNSCC and how hsa-let-7e-5p regulates the progression and metastasis of HNSCC are unclear.

In this study, the levels of hsa-let-7e-5p expression in paired HNSCC and adjacent non-tumor tissues and HNSCC cells were examined. Furthermore, the effect of altered hsa-let-7e-5p expression on HNSCC cell proliferation, wound healing, migration and invasion *in vitro* and the impact of hsa-let-7e-5p over-expression on the HNSCC growth *in vivo* were investigated. The results indicated that hsa-let-7e-5p expression was down-regulated in HNSCC tissues and cells and hsa-let-7e-5p acted as a tumor suppressor to inhibit the progression and metastasis of HNSCC by targeting CCR7 expression.

## Materials and Methods

### Cell culture and transfection

HNSCC PCI-37B and PCI-37A cells were derived from a patient with lymph node metastatic and primary HNSCC, respectively and were gifts from the University of Pittsburgh Cancer Institute. PCI-37B cells express higher levels of CCR7 mRNA transcripts and have a high metastatic ability while PCI-37A cells express lower levels of CCR7 mRNA transcripts with a low metastatic ability [12]. The cells were cultured in DMEM medium (Invitrogen, Carlsbad, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, Carlsbad, USA), 100 U/ml of penicillin and 100 µg/ml of streptomycin at 37 °C in a 5% CO<sub>2</sub> incubator.

PCI-37B cells (3×10<sup>5</sup> cells/well) were cultured in 6-well plates overnight and transfected with control scramble or hsa-let-7e-5p mimic (Genepharma, Suzhou and Wanleibio, Shenyang, China, respectively) using Lipofectamine 2000 (Invitrogen). Similarly, PCI-37A cells were transfected with control scramble or hsa-let-7e-5p-specific inhibitor (Genepharma). Two days after transfection, the efficacy of hsa-let-7e-5p over-expression and silencing was examined by qRT-PCR.

### Clinical human tumor tissues

Surgical HNSCC tumor and matched adjacent non-tumor tissues were obtained from 15 patients, who underwent surgical resection prior to chemotherapy or radiotherapy at the Department of

Oral and Maxillofacial Surgery, School and Hospital of Stomatology, China Medical University (Table 1). Written informed consent was obtained from individual patients before surgery and the experimental protocol was approved by the Ethical Committee of China Medical University. The tissues were immediately stored in liquid nitrogen before process.

### Total RNA extraction and qRT-PCR

Total RNA was extracted from HNSCC cells and tissues using the high purity total RNAs rapid extraction kit (BioTeke, Beijing, China) following the manufacturer's protocol. After being qualified and quantified, the RNA samples were reversely transcribed into cDNAs using the High Capacity cDNA Reverse Transcription Kit (ThermoFisher). The relative levels of hsa-let-7e-5p to control U6 transcripts in each sample were tested by qRT-PCR using the SYBR Green mixture (Solarbio, Beijing, China) and specific primers in Superior 5-color Quantitative Real-Time PCR system (Bioneer, KOR). The primer sequences are shown in Table 2. The data were analyzed by the 2<sup>-ΔΔCt</sup> method.

**Table 1.** Clinical and pathological features of 15 HNSCC patients.

Patient number	age(year)/sex	Tumor site	Differentiation degree of the tumor	Metastatic lymph node	TNM
1	59/M	Buccal	Medium	Negative	T2N0M0
2	54/M	Tongue and Soft Palate	High	Negative	T2N0M0
3	64/F	Buccal	Medium	Negative	T2N0M0
4	50/M	Tongue	Medium	Positive	T2N1M0
5	66/F	Buccal	Medium	Positive	T4N1M0
6	54/F	Tongue	Medium	Negative	T2N0M0
7	51/M	Mouth Floor	Poorly	Negative	T2N0M0
8	47/M	Soft Palate	Medium	Negative	T1N0M0
9	68/M	Tongue	Medium	Negative	T2N0M0
10	61/M	Gingival	Medium	Positive	T1N2bM0
11	72/M	Tongue	Poorly	Negative	T2N0M0
12	63/M	Tongue	Poorly	Positive	T2N2cM0
13	61/F	Tongue	Poorly	Negative	T2N0M0
14	58/M	Tongue	Medium	Negative	T2N0M0
15	50/M	Tongue	Medium	Negative	T2N0M0

**Table 2.** Oligonucleotide primer sets for real-time PCR

name	Sequence(5'-3')	Length	Tm	size
hsa-let-7e-5p F	AGCAAGCTTTGGCACCCACC CGTAGAAC	28	55.0	400
hsa-let-7e-5p R	TAAGGATCCGATGCGAGGGAC AAGGACAGAA	30	55.0	
U6 F	CTCGCTTCGGCAGCAC	17	60.4	94
U6 R	AACGCTTCACGAATTTGCGT	20	59.7	
CCR7-3'UTR-WT F	CTAGCTAGCTGGACTAGAGG GACCTC	26	55	374
CCR7-3'UTR-WT R	ACGCGTCGACTCATTGAGAG GACTC	25		
CCR7-3'UTR-MT F	AGCTTGGTCAACCAATGCCA	20	58	374
CCR7-3'UTR-MT R	TTGACCAAGCTGGGATTGGG	20		

## Bioinformatics analysis

The potential targets of hsa-let-7e-5p were predicted by bioinformatic analysis using online websites (targetScanSites, picTarSites, miRandaSites).

## Dual-luciferase reporter assay

The wild type (WT) and mutant type (MT) of CCR7 3'UTR sequences were generated by PCR amplification from human genomic DNA using specific primers (Table 2) and cloned into pmirGLO- reporter (Promega), respectively. PCI-37B cells were transfected with either plasmid for the WT or MT of CCR7 3' UTR luciferase reporter, together with control scramble or hsa-let-7e-5p mimic as well as the plasmid for Renilla luciferase. Two days later, the luciferase activities were measured using Dual Luciferase Reporter Assay System (Promega). The firefly luciferase activity was normalized to the renilla luciferase activity.

## Western blot

The cells in different groups were harvested and lysed in RIPA lysis buffer, followed by centrifuging at 4°C 12,000 g for 10 min. The concentrations of total proteins in individual lysate samples were measured using the BCA protein concentration assay kit (Wanleibio, Shenyang, China). Individual lysates (40 µg/lane) were separated by sodium dodecyl sulfate polyacrylamide-gel-electrophoresis on 10% gels and transferred onto polyvinylidene difluoride membrane (Millipore, USA). The membranes were treated with 5% fat-free dry milk in TBST and incubated with rabbit anti-CCR7 (Bioyanbio, Zhenjing, China) and anti-β-actin antibodies (Wanleibio, Shenyang, China) at 4°C overnight. The membranes were treated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Wanleibio, Shenyang, China) and visualized by the enhanced chemiluminescent reagent, followed by imaging in the Gel Imaging System (LiuYi, Beijing, China). The relative levels of CCR7 to β-actin protein expression were analyzed using Gel-Pro-Analyzer software 6.0 (Media Cybernetics US).

## Cell proliferation assay

The different groups of HNSCC cells (3×10<sup>3</sup> cells/well) were cultured in triplicate in 96-well plates for 4 days. The cell proliferation in individual groups was examined longitudinally using the CCK8 proliferation assay kit. Briefly, during the last 2-h culture, the cells were treated with the CCK8 reagent and the absorbance of individual wells of cells were measured at 450 nm using a microplate spectrometer readers (Biotek, US).

## Wound-healing assay

The cells in different groups were cultured in

triplicate in 6-well plates up to 90% confluency, and the monolayer of cells was wounded using a micropipette tip. We used Mitomycin C and medium without FBS to culture cells to discard the possible influence of proliferation. After being washed, the cells were cultured for 0 h, 12 h and 24 h. The wound distance was photographed and the percent migration of individual groups of cells was calculated.

## Cell migration and invasion assay

The different groups of cells (1×10<sup>4</sup> cells/well or 4×10<sup>3</sup> cells/well) were cultured in triplicate in 200 µl of serum-free medium in the upper chambers of 24-well transwell chambers (8 µm pore, Corning, US). The lower chambers were added with 800 µl of DMEM containing 20% FBS. One day later, the non-migrated HNSCC cells on the surface membrane of the upper chambers were removed using a cotton swab and the migrated cells on the bottom surface of the upper chambers were fixed and stained with 0.05% crystal violet, followed by photographed under an inverted microscope (200×) (AE31, Motic, China). The migrated cells in five random fields were counted in blinded manner.

Similar procedures were used for testing invasion, except that the upper chambers were coated with matrigel and 2×10<sup>4</sup> cells/well or 8×10<sup>3</sup> cells/well were used.

## Lentivirus transduction

PCI-37B cells were cultured in 6-well plates up to 70% of confluency and transduced with lentivirus for LV-hsa-let-7e-5p mimic or control miRNA Gene-pharma for 24 h. The cells were used for subsequent experiments.

## Xenograft tumor model

Female BALB/c nude mice (6-week-old) were from Wanleibio and housed in a specific pathogen-free facility with free access to autoclaved foods and water. The mice were randomized and inoculated subcutaneously with 1×10<sup>7</sup> PCI-37B/hsa-let-7e-5p or control PCI-37B/con cells into their right flank. The tumor growth in individual mice was monitored every three days up to 21 days post inoculation. The tumor volumes were calculated by the formula, volume = length × width<sup>2</sup> × 0.5. At the end of the experiment, the mice were sacrificed and their tumors were dissected and measured. One piece of tumor sample was used for total RNA extraction to quantify the expression of hsa-let-7e-5p using qRT-PCR.

## Statistical analysis

Data are expressed as mean ± standard deviations (SD). The difference among the groups was statistically analyzed by one-way analysis of variance

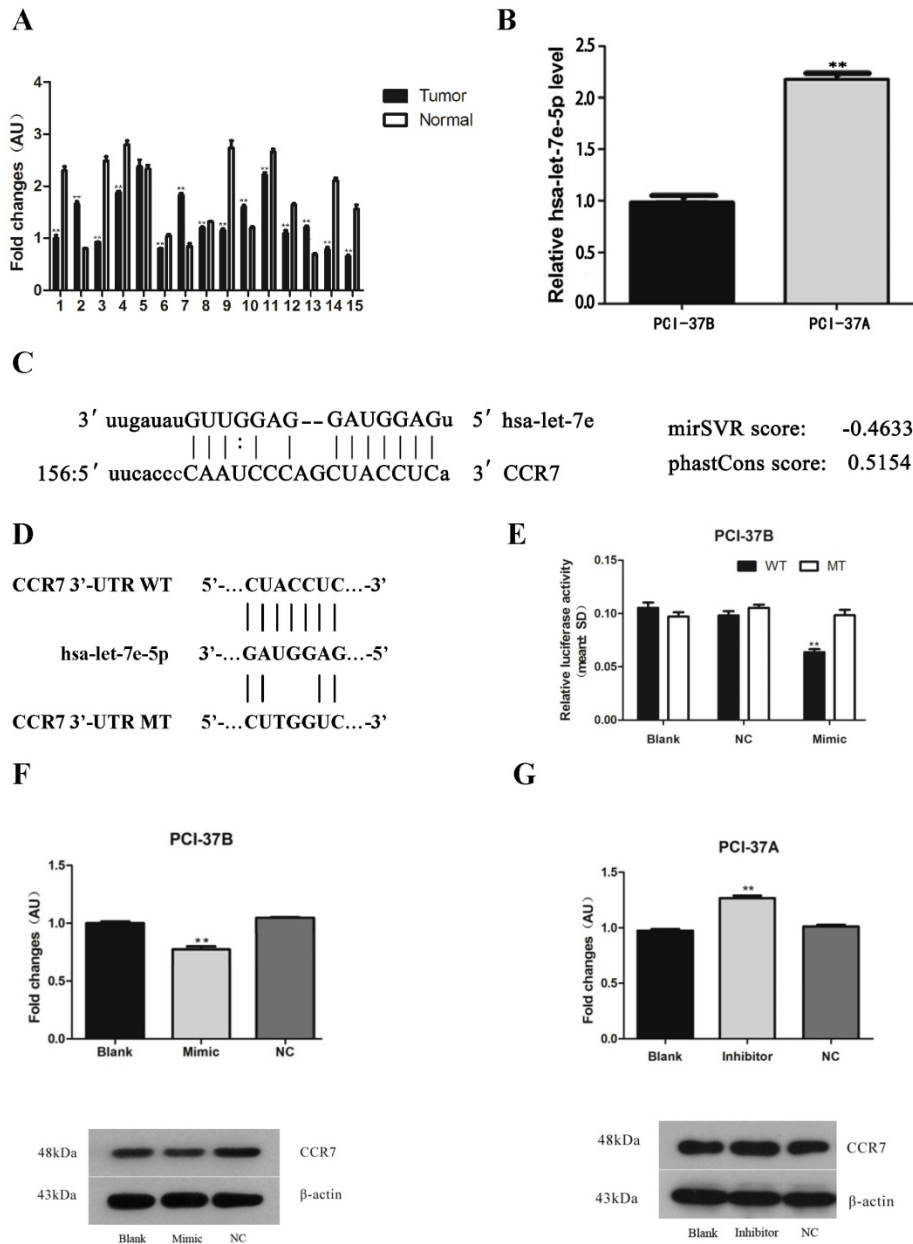
and the difference between the groups was analyzed by Student's T test using SPSS 13.0 software. A p-value of <0.05 was considered statistically significant.

**Results**

**Hsa-let-7e-5p is deregulated and targets CCR7 in HNSCC**

To study the role of hsa-let-7e-5p in the development and progression of HNSCC, the relative levels of hsa-let-7e-5p transcripts in 15 paired of HNSCC and

adjacent non-tumor tissues were examined by qRT-PCR. The relative levels of hsa-let-7e-5p transcripts in 10 out of 15 HNSCC tissues were significantly lower than that in the adjacent non-tumor tissues, indicated that hsa-let-7e-5p expression was down-regulated in HNSCC (p<0.01, Fig. 1A). Similarly, the relative levels of hsa-let-7e-5p transcripts in the highly metastatic PCI-37B cells were also significantly lower than that in the lowly metastatic PCI-37A cells (p<0.01, Fig. 1B).



**Figure 1. Hsa-let-7e-5p expression is down-regulated in HNSCC tissues and cells and targets CCR7.** The relative levels of hsa-let-7e-5p expression in 15 paired of HNSCC and adjacent non-tumor tissues (A) and cells (B) were determined by qRT-PCR. (C) The potential sequence in the 3'UTR CCR7 for hsa-let-7e-5p binding was predicted by bioinformatics. (D) The sequences of the wild-type (WT) and mutant (MT) 3'UTR CCR7 for hsa-let-7e-5p binding. (E) Dual-luciferase reporter assay revealed that transfection with hsa-let-7e-5p mimic significantly reduced the WT, but not the MT, 3'UTR CCR7-regulated luciferase expression in PCI-37B cells. (F) Transfection with hsa-let-7e-5p mimic significantly decreased CCR7 protein expression in PCI-37B cells. (G) Transfection with hsa-let-7e-5p inhibitor significantly increased CCR7 protein expression in PCI-37A cells. Data are representative images or expressed as the mean ± SD of each group from three separate experiments. \*\*p < 0.01.

To explore how hsa-let-7e-5p acts in HNSCC, the potential targets of hsa-let-7e-5p were predicted by bioinformatics analysis. The 3'UTR of CCR7 contained the sequence for hsa-let-7e-5p binding (Fig. 1C). Next, the mutant 3'UTR of CCR7 was synthesized (Fig. 1D) and the potential binding of hsa-let-7e-5p to the 3'UTR of CCR7 and its function were validated by dual-luciferase reporter assays. As shown in Figure 1E, co-transfection with hsa-let-7e-5p mimic and the plasmid for wild-type of CCR7 3'UTR-regulated luciferase significantly reduced the luciferase activities in PCI-37B cells, relative to the controls transfected with hsa-let-7e-5p mimic and the plasmid for the mutant CCR7 3'UTR-regulated luciferase and the cells transfected with the plasmid and control miRNA (NC) or the cells transfected with the plasmid alone ( $p < 0.01$ ). Western blot analysis indicated that transfection with hsa-let-7e-5p mimic significantly decreased the levels of CCR7 protein expression in PCI-37B, whereas transfection with hsa-let-7e-5p inhibitor significantly increased the CCR7 protein expression in PCI-37A (Fig. 1F and 1G). Collectively, such data indicate that down-regulated hsa-let-7e-5p expression is associated with the progression of HNSCC and suggest that hsa-let-7e-5p may bind to the 3'UTR of CCR7 to decrease its protein expression in HNSCC cells.

### Altered hsa-let-7e-5p expression modulates the tumor-behaviors in HNSCC cells

To explore the regulatory function, HNSCC PCI-37B and PCI-37A cells were transfected with control scramble miRNA, hsa-let-7e-5p mimic or inhibitor, respectively. The proliferation, wound healing, migration and invasion of the different groups of cells were examined. In comparison with the unmanipulated (Blank) cells, transfection with control scramble miRNA did not alter the proliferation, wound healing, migration and invasion of both PCI-37B and PCI-37A cells (Fig. 2). Transfection with hsa-let-7e-5p mimic significantly reduced the proliferation, wound healing, migration and invasion of PCI-37B cells ( $p < 0.01$  for all, Fig. 2A, 2C, 2E, and 2G). In contrast, transfection with hsa-let-7e-5p inhibitor significantly enhanced the proliferation, wound healing, migration and invasion of PCI-37A cells ( $p < 0.01$  for all, Fig. 2B, 2D, 2F and 2H). Hence, altered hsa-let-7e-5p expression changed the tumor behaviors of HNSCC cells *in vitro*.

### Hsa-let-7e-5p over-expression inhibits xenograft tumor growth *in vivo*

Finally, the impact of hsa-let-7e-5p over-expression on tumor growth was determined. Individual BALB/c nude mice were inoculated sub-

cutaneously with the unmanipulated, the scramble miRNA-transfected or hsa-let-7e-5p-transfected PCI-37B cells and the tumor growth in individual groups of mice was monitored (Fig. 3A and 3B). Induction of hsa-let-7e-5p over-expression significantly inhibited the growth of inoculated HNSCC at 15 days post inoculation and later ( $p < 0.05$  or  $p < 0.01$ ). Further qRT-PCR revealed that the relative levels of hsa-let-7e-5p transcripts in the hsa-let-7e-5p over-expressing tumors were significantly higher than that of the controls ( $p < 0.01$ , Fig. 3C). Therefore, increased hsa-let-7e-5p expression significantly inhibited the growth of inoculated HNSCC *in vivo*.

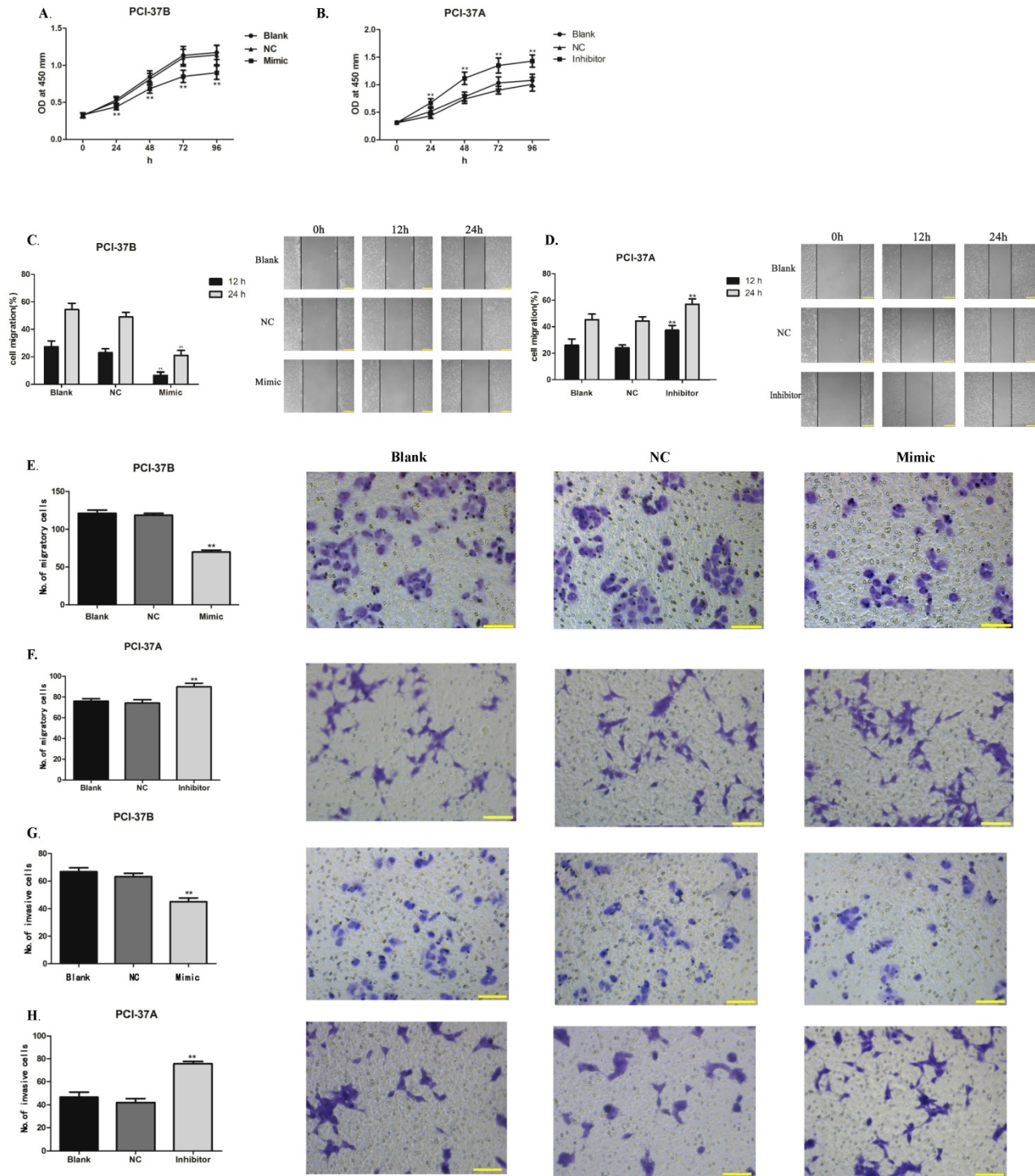
## Discussion

Previous studies have shown the levels of has-let-7e-5p expression in different types of malignant tumors remain controversial [6-10]. There is little information on the expression pattern and potential role of has-let-7e-5p in HNSCC. In this study, we found that has-let-7e-5p transcription was down-regulated in HNSCC tissues and highly metastatic HNSCC cells. These data suggest that has-let-7e-5p may act as a tumor suppressor and its down-regulated expression may promote the development and progression of HNSCC, extending previous observations in NSCLC [13], breast cancer [5] and glioma [14]. However, our data were in disagreement with other reports that up-regulated has-let-7e-5p expression is associated with aggressiveness of oral tongue and esophageal squamous cell carcinoma, particularly for young patients [11]. Actually, we found that has-let-7e-5p expression was significantly up-regulated in 4 out of 15 HNSCC. The difference may stem from different population of patients between our study and those of others. These contradictory findings suggest that has-let-7e-5p expression may be regulated by specific tumor type, stage and its host age and has-let-7e-5p may have different roles during the pathogenesis of different types of malignant tumors by targeting different gene expression.

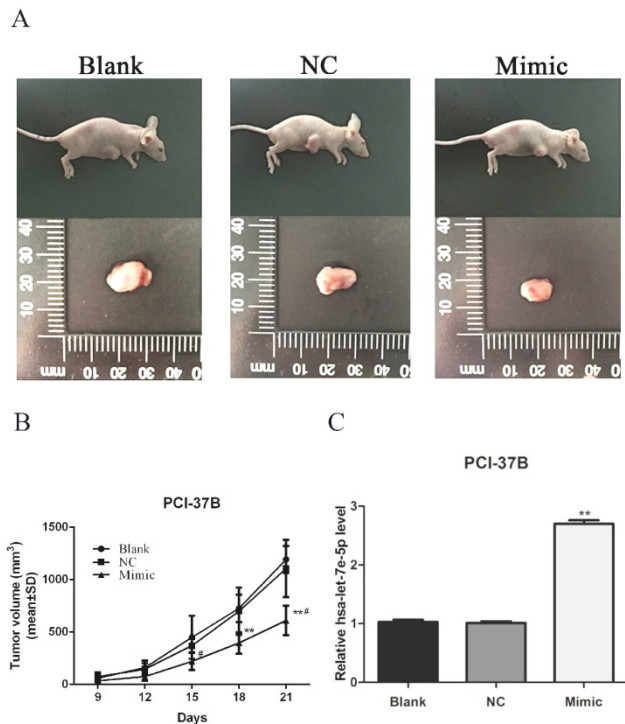
MicroRNAs function as tumor suppressors or oncogenes to inhibit or promote the development and progression of malignant tumors. While hsa-let-7e-5p can inhibit the proliferation and metastasis of glioma stem cells [14] and colorectal cancer [15], hsa-let-7e-5p, together with miR-99b and miR-125a, acts as a pro-metastasis oncomir to promote ESCC cell migration and invasion *in vitro* [10]. We found that induction of hsa-let-7e-5p over-expression inhibited the proliferation, wound healing, migration and invasion of highly aggressive PCI-37B cells while inhibition of endogenous hsa-let-7e-5p expression by transfection with hsa-let-7e-5p inhibitor enhanced the

proliferation, wound healing, migration and invasion of less aggressive PCI-37A cells. Furthermore, hsa-let-7e-5p over-expression retarded the growth of inoculated PCI-37B HNSCC in mice. There independent lines of evidence demonstrated that hsa-let-7e-5p acted as a tumor suppressor to inhibit the development and progression of HNSCC. We are

interested in further investigating the impact of altered hsa-let-7e-5p expression in the lymph node and distant metastasis of HNSCC *in vivo* in the future. If validated, functional increase in the levels of hsa-let-7e-5p expression may be valuable to inhibit the progression of HNSCC.



**Figure 2. Altered hsa-let-7e-5p expression modulates the behaviors of HNSCC cells *in vitro*.** PCI-37B and PCI-37A cells were unmanipulated or transfected with control scramble, hsa-let-7e-5p mimic or inhibitor, respectively. The proliferation (A, B), wound healing (C, D), migration (E, F) and invasion (G, H) of each group of cells were examined, respectively. Data are representative images or expressed as the mean  $\pm$  SD of each group from three separate experiments (magnification  $\times$  100 for wound healing;  $\times$  200 for migration and invasion). Bar = 200  $\mu$ m. \*\* P < 0.01.



**Figure 3. Induction of hsa-let-7e-5p over-expression inhibits the growth of inoculated PCI-37B HNSCC in mice.** Individual BALB/c nude mice were injected subcutaneously with unmanipulated, lentivirus-mediated control miRNA-expressing, or has-let-7e-5p over-expressing PCI-37B cells ( $1 \times 10^7$  cells/mouse). The dynamic growth of inoculated tumors in each group was monitored up to 21 days post inoculation (A). Subsequently, the tumors were dissected out and imaged (B). \*  $P < 0.05$ , \*\*  $P < 0.01$  vs. the Blank. #  $P < 0.05$  vs. the NC. The relative levels of has-let-7e-5p transcripts in each group of tumors were examined by qRT-PCR (C). Hsa-let-7e-5p expression was assessed by real-time PCR. \*\*  $P < 0.01$  vs. the Blank or NC. Data are representative images or present as the mean  $\pm$  SD of each group (n=6 per group) from three separate experiments.

To understand the molecular mechanisms underlying the action of hsa-let-7e-5p in inhibiting the progression of HNSCC, we performed bioinformatics to predict the potential targets of hsa-let-7e-5p. We found that the 3'UTR HMGA2, STAT3, and CCR7 contained the potential binding sequence of hsa-let-7e-5p. Further luciferase reporter assay indicated that transfection with hsa-let-7e-5p mimic significantly reduced the WT 3'UTR, but not the MT 3'UTR of CCR7-regulated luciferase expression in PCI-37B cells. Functionally, hsa-let-7e-5p over-expression decreased the levels of CCR7 protein expression in PCI-37A cells while inhibition of endogenous hsa-let-7e-5p expression increased the levels of CCR7 protein expression in PCI-37A cells. Together, such findings indicated that hsa-let-7e-5p inhibited CCR7 expression by binding to the 3'UTR CCR7 and the CCR7 was one of its targets in HNSCC cells. CCR7 is a member of the G-protein-coupled receptors (GPCR) family, and substantially expressed on several types of immune cells. CCR7 is crucial for chemoattraction of immune cells and tumor cells. Our data were consistent with a recent report and support

the notion that high levels of CCR7 expression promote the invasion and metastasis of HNSCC [16] and esophageal squamous cell carcinoma [17]. In addition, our previous studies and those of others have shown that CCR7 can regulate the metastasis of HNSCC through the signaling pathways, including the CCR7-PI3K-Cdc42-Rac [18], CCR7-PKC $\alpha$ -NF- $\kappa$ B [19], CCR7-PI3K-Akt-mTOR-NF- $\kappa$ B [20, 21], CCR7-JAK2-STAT3 [22], CCR7-MAPKs [23], CCR7-RhoA/ROCK-Pyk2 cofilin [24-26]. Moreover, the CCR7-related signaling can up-regulate some stimulators to enhance the proliferation and angiogenesis of malignant tumors [27]. It is possible that the down-regulated hsa-let-7e-5p expression to enhance CCR7 expression may promote the progression and metastasis of HNSCC through similar signal pathways. Thus, such findings may shed new light on regulating CCR7 expression and HNSCC metastasis. We recognized that our study had limitations, including small sample size, the lack of further functional studies on whether over-expression of CCR7 can antagonist the hsa-let-7e-5p mediated inhibition to restore the progression of HNSCC. Therefore further studies in a bigger population are warranted to validate the role of hsa-let-7e-5p in the development and progression of HNSCC.

In conclusion, the results revealed that hsa-let-7e-5p expression was down-regulated in HNSCC tissues and highly aggressive cells. The hsa-let-7e-5p bound to the 3'UTR CCR7 and inhibited its expression. Hsa-let-7e-5p inhibited the proliferation, wound healing, migration and invasion of HNSCC cells *in vitro* and the growth of HNSCC tumors *in vivo*. Collectively, such novel data indicated that hsa-let-7e-5p inhibited the progression and metastasis of HNSCC by targeting CCR7. Such findings may provide new insights into regulating CCR7 expression and HNSCC metastasis.

## Abbreviations

3'UTR: 3'untranslated region; CCR7: Chemokine receptor 7; Blank: Blank control; NC: Negative control; qRT-PCR: quantitative real-time PCR; WT: the wild type; MT: mutant type.

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## Competing Interests

The authors have declared that no competing interest exists.

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