**ORIGINAL ARTICLE**

**IncRNA ZNF667-AS1 Suppresses Epithelial Mesenchymal Transformation by Targeting TGF-β1 in Oral Squamous Cell Carcinoma**

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**SUMMARY**

**Background:** IncRNAs perform complex functions and play an essential role in all stages of tumor progression. However, there are few studies that discuss the function of IncRNA ZNF667-AS1 in oral squamous cell carcinoma (OSCC). This study aimed at analyzing the expression and biological behavior of IncRNA ZNF667-AS1 in OSCC.

**Methods:** IncRNA ZNF667-AS1 expression level in OSCC tissues and cell lines was explored by real-time PCR. The role of IncRNA ZNF667-AS1 on prognosis was elucidated. Cell proliferation assay, plate colony formation assay, wound-healing assay, and transwell migration assay were used to detect cell proliferation ability, cell clone formation ability, migration ability, and invasion ability, respectively. The effect of IncRNA ZNF667-AS1 on epithelial mesenchymal transformation (EMT) process was evaluated by western blot and real-time PCR.

**Results:** The expression levels of IncRNA ZNF667-AS1 were decreased in malignant tumor tissues. The OSCC patients with high expression of IncRNA ZNF667-AS1 had a longer survival time. IncRNA ZNF667-AS1 inhibited cell proliferation, cell clone formation ability, invasion and migration. Furthermore, IncRNA ZNF667-AS1 could inhibit the EMT process by suppressing transforming growth factor-β1 (TGF-β1) expression, and TGF-β1 treatment could partially restore the inhibitory effect.

**Conclusions:** IncRNA ZNF667-AS1 may act as an antioncogene inhibiting the ability of proliferation, cell clone formation, invasion and migration, and suppress the process of EMT by targeting TGF-β1. IncRNA ZNF667-AS1 could be a potential therapeutic target and a new predictive biological marker of OSCC.


**KEY WORDS**

IncRNA ZNF667-AS1, oral squamous cell carcinoma, progression, prognosis, epithelial mesenchymal transformation

**INTRODUCTION**

Oral squamous cell carcinoma (OSCC) is a common tumor which occurs in oral squamous cell coverage and adjacent sites [1]. It is the main type of oral cancer, taking up about 90% of the total incidence rate of oral cancer [2]. An investigation showed that tobacco, alcohol, areca nut, and human papillomavirus were the important
Long noncoding RNAs (lncRNAs) are a group of RNA molecules with a length over 200 bp. lncRNAs do not have coding function and lack of open reading frame (ORF). lncRNAs have complex functions and play an essential role in all stages of tumor progression. More and more evidence has suggested that a variety of lncRNAs in OSCC, including IncRNA AC007271.3, IncRNA CASC9, and IncRNA HOXA11-AS, are closely related to the early diagnosis, targeted therapy, and the prognosis of OSCC. ZNF667-AS1, also known as MORT, is an antisense chain of ZNF667 which belongs to the zinc finger proteins-C2H2 family. A number of studies have suggested that IncRNA ZNF667-AS1 was low-expressed in various tumor tissues. Our previous studies showed that the expression level of IncRNA ZNF667-AS1 was reduced in esophageal squamous cell carcinoma (ESCC) tissues, and its low expression was related to abnormal methylation status. However, there are few studies that discuss the function of IncRNA ZNF667-AS1 in OSCC. In epithelial mesenchymal transformation (EMT) processes, epithelial cells no longer have the intrinsic morphology, and turn into mesenchymal phenotype in which the ability of adhesion is decreased. Primary tumor cells could improve the ability of invasion and migration by EMT, eventually leading to tumor metastasis. EMT produces invasive cells that can enter into the blood circulation and migrate to other organs throughout the body. EMT is the momentous course in cancer progression, which supplies a favorable microenvironment for tumor cells to survive. Appropriate cell environment, cytokines, and extracellular signals could induce EMT. Multiple studies have indicated that lncRNAs could regulate EMT in various malignant tumors. However, the influence of IncRNA ZNF667-AS1 in the OSCC EMT process is unknown. In this work, the expression and the prognosis of IncRNA ZNF667-AS1 in OSCC were explored. Then, we analyzed the molecular mechanisms of IncRNA ZNF667-AS1 in modulating the ability of proliferation, cell clone formation, invasion and migration in OSCC cell lines. In addition, we tried to clarify the relationship between IncRNA ZNF667-AS1 and EMT, exploring the possible mechanisms. This study aimed at clarifying the mechanisms of action of IncRNA ZNF667-AS1 on the OSCC, which could provide an experimental basis for future clinical application of IncRNA ZNF667-AS1.

**MATERIALS AND METHODS**

**The information of clinical patients**

Surgical specimens of 121 patients with OSCC were obtained from the biological specimen bank of the Fourth Hospital of Medical University between June 2014 and December 2016. Inclusion criteria: (1) confirmation of OSCC based on postoperative pathological diagnosis; (2) did not receive chemoradiotherapy before the operation; (3) no combination of other cancers; (4) complete clinical information. Exclusion criteria: (1) combination of other malignant tumors; (2) preoperative anticancer therapy, such as chemoradiotherapy; (3) incomplete clinical information. The primary tumor tissue and the adjacent normal tissue which was more than 2 cm away from the edge of primary tumor were taken from each OSCC patient. After the specimens were removed, some of them were embedded in paraffin for hematoxylin-eosin (HE) staining, and the rest was stored at -80°C for total RNA extraction. After HE staining, the diagnosis of OSCC was confirmed by three pathologists, and there was no cancer cell infiltration in the corresponding adjacent tissues. The Medical Ethics Committee of the Fourth Clinical Hospital of Hebei Medical University approved this study, and informed consent was obtained from each of the 121 patients or the family members. The clinicopathological data consisted of age, gender, tumor size, TNM stage, and lymph mode metastasis.

**Cell culture, treatment and transfection**

Four OSCC cell lines (Tca-83, CAL-27, HSC-2, and SCC-25) were cultured in Dulbecco’s Modified Eagle’s Medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% fetal bovine serum. The culture medium of the four OSCC cell lines was changed every 24 hours. For transforming growth factor-β1 (TGF-β1) treatment, the cells were treated with 10 ng/mL of TGF-β1 (Sigma Company) for 48 hours. The cells in good condition were digested with trypsin and counted. The cells were spread on a 6-well plate (2 x 10^5 cells/well). The cells were transfected when they had grown to 70% - 80% in each well. FuGENE HD Transfection Reagent (Promega, Madison, WI, USA) was used for cell transfection experiments. The OSCC cells were transfected with pcDNA3.1-ZNF667-AS1 or ZNF667-AS1-specific shRNA plasmid for 24 - 48 hours. The shRNA expression vector was made by Shanghai GenePharma Company.

**Real-time polymerase chain reaction (real-time PCR)**

According to the methods previously described, the total RNA of the cells was extracted, and the cDNA was synthesized. The expressions of IncRNA ZNF667-AS1 (Forward: 5′-CATCACTACCATCCATCCTA-3′, Reverse: 5′-CCAGGCAGAGAAGGATAA-3′),
IncRNA ZNF667-AS1 Suppresses EMT in OSCC

E-cadherin (Forward: 5'-CGAGAGCTACA CTTCACGG-3', Reverse: 5'-GGCCTTTTGTAGTAACTCACAC-3'), N-cadherin (Forward: 5'-CAACTTGCCAGAAACTCCAGG-3', Reverse: 5'-ATGAAACCGGGCTATCTGTC-3'), Vimentin (Forward: 5'-CGCCTOSCGGAT GAATTCAG-3', Reverse: 5'-TCAGGGAGGAAAAGTTTGGA-3'), β-catenin (Forward: 5'-ATGGCTTGGAATGAGAC-3', Reverse: 5'-AAGTGAGTCAGCCG GTGCTA-3'), Twist1 (Forward: 5'-ACCATCTCACACCTCG-3', Reverse: 5'-GATTGTCCCGACCTCTTG-3'), and TGF-β1 (Forward: 5'-AGAACGCTCAACC CCAGTGCTA-3', Reverse: 5'-TGTGTGATGCTTTGGGTTCGTCA-3') mRNA were detected by GoTaq qPCR Master Mix (Promega, USA). The 2^−ΔΔCt method was used to calculate the relative expression of IncRNA ZNF667-AS1, E-cadherin, N-cadherin, Vimentin, β-catenin, Twist1, and TGF-β1 mRNA. GAPDH (Forward: 5'-AGAAGTCACCCGC GTGCTA-3', Reverse: 5'-ACCATCCTCACACCTCTG-3') was employed as control for mRNA. Each of the above experiments were performed 3 times.

Proliferation assay
Different group OSCC cells were inoculated into 96-well plates with the density for each well of 5 x 10^4 cells. When the cells grew to complete fusion, a 200 μL pipette tip was used vertically to scratch the cells. The scratched cells were washed off using PBS, then 2 mL serum-free medium was added. The scratch spacing was observed and the cell migration percentage was calculated at 0, 12, and 24 hours after scratch. Each of the above experiments were performed 3 times.

Wound-healing assay
Different OSCC cells were inoculated into 6-well plates at a density for each well of 5 x 10^5 cells. When the cells grew to complete fusion, a 200 μL pipette tip was used vertically to scratch the cells. The scratched cells were washed off using PBS, then 2 mL serum-free medium was added. The scratch spacing was observed and the cell migration percentage was calculated at 0, 12, and 24 hours after scratch. Each of the above experiments were performed 3 times.

Cell invasion assay
According to the methods previously described, the cell invasion assay was performed and analyzed [19]. Different OSCC cells were suspended in serum-free medium. Then, 50 μL Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) was added to the upper compartment. Cell concentration was adjusted and inoculated into the chamber with the density of each well at 1 x 10^5 cells. The matrix glue and the cells in the upper chamber were wiped off after 24 hours incubation. The cells were fixed by 4% paraformaldehyde for 20 minutes and stained for by 1% crystal violet 20 minutes. The number of invasive cells was calculated from 5 visual fields (at 200 x magnification). The above experiments were performed 3 times.

Western blot analysis
According to the methods previously described, the total protein of the cells was extracted, and the cDNA was synthesized and quantified [19]. The protein of different groups was run on 10% - 12% SDS-PAGE at 30 μg/well. The separated protein was moved to PVDF membrane and blocked with sealing fluid for 1 hour. E-cadherin (1:1,000 dilution, Abcam, UK) or N-cadherin (1:1,000 dilution, Abcam, UK) or Vimentin (1:1,000 dilution, Abcam, UK) or β-catenin (1:1,000 dilution, Abcam, UK) or Twist1 (1:1,000 dilution, Abcam, UK) or TGF-β1 (1:1,000 dilution, Abcam, UK) or GAPDH (1:500 dilution, Abcam, UK) were incubated at 4°C for more than 12 hours. Secondary antibodies were added for 2 hours and eventually the bands were analyzed by chemiluminescence analysis and ImageJ. All the above experiments were performed 3 times.

Statistical analysis
SPSS 21.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis. Data were expressed as mean ± SD. The relation between IncRNA ZNF667-AS1 expression and other factors was statistically analyzed with χ² or Fisher’s exact test. The overall survival of OSCC patients was analyzed by Kaplan-Meier method. The prognostic factors of OSCC patients were analyzed by univariate and multivariate analysis. The one-way ANOVA was used to calculate the differences between groups in cell proliferation, and invasion and migration. When the p-value is less than 0.05, the difference was considered as statistically significant.
Table 1. Relationship between the expression level of ZNF667-AS1 and clinical pathological features in OSCC patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ZNF667-AS1</th>
<th>( \chi^2 )</th>
<th>p</th>
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<td></td>
<td></td>
<td>low55</td>
<td>high66</td>
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<td>19</td>
<td>28</td>
<td>0.784</td>
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<td>( \geq 60 )</td>
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<td>38</td>
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<tr>
<td>Female</td>
<td>46</td>
<td>20</td>
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<td>9</td>
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<tr>
<td>Yes</td>
<td>81</td>
<td>46</td>
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<td>Well-moderate</td>
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</table>

Table 2. Univariate and multivariable analyses of prognostic factors in OSCC for 5-year survival.

<table>
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<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
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<td></td>
<td>HR</td>
<td>p-value</td>
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<tr>
<td>Expression of ZNF667-AS1 high vs. low</td>
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<td>Gender male vs. female</td>
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<td>0.967</td>
</tr>
<tr>
<td>Age (years) &lt; 60 vs. ( \geq 60 )</td>
<td>0.652</td>
<td>0.081</td>
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<tr>
<td>Histological grade well-moderate vs. poor</td>
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<td>0.137</td>
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<tr>
<td>TNM stage I and II vs. III and IV</td>
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<td>&lt; 0.001</td>
</tr>
<tr>
<td>Metastatic state of lymph node yes vs. no</td>
<td>3.414</td>
<td>&lt; 0.001</td>
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RESULTS

IncRNA ZNF667-AS1 is low expressed in OSCC cell lines and tissues and associated with overall survival

As shown in Figure 1A, ZNF667-AS1 had different degrees of expression in four kinds of OSCC cells. The results of real-time PCR demonstrated that ZNF667-AS1 was significantly decreased in OSCC tissues (Figure 1B; \( p < 0.05 \)). The clinicopathological features of 121 patients with OSCC were further analyzed (Table 1, Figure 1C). Comparing with OSCC patients with stage I and II the level of ZNF667-AS1 expression was obviously reduced in those with stage III and IV (Figure 1D; \( p < 0.05 \)). The level of ZNF667-AS1 expression in OSCC patients with lymph node metastasis was lower than those without metastasis (Figure 1E; \( p < 0.05 \)). However, gender, age, and differentiation degree were not related to the ZNF667-AS1 expression (\( p > 0.05 \)). Low expression of ZNF667-AS1 in OSCC patients exhibited poor overall survival (Figure 1F; \( p < 0.05 \)). The results suggested that ZNF667-AS1 may function as a tumor-suppressor, and low expression of ZNF667-AS1 in OSCC patients indicates poor prognosis.

The prognostic significance of ZNF667-AS1 expression in OSCC patients was evaluated by univariate and multivariate analysis. Univariate analysis indicated that the expression of ZNF667-AS1 (\( p < 0.001 \)), TNM stage (\( p < 0.001 \)) and lymph node metastasis (\( p = 0.004 \)) influence the prognosis of OSCC patients (Table 2). Multivariate analysis indicated that the prognosis of OSCC patients can be judged by the expression of ZNF667-AS1 (\( p < 0.001 \)) and lymph node metastasis (\( p = 0.001 \)) independently (Table 2).
Figure 1. ZNF667-AS1 is downregulated in OSCC and associated with better overall survival.

(A) Relative expression of ZNF667-AS1 in four OSCC cell groups. (B) Relative expression of ZNF667-AS1 in OSCC tissues and corresponding normal tissues. (C) Relative expression of ZNF667-AS1 in OSCC patients with different clinicopathological factors. (D) Relative expression of ZNF667-AS1 in OSCC patients with different TNM stage. (E) Relative expression of ZNF667-AS1 in OSCC patients with different TNM stage. (F) High expression of ZNF667-AS1 is associated with better overall survival.

* p < 0.05.
Figure 2. Effects of ZNF667-AS1 on proliferation and cloning formation of OSCC cells.

The transfection efficiency of overexpressing ZNF667-AS1 on SCC-25(A) and HSC-2 cells (B). The transfection efficiency of knockdown of ZNF667-AS1 on Tca-83 (C) and CAL-27 cells (D). Overexpression of ZNF667-AS1 inhibits the ability of proliferation in SCC-25(E) and HSC-2 cells (F). Overexpression of ZNF667-AS1 inhibits the ability of cloning formation in SCC-25 (G) and HSC-2 cells (H). Knockdown of ZNF667-AS1 enhances the ability of proliferation in Tca-83 (I) and CAL-27 cells (J). Knockdown of ZNF667-AS1 enhances the ability of cloning formation in Tca-83 (K) and CAL-27 cells (L).

* p < 0.05.

The role of lncRNA ZNF667-AS1 in OSCC cell proliferation and cloning formation

According to the different expression levels in the four OSCC cells, lncRNA ZNF667-AS1 expression in SCC-25 and HSC-2 cells were lower than other cells. SCC-25 and HSC-2 cells were chosen for follow-up experiments. As shown in Figure 2A and 2B, after treatment with pcDNA3.1-ZNF667-AS1, SCC-25 and HSC-2 cells showed overexpression of ZNF667-AS1 compared with NC group and pcDNA3.1-NC group. Cloning formation assay and proliferation assay showed that compared with the control group, pcDNA3.1-ZNF667-AS1 group inhibited the ability of cloning formation and the proliferation of SCC-25 and HSC-2 cells (Figure 2E - 2H). As shown in Figure 2C and 2D, after treatment with ZNF667-AS1-specific shRNA plasmid, SCC-25 and HSC-2 cells showed low expression of ZNF667-AS1 compared with those after treatment with shRNA. Cloning formation assay and proliferation assay showed that compared with the control group, ZNF667-AS1-specific shRNA group promoted cloning formation and the proliferation of SCC-25 and HSC-2 cells (Figure 2I - 2L). The above results showed that IncRNA ZNF667-AS1 inhibited cell proliferation ability and cloning formation ability in OSCC cells.

The function of IncRNA ZNF667-AS1 in OSCC cell migration and invasion

Overexpression of ZNF667-AS1 repressed the ability of migration and invasion in SCC-25 (Figure 3A, 3E) and HSC-2 cells (Figure 3B, 3F) significantly. Meanwhile, down regulation of ZNF667-AS1 expression could re-
IncRNA ZNF667-AS1 Suppresses EMT in OSCC

Clin. Lab. 8/2021

verse inhibitory functions of ZNF667-AS1 on cell migration ability and cell invasion ability in Tca-83 (Figure 3C, 3G) and CAL-27 cells (Figure 3D, 3H). The above findings indicated that IncRNA ZNF667-AS1 repressed OSCC cell migration and invasion.

**IncRNA ZNF667-AS1 could affect the EMT process in OSCC**

Epithelial cells could obtain metastasis ability and invasion ability through the EMT process, which promotes tumorigenesis. In order to analyze the function of IncRNA ZNF667-AS1 on EMT, we detected the expression of EMT-related markers (E-cadherin, N-cadherin, Vimentin, β-catenin, Twist1). Real-time PCR and western blot displayed that after over-expressing ZNF667-AS1, the expression of N-cadherin, Vimentin, β-catenin, and Twist1 at both mRNA and protein level were obviously reduced, and E-cadherin expression was elevated in SCC-25 (Figure 4A, p < 0.05) and HSC-2 cells (Figure 4B, p < 0.05). However, knocking down ZNF-667-AS1 obtained the contradictory results (Figure 4C - 4D, p < 0.05). The above findings showed that IncRNA ZNF667-AS1 repressed the EMT processes.

We further investigated the possible mechanism of ZNF667-AS1 inhibiting EMT processes. After over-expressing ZNF667-AS1, TGF-β1 mRNA and protein expression decreased (Figure 5A - 5B). Furthermore, we explored the effect TGF-β1 on EMT processes. After treatment with TGF-β1, N-cadherin, Vimentin, β-catenin and Twist1 were obviously elevated and E-cadherin was inhibited, which indicated that TGF-β1 promotes the process of EMT (Figure 5C - 5D). Meanwhile, TGF-β1 could partly reverse the inhibitory effect of ZNF667-AS1 on EMT processes. Thus, we speculate that ZNF-667-AS1 inhibits EMT processes by down regulating TGF-β1.

**Figure 3. Effects of ZNF667-AS1 on migration and invasion of OSCC cells.**

Overexpression of ZNF667-AS1 inhibits the ability of migration in SCC-25(A) and HSC-2 cells (B). Knockdown of ZNF667-AS1 enhances the ability of proliferation in Tca-83 (C) and CAL-27 cells (D). Overexpression of ZNF667-AS1 inhibits the ability of invasion in SCC-25 (E) and HSC-2 cells (F). Knockdown of ZNF667-AS1 enhances the ability of clone formation in Tca-83 (G) and CAL-27 cells (H). * p < 0.05.
Figure 4. ZNF667-AS1 affects the EMT process in OSCC.

The mRNA and protein expression levels of EMT markers are decreased after ZNF667-AS1 overexpression both in SCC-25(A) and HSC-2 cells (B). The mRNA and protein expression levels of EMT markers are increased after knockdown of ZNF667-AS1 both in Tca-83 (C) and CAL-27 cells (D). * p < 0.05.

DISCUSSION

Various studies have shown that tumorigenesis and progression of OSCC are associated with multiple gene mutations, chromosomal translocations, deletions or epigenetic changes [20,21]. IncRNA, as a major participant in epigenomic regulation, usually lacks the capacity of protein coding and regulates cell biological functions in various pathways, thus affecting the development of tumors [22]. Huang suggested that lncRNA NEAT1 improves OSCC cell proliferation ability and invasion ability by targeting miR-365 or RGS20 [23]. Other studies have shown that lncRNA PLAC2 promotes the proliferation ability and invasion ability of OSCC cells by activating the Wnt/β-catenin pathway [24]. However, the function of lncRNA ZNF667-AS1 on OSCC remains unclear. The results of this study showed that ZNF667-AS1 was low-expressed in both OSCC cells and tumor tissues and related to TNM stage and lymph node metastasis. As TNM stage and lymph node metastasis are closely related to prognosis, we speculate that ZNF667-AS1 may be related to prognosis. The results showed that low expression of ZNF667-AS1 showed poor prognosis. This is consistent with the study of ZNF667-AS1 in cervical cancer [25], ESCC [13], and laryngeal squamous cell carcinoma (LSCC) [26]. These results suggest that ZNF667-AS1 expression is low in OSCC patients, and it may be one of the ideal prognostic indicators.

ZNF667-AS1 regulates the development of a variety of tumors as a tumor suppressor. Zhao et al. showed that ZNF667-AS1 inhibited the proliferation ability of cervical cancer cells [25]. Other researches showed that ZNF667-AS1 restrained the ability of proliferation, invasion and migration in both ESCC cells [13] and LSCC cells [26]. To further study the function of ZNF667-AS1 on OSCC tumorigenesis, SCC-25 and HSC-2 were used for further experiments. After transfecting the ZNF667-AS1 plasmid, the ability of proliferation, invasion and migration of SCC-25 and HSC-2 cells were significantly down-regulated, while knockdown of ZNF667-AS1 reversed this process. This is consistent
IncRNA ZNF667-AS1 Suppresses EMT in OSCC

Figure 5. ZNF667-AS1 may inhibit the EMT process partly by repressing TGF-β1 in OSCC cells.

The mRNA (A) and protein (B) expression level of TGF-β1 is decreased after ZNF667-AS1 overexpression in SCC-25 cells. ZNF667-AS1 overexpression inhibits the EMT process, whereas TGF-β1 treatment enhances the EMT process both in mRNA (C) and protein (D) levels. * p < 0.05.

with the results of other cancer types. These results in vitro demonstrated that ZNF667-AS1 could play a role as a tumor suppressor gene, while the molecular mechanism of ZNF667-AS1 is unclear. IncRNAs can influence tumor growth and metastasis by regulating EMT [27], NF-κB [28], JAK/STAT3 [29], PI3K-Akt [30], and other signal pathways, ultimately promoting tumorigenesis and distant metastasis of other organs. EMT is an essential pathway of tumor migration and invasion. The feature of EMT is that cell polarity is losing and epithelial biomarkers decrease, including E-cadherin, cytokeratin and so on; besides, interstitial bio-

Clin. Lab. 8/2021 9
markers increased, for example Vimentin, some cadherins, fibronectins, and matrix metalloproteinase [14-16]. Our findings displayed that overexpression of ZNF667-AS1 promoted E-cadherin expression which is the epithelial cell marker, while it suppressed mesenchymal cell markers, including N-cadherin, Vimentin, β-catenin, and transcription factor Twist1. Knockdown of ZNF667-AS1 could reverse this process. These results reveal that ZNF667-AS1 can suppress the ability of invasion and metastasis in OSCC cells by inhibiting EMT. Although several articles have reported the inhibitory effect of IncRNA ZNF667-AS1 in tumors, there are few studies on its effect on EMT and the mechanism. In this paper, we preliminarily explored the mechanism by which ZNF667-AS1 affects the EMT process of OSCC. Previous studies had shown that TGF-β1 can induce the EMT process of breast cancer cells [31]. Therefore, we considered whether ZNF667-AS1 can affect the EMT process through TGF-β1. First of all, the mRNA and the protein level of TGF-β1 were decreased after ZNF667-AS1 overexpression. Furthermore, the effect of TGF-β1 on EMT had been explored. The results indicated that TGF-β1 could promote the process of EMT, TGF-β1 could partly reverse the inhibitory effect of ZNF667-AS1 on EMT processes. But the inhibitory effect could not be reversed completely. We speculate that there may still be other mechanisms affecting the inhibition of ZNF667-AS1 on EMT. Thus, we speculate that ZNF667-AS1 inhibits EMT processes by down regulating TGF-β1. Other mechanisms of IncRNA ZNF667-AS1 affecting the EMT process should be further studied.

CONCLUSION

In brief, the above findings displayed that IncRNA ZNF667-AS1 had low-expression in OSCC cells and tissues. IncRNA ZNF667-AS1 could restrain the ability of proliferation, cell clone, invasion and migration of OSCC cells and regulate the EMT process by low-regulating TGF-β1. These above results provide a new therapeutic target for OSCC.

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Declaration of Interest:
There are no conflicts of interest with other institutions or individuals.

References:


