Review Article

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Evaluation of Changes in miR-7113-3p, miR-6721-5p, and MAP2K1 gene expressions in tumor and normal tissues of patients with oral cancer

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ABSTRACT

Background: Squamous cell carcinoma of the oral cavity (OSCC) is one of the most common cancers in the Head and Neck Squamous Cell Cancer (HNSCC) group. The increasing frequency of oral carcinomas and their late-stage appearance is a major worldwide health concern. MicroRNAs (miRNAs) play an important role in cancer growth and progression, as the available relevant data indicate. However, no information is available about the parts miR-7113-3p and miR-6721-5p taken in OSCC. In the present study, the expression of MAP2K1, miR-7113-3p, and miR-6721-5p was examined to determine their possible biological role in the advancement of oral squamous cell carcinoma.
Methods: Quantitative Real-Time PCR was applied to investigate the mRNA expression of MAP2K1, miR-7113-3p, and miR-6721-5p in fresh frozen OSCC tissues and adjacent normal fresh frozen tissues of 30 patients and then, the relationship between MAP2K1 Expression and clinical parameters was studied.
Results: MAP2K1 expression dramatically increased in tumor tissues compared to the normal tissues, while miR7113-3p and miR-6721-5p expression significantly decreased. Furthermore, a statistical correlation of p=0.04 was also observed between increased MAP2K1 expression and Perineural invasion. In addition, the downregulation of miR-7113-3p was positively correlated with the overexpression of MAP2K1 (p=0.0218), and a negative correlation was observed between the downregulation of miR-6721-5p and overexpression of MAP2K1 (p=0.7771).
Conclusion: Based on the findings, miR-7113-3p, and miR-6721-5p might be prospective biomarkers for OSCC patients and can be utilized to detect OSCC at an early stage of its diagnosis. MAP2K1 overexpression is linked to the development of OSCC and Perineural invasion.

Keywords: OSCC, MAP2K1 Target gene, miR-7113-3p, miR-6721-5p, Quantitative real-time PCR

INTRODUCTION:

The OSCC is one of the deadliest head and neck tumors since it has a high risk of recurrence and invasion (1). Oral cancer has held the sixth rank among all human malignancies worldwide, and according to the literature, its mortality rate is so high (2). Regardless of therapy advances, OSCC has a poor prognosis, and its diagnosis and prediction using current biomarkers have remained challenging. (3).

Determination of genetic pathways that contribute to the pathogenesis of OSCC may aid in the development of therapeutic and diagnostic targets, which both have received insufficient experimental consideration (4). A study published in 2017 by a group of Chinese researchers found that the levels of MAPK-signaling protein components tend to be higher in patients with oral cancer. (5). MAP2K1, a gene related to the MAPK signaling pathway, is overexpressed in numerous cancers and may be linked to a prognostic biomarker of head and neck squamous cell carcinoma (6).

Because of their oncogenic and tumor-suppressive functions, microRNAs can be used as potential diagnostic and prognostic biomarkers for a wide range of types of cancer (7). The microRNAs (miRNAs) are short, non-coding RNA molecules of 15-22 nucleotides that modulate gene expression by silencing the target mRNA. The miRNA family plays an important regulatory role in various fundamental biological processes such as cell division, growth, and apoptosis (8). In recent years, many researchers have conducted extensive studies on the abnormal expression of microRNAs in various disorders, including cancer. In a majority of cases, their expression is repressed compared to normal tissues. The first study to suggest a correlation between microRNAs and cancer revealed the detection of miR-15a and miR16-1 which were frequently deleted in genomic regions in Chronic lymphocytic leukemia, between exon 2 and exon 5 of the Leu2 gene (9). DNA methylation is a major regulator of microRNA expression in OSCC, as it is in many other cancers. The microRNAs exhibit distinct expression patterns because tumor cells express themselves differently compared with normal cells. This vast spectrum of alterations in microRNA expression has also been noted between oral cancer cells and normal cells. In light of these findings, microRNAs may be beneficial as biomarkers for early-stage diagnosis of oral cancer, as well as in the introduction of cancer treatments and therapies based on miRNAs (10).

A previous study confirmed that has-miR-7113-5p targeted WNT10B. According to microarray studies, this miRNA was downregulated in PTSD (11). Furthermore, the researchers discovered that miR-6721 was linked to aberrant expression in patients with low cell-free DNA (cfDNA) fetal fractions (12). Interestingly, alterations in the expression of miR-7113-3p and miR-6721-5p, as well as their correlation with the target gene MAP2K1 in OSCC, have not yet been examined. (To select miR-7113-3p and miR-6721-5p, we consulted the bioinformatics databases Mirwalk (http://mirwalk.uni-hd.de) and miRDB (http://mirdb.org)).

Therefore, the present study aimed to examine the changes in the level of miR-7113-3p, miR-6721-5p, and MAP2K1 expression in tumor tissues and their adjacent normal tissues of OSCC patients. In the final analysis, by assessing the association of MAP2K1 with clinical and pathologic features, it was determined whether the MAP2K1 gene was involved in OSCC malignancy progression.

2. Materials and Methods:

2.1. Patients and Sample Collection:

Ethical approval of this study was obtained from the ethics committee of Islamic Azad University, East Tehran Branch, Tehran, Iran (IR.IAU.ET.REC.1400.036). The study utilized 30 pairs of tumor and adjacent normal tissue specimens collected between October 2020 and January 2021 at Imam Khomeini Hospital, Tehran, Iran. All patients who received adjuvant treatments, including chemo and radiation, were excluded from the study. Patient consent was obtained through written consent forms, and the clinicopathologic findings were derived from recorded data, which included Histological Grade, Lymphatic/Vascular Invasion, Perineural Invasion, Tu-

mor Size, and Lymph Node Invasion (Table1). The samples collected from the Tumor Bank of Cancer Institute (Imam Khomeini Hospital, Tehran, Iran) were approved by an orthodontic specialist and a pathologist. We immediately preserved fresh tissue samples in liquid nitrogen and stored them at -80°C until RNA extraction.

2.2. RNA Extraction and Quantitative Real-Time PCR (QRT-PCR):

After following the manufacturer's instructions, TRIZOL reagent was used to extract RNA (Invitrogen, Sigma, USA). Electrophoresis in 1.5% agarose gel and a Nanodrop ND-2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to confirm the quality and quantity of extracted RNAs, respectively (the light absorption ratio of 260 nm to 280 nm in pure RNA is around 1.9 to 2.0 and it has a 28S to 18S bond strength of 2:1). Total extracted RNA was reverse-transcribed using BioFACT's cDNA Synthesis kit to synthesize complementary DNA (cDNA) (Daejeon, South Korea), according to the manufacturer's protocol. Additionally, cDNA for miRNAs was synthesized using appropriate stem-loop RT primers and the MiR-Amp kit (Pars Genome, Iran). The SYBR Green RT-PCR Kit (BioFact, Daejeon, South Korea) was used to conduct the quantitative real-time PCR analysis on a Roche ExicyclerTM 96 thermocycler. The following thermal cycling profile was employed for quantitative real-time PCR on miRNAs and MAP2K1: 95°C for 2 min, followed by 40 cycles of 95°C for 15 s for denaturation, 60°C for 30 s for annealing, and 72°C for 20 s for elongation. By employing ACTB as a housekeeping gene, the expression of MAP2K1 was normalized. In addition, the expression of miR-7173-3p and miR-6721-5p was standardized using U6 as an endogenous control. After completion of the preceding stages, the received information was checked for the Melting curve and the obtained diagrams were examined for dimer formation.

The findings of the melting curve of these samples revealed that the microRNAs product was proprietary and had its TM, as well as a single peak, thus confirming the correctness of the primers and the accuracy of Real-Time PCR. Finally, the CT number was calculated using the provided data. Primers were designed using Oligo Analyzer and the Primer3plus program, evaluated for optimal properties through the BLAST program, and synthesized by BIONEER (Daejeon, South Korea). A summary of the primer sequences can be found in Table 1.

3. Statistical Analysis:

The results were provided as the mean ± SEM of three identical experiments carried out in triplicate. GraphPad Prism software 9.0.0 (GraphPad Software, Inc., San Diego, CA, USA) and SPSS software were used to analyze the data (version 21.0; SPSS, Inc., Chicago, IL, USA). To determine the normal distribution of sample data, the One-Sample Kolmogorov-Smirnov Test was performed. The independent-sample Kruskal-Wallis tests were used to evaluate the association between MAP2K1 levels and clinicopathological features in OSCC patients. Further-

Table 1. Real-time quantitative PCR primers.

Genes	5′-3′ Primer Sequence
MAP2K1	F: GGTGTTCAAGGTCTCCCACAAG R: CCACGATGTACGGAGAGTTGCA
MiR-6721-5p	F: CGGGCTGGGCAGGGGCTTATT R: CGCAGGGTCCGAGGTATTC
MiR-7113-3p	F: TCCAGGGAGACAGTGTGTGA R: CCAGTGCAGGGTCCGAGGTA
ACTB	F: GATCAAGATCATTGCTCCTCCTG R: CTAGAAGCATTTGCGGTGGAC
U6	F: CTCGCTTCGGCAGCACA R: AGAGCAGGGTCCGAGGT

more, the one-way analysis of variance (ANOVA) was employed to compare the level of MAP2K1 expression in different tumor sizes and clinical stages. The correlation between miR-7113-3p, miR-6721-5p, and MAP2K1 expression was investigated by applying Pearson correlation and regression analysis. Gene expression differences were calculated using Genex6 software. To analyze the relationship between levels of variables and disease probability, the odds ratio method was employed. This parameter was calculated using logistic regression in SPSS software. Finally, the diagnostic value was evaluated using the receiver operating characteristic (ROC) curve. A p-value of less than 0.05 (?0.05) was regarded as statistically significant.

4. Results:

4.1. miR-7113-3p and miR-6721-5p Expression Were Downregulated in OSCC:

miR-7113-3p and miR-6721-5p Expression Were Downregulated in OSCC:

The expression patterns of miR-7113-3p and miR-6721-5p were examined in 30 paired OSCC tissues and adjacent normal oral tissues using RT-qPCR. Then, miR-7113-3p and miR-6721-5p expression levels were both reduced, as shown in Fig. 1. (4.24-folds and 1.85-folds, respectively), in OSCC tissues compared to normal tissues (p=0.00000 and p=0.00001, respectively).

4.2. MAP2K1 gene expression was upregulated in OSCC:

In the current study, RT-qPCR was used to evaluate the expression of MAP2K1 as a possible target for miR-7113-3p and miR-6721-5p in 30 paired OSCC tissues and adjacent normal oral tissues. Mirwalk and miRDB algorithms were used to discover potential co-targets of miR-7113-3p and miR-6721-5p in OSCC. Online bioinformatics databases confirmed that MAP2K1 may be an acceptable direct target for the corresponding miRNAs. MAP2K1 expression was observed to be considerably higher (3.087-folds) in tumor tissues compared to adjacent normal oral tissues (p=0.00000) (Fig. 2).

4.3. The Correlation between MAP2K1 Expression and miR-7113-3p, miR-6721-5p in OSCC Patients:

The Pearson correlation analysis was used to examine the connection between miR-7113-3p and miR-6721-5p levels and MAP2K1 expression in OSCC. An inverse and significant correlation were observed between miR-7113-3p downregulation and MAP2K1 target gene over-

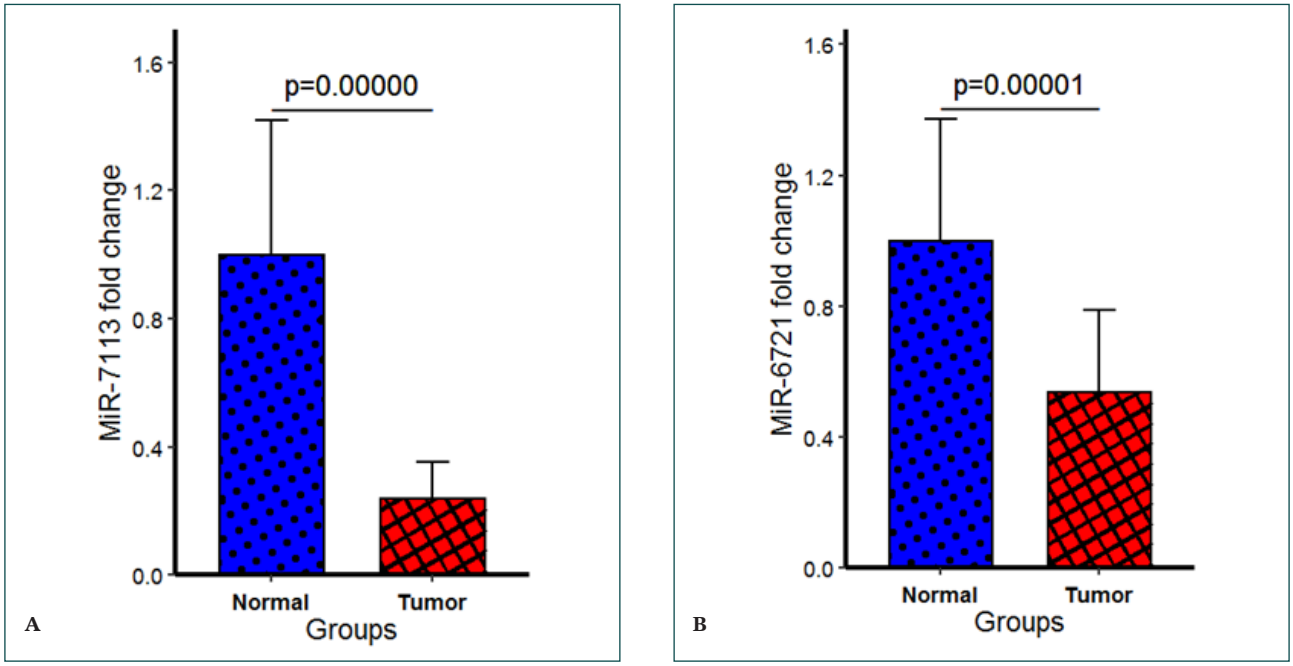


Figure 1. Downregulated expressions of miR-7113-3p (A) and miR-6721-5p (B) in oral squamous cell carcinoma. Data are shown as means±SD of three separate experiments. Transcript levels were normalized to U6 expression. (*p<0.05) (n=30).

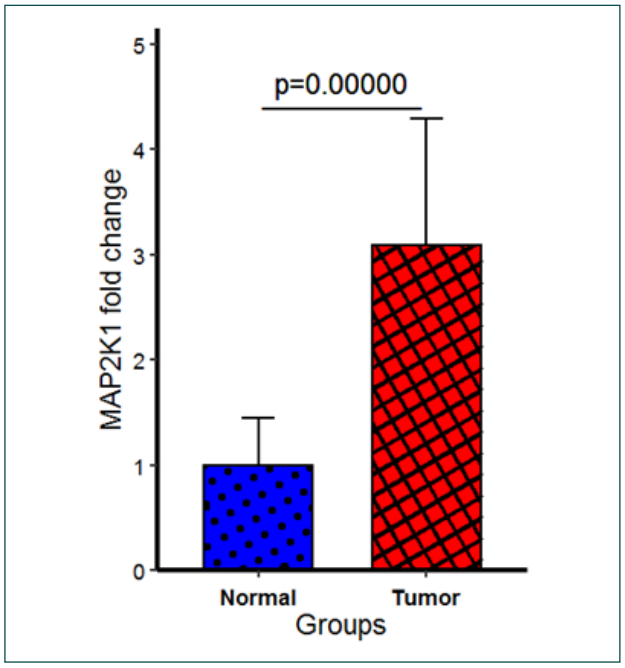


Figure 2. Quantitative RT-PCR analysis of MAP2K1 expression in OSCC tissues and adjacent normal tissues (n=30). Transcript levels were normalized to ACTB expression. Data are presented as means ± SD. (*p<0.05).).

expression in OSCC (r=-0.295, p=0.021). A direct and nonsignificant correlation was also identified between miR-6721-5p downregulation and MAP2K1 overexpression (r=0.037, p=0.777) (Fig. 3).

addition, MAP2K1 expression was significantly correlated with miR-7113-3p, while MAP2K1 expression and miR-6721-5p were not significantly correlated (P=0.021 and P=0.777 respectively).

4.4. Potential Diagnostic Values of MAP2K1 in OSCC:

Based on ROC curve analysis, MAP2K1 was evaluated for its potential to diagnose OSCC. The area under the curve (AUC) of MAP2K1 was 0.9466 (95%CI=0.8934-0.9999; P=0.00000). The best cutting point is indicated by the threshold and its sensitivities and specificities are also provided. To choose the best cutting point, a value of J or the Youden index is employed (J=0.8333). The optimal MAP2K1 cutting point is Δct = 6.8125, with a sensitivity of 0.8667 and a specificity of 0.9667.

4.5. Potential Diagnostic Values of miR-7113-3p and miR-6721-5p in OSCC:

The potential diagnostic value of mir-7113-3p and mir-6721-5p for OSCC was assessed by ROC curve analysis. According to the following tables, the value of AUC for miR-7113-3p is 0.9666 (95%CI=0.9284-1; P=0.00000) and for miR-6721-5p is equal to 0.8261 (95%CI=0.7155-0.9367; P=0.00000).

4.6. The Association Between MAP2K1 Expression and Clinicopathological Features:

The association between MAP2K1 expression levels and some other clinicopathological parameters of OSCC patients was investigated in Table 2 to gain a better knowledge of its possible function in the development of oral cancer. It was found that MAP2K1 expression was noticeably associated with tumor Perineural invasion (p=0.041). According to the presence of Perineural invasion in 30 patients, 37% were positive (n=11), and 63% were negative (n=19). MAP2K1 expression increased in all patients considering the small number of patients with Perineural invasion. Thus, the presence of Perineural invasion was significantly associated with MAP2K1 upregulation (p<0.05). Other clinicopathological features including necrosis (0.345), age (p=0.702), tumor size (p=0.904), vascular invasion (p=0.627), pathological grading (p=0.865), clinical stage (p=0.139), lymph node status (p=0.601), depth invasion (p=0.865), clinical metastasis (0.614), family history (0.456), gender (p=0.159) and smoking status (p=0.443) did not have statistically significant associations with increased MAP2K1 expression.

5. Discussion:

Head and Neck Squamous Cell Cancer (HNSCC) is a serious public health problem globally, with a high fatality rate. The most frequent kind of HNSCC is oral squamous cell carcinoma (OSCC), which remains a concern for head and neck specialists despite major advances in diagnostic techniques and treatments (13). Oral cancer is a multifactorial disease caused by a combination of genetic abnormalities and environmental factors, the most important of which are tobacco and alcohol use (14). Epigenetic alterations, such as DNA methylation, histone modifications, and non-coding RNA modifications (miRNAs), have been shown to play an important

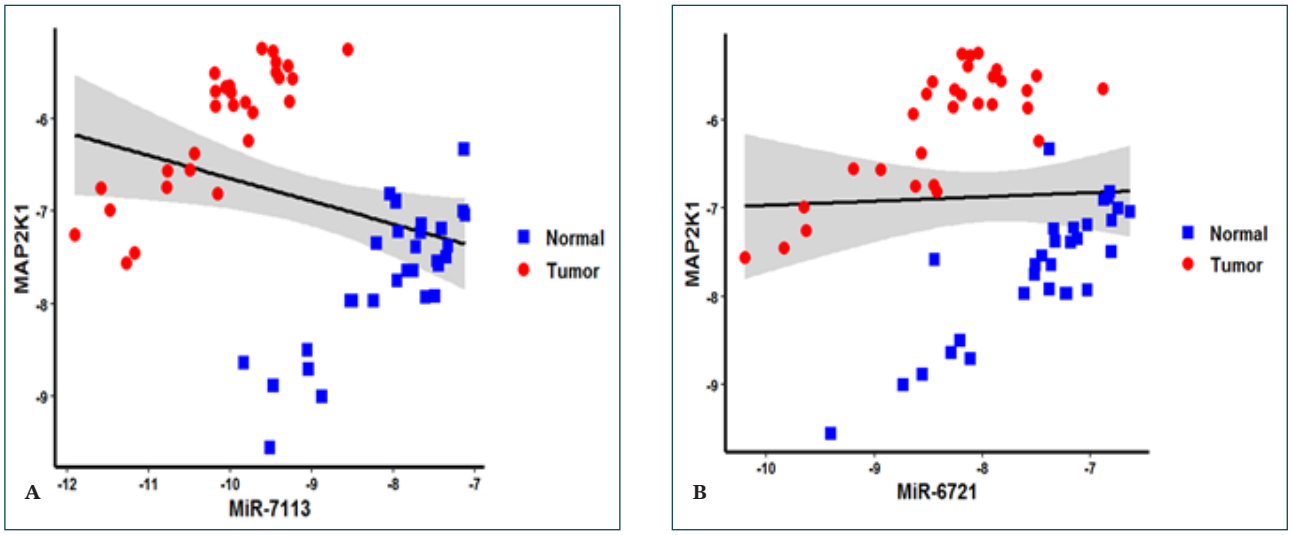
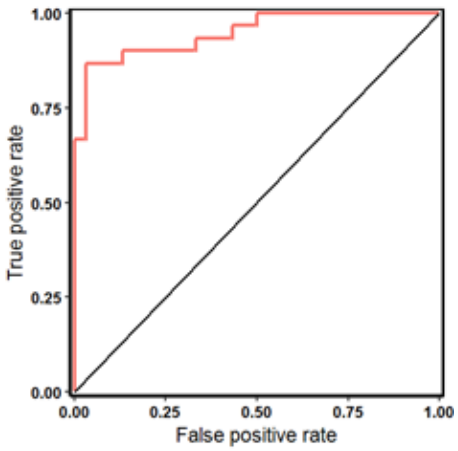


Figure 3. Pearson's correlation analysis between MAP2K1 mRNA expression and miR-7113-3p (A) and miR-6721-5p (B) levels in OSCC patients. Data are presented as means ± SE. In addition, MAP2K1 expression was significantly correlated with miR-7113-3p, while MAP2K1 expression and miR-6721-5p were not significantly correlated (P=0.021 and P=0.777 respectively).

MAP2K1:



AUC	CI_lower	CI_high
0.946667	0.893397	0.999937

threshold	sensitivities	specificities	J
6.8125	0.866667	0.966667	0.833333

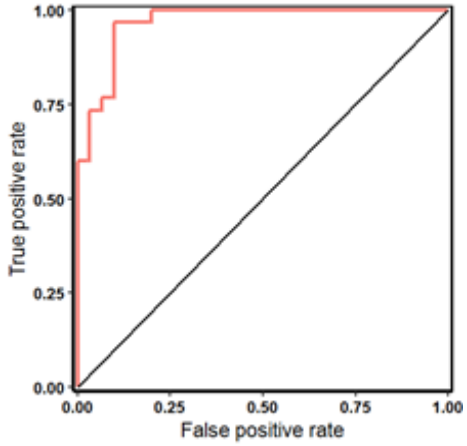
Figure 4. ROC curve analysis related to MAP2K1 expression can distinguish patients with OSCC from healthy controls (P=0.0000).

regulatory role in the development and progression of oral cancer (15).

MiRNAs seem to be essential in the epigenetic regulation of cellular processes such as cell cycle regulation, differentiation, apoptosis, and migration. MiRNA dysregulation leads to tumor-related events throughout cancer development (16). In this way, miRNAs can control gene expression involved in cancer biology as oncogenes or tumor suppressors (17).

Numerous recent studies have shown that microRNA expression alters in oral squamous cell carcinoma and some microRNAs function as tumor suppressors or tumor promoters during tumorigenesis. Tumor suppressor miRNAs like miR-26-a, miR-99a-5p, miR-375, and miR-139-5p were down-regulated in oral cancer and inhibited oncogenes, whereas OncomiRs like miR-21, miR-31, miR-93, miR-211, and miR-373 were up-regulated in oral cancer and inhibited tumor suppressors (18). Furthermore, a study on the expression of numerous microRNAs indicated that miR-31 may be an ideal candi-

miR-7113-3p:

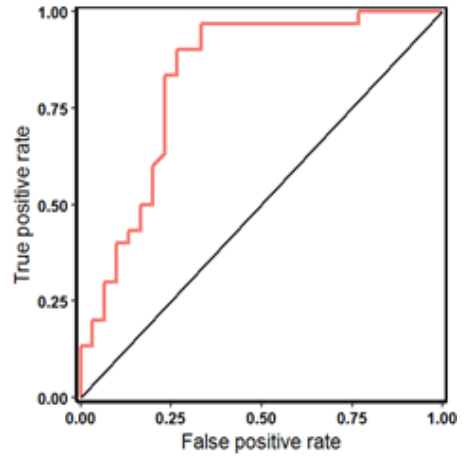


AUC	CI_lower	CI_high
0.966667	0.92843	1

threshold	sensitivities	specificities	J
9.1475	0.966667	0.9	0.866667

Figure 5. Analysis of the ROC curve for miR-7113-3p expression (P=0.0000).

miR-6721-5p:



AUC	CI_lower	CI_high
0.826111	0.715481	0.936742

threshold	sensitivities	specificities	J
7.465	0.966667	0.666667	0.633333

Figure 6. Analysis of the ROC curve for miR-6721-5p expression (P=0.00001).

date for clinical application in oral cancer due to its high sensitivity in tissue, saliva, and plasma (19).

There have been some important candidate miRNAs involved in the progression of oral cancer as previous studies have demonstrated. Downregulation of miR-125a, miR-184, and miR-16, as well as upregulation of miR-96, were noted in both oral tumors and surgical margins, suggesting that combinatorial regulation of these miRNAs and target transcription factors contributes to oral tumorigenesis and is useful in detecting minimal residual disease after surgery (20).

While miR-7113-3p and miR-6721-5p have frequently been reported to contribute to a variety of cancers, no study has evaluated their expression in OSCC. For instance, miR-7113 was upregulated by AnAc in MDA-MB-231 cells and targets the host gene NDUFS8 to cause breast cancer (21). According to Qiang. Guo', has-miR-7113-3p participates in the LINC00973-miRNA-mRNA cRNA network and enhances in non-small-cell lung cancer tissues (NSCLC) (22). According to the findings, the circ 0034467_ miR-6721-5p - SLC19A1 regulatory network may serve as a key regulator in prostate cancer

Table 2. The clinicopathological characteristics and MAP2K1 expression of OSCC patients.

Clinicopathological Characteristic	Total cases (n=30)	p-value
Gender		
Female	7	0.159
male	23	
Family history		
Yes	6	0.456
No	24	
Smoking status		
Non-smokers	22	0.443
Smokers	7	
Ex-smokers	1	
Vascular invasion		
Yes	6	0.627
No	24	
Perineural invasion		
Yes	11	*0.041
No	19	
Age (years)		
>40	27	0.702
<40	2	
Tumor Size (cm)		
<2	5	0.904
2-5	14	
>5	11	
Pathological grading		
I	17	0.865
II	13	
Clinical stage		
I	3	0.139
II	3	
III	7	
IV	17	
Lymph node metastasis		
Yes	5	0.601
No	24	
Unknown	1	
Depth invasion		
Yes	9	0.865
No	21	
Necrosis presence		
Yes	7	0.345
No	23	
Clinical Metastasis		
Yes	1	0.614
No	29	

(23). Additionally, one study demonstrated that miR-6721-5p was downregulated by HOXC6, another gene related to cancer progression (24). Based on these results, miR-7113-3p and miR-6721-5p may represent potential biomarkers in OSCC and different cancers by exerting oncogenic or tumor-suppressive functions. It is, however, necessary to conduct more research to verify these findings.

The current study aimed to discover new diagnostic or prognostic biomarkers for OSCC. The miR-7113-3p and miR-6721-5p are significantly down-regulated in OSCC tissues compared to normal tissues, according to our analyses.

The role of MAP2K1 in tumorigenesis and cancer progression has been noted previously as a candidate for further studies. Activated MAP2K1 promotes cancer cell proliferation and confers drug resistance. The results of Zhe Jin's study suggested that blocking MAP2K1 and miR-330-3p inhibited the ability of HepG2 cells to migrate. In this study, miR-330-3p suppressed the migration of liver cancer cells by interacting with MAP2K1 (25). In addition, Jiacong You observed MAP2K1 overexpression in non-small cell lung cancer and discovered that miR-449a regulated MAP2K1 expression by directly targeting its 3'UTR (26). MAP2K1 mutations have been identified at a lower frequency in several cancers, including lung adenocarcinoma, melanoma, and gastric cancer. About 1% of HNSCC cases exhibit MAP2K1 mutations, just like lung cancer (27).

MAP2K1 has been shown to regulate tumorigenic development in OSCC. It is primarily responsible for cancer proliferation, chemoresistance, invasion, and migration in oral cancer (28). Further studies demonstrated that MAP2K1 activation increased CD44 expression and promoter activity, whereas CD44 attenuation decreased both in vitro migration and in vivo oral tumor formation (29). Another study indicated that MAP2K1 activation frequently occurs in oral malignancies and is linked to tumor cell proliferation, migration, and invasion through regulating antiapoptotic and proliferative pathways (30). These findings confirmed what we discovered in the present study.

Based on bioinformatics analysis, MAP2K1 is a direct target of miR-7113-3p and miR-6721-5p. A significant increase was also observed in MAP2K1 gene expression in tumor tissues, particularly in comparison to adjacent normal tissues from OSCC patients, which supports previously reported results. However, miR-7113-3p and miR-6721-5p expression levels significantly decreased. In the current study, we correlated the expression level of miR-7113-3p and miR-6721-5p to MAP2K1 mRNA and showed a significant inverse correlation between miR-7113-3p downregulation and MAP2K1 target gene overexpression in OSCC ($r=-0.295$, $p=0.021$). It was also shown that there is a non-significant association between miR-6721-5p downregulation and MAP2K1 overexpression ($r=0.037$, $p=0.777$).

Perineural invasion (PNI) is a form of tumor progression in which cancer cells encroach along nerves (31). Perineural invasion is well known to be associated with a poor outcome in cancers of the colorectal, pancreas, and salivary glands. The perineural invasion has been reported to occur in 2–82 % of oral squamous cell carcinoma and Perineural invasion and prognostic factors were shown to be correlated (32). According to the present study, there was a significant association between MAP2K1 overexpression and Perineural invasion status in OSCC tumors ($p=0.041$) and no remarkable association was found between vascular and depth invasions with MAP2K1 overexpression ($p=0.627$ and $p=0.865$ respectively). Furthermore, MAP2K1 expression increased in tumors in late stages (grade II), although no significant correlation was found ($p=0.139$). Moreover, the overexpression of MAP2K1 was not correlated with necrosis presence ($p=0.345$), clinical metastasis ($p=0.614$), tumor size ($p=0.904$), pathological grading ($p=0.865$), smoking status ($p=0.443$) and family history ($p=0.456$). To confirm these results and gain a better understanding of the relationship between the MAP2K gene and miR-7113-3p and miR-6721-5p expression in oral cancer malignancy, and modify the aggressive behaviors of oral cancer cells in clinical trials, further research on the expression of the MAP2K1 protein is required.

6. Conclusion:

The present study, to the best of our knowledge, is the first work that evaluated miR-7113-3p and miR-6721-5p expression in OSCC and showed increased expression of the MAP2K1 gene and decreased expression of miR-7113-3p and miR-6721-5p in tumor tissues, compared to normal adjacent tissues of OSCC patients. As potential diagnostic and prognostic biomarkers for OSCC patients, miR-7113-3p and miR-6721-5p have the potential to become powerful biomarkers shortly, and they may even contribute to the early diagnosis and prognosis of this disease.

REFERENCES

1. Zhao W, Cui Y, Liu L, Qi X, Liu J, Ma S, et al. Splicing factor derived circular RNA circUHRF1 accelerates oral squamous cell carcinoma tumorigenesis via feedback loop. Cell Death & Differentiation. 2020;27(3):919-33.
2. Roi A, Roi CI, Negruțiu ML, Riviș M, Sinescu C, Rusu L-C. The challenges of OSCC diagnosis: Salivary Cytokines as potential biomarkers. Journal of Clinical Medicine. 2020;9(9):2866.
3. Ries J, Baran C, Wehrhan F, Weber M, Motel C, Kesting M, et al. The altered expression levels of miR-186, miR-494 and miR-3651 in OSCC tissue vary from those of the whole blood of OSCC patients. Cancer Biomarkers. 2019;24(1):19-30.
4. Chang K-W, Liu C-J, Chu T-H, Cheng H-W, Hung P-S, Hu W-Y, et al. Association between high miR-211 microRNA expression and the poor prognosis of oral carcinoma. Journal of dental research. 2008;87(11):1063-8.
5. Peng Q, Deng Z, Pan H, Gu L, Liu O, Tang Z. Mitogen-activated protein kinase signaling pathway in oral cancer. Oncology letters. 2018;15(2):1379-88.
6. Lawal B, Lee C-Y, Mokgautsi N, Sumitra MR, Khedkar H, Wu AT, et al. mTOR/EGFR/iNOS/MAP2K1/FGFR/TGFB1 are druggable candidates for N-(2, 4-difluorophenyl)-2′, 4′-difluoro-4-hydroxybiphenyl-3-carboxamide (NSC765598), with consequent anticancer implications. Frontiers in oncology. 2021;11:656738.
7. Baghaei F, Abdollahi A, Mohammadpour H, Jahanbin M, Naseri Taheri F, Aminishakib P, et al. PTEN and miR-26b: Promising prognostic biomarkers in initiation and progression of Oral Squamous Cell Carcinoma. Journal of Oral Pathology & Medicine. 2019;48(1):31-5.
8. Shan C, Chen X, Cai H, Hao X, Li J, Zhang Y, et al. The emerging roles of autophagy-related microRNAs in cancer. International Journal of Biological Sciences. 2021;17(1):134.
9. Acunzo M, Romano G, Wernicke D, Croce CM. MicroRNA and cancer–a brief overview. Advances in biological regulation. 2015;57:1-9.
10. Yoshizawa JM, Wong DT. Salivary microRNAs and oral cancer detection. MicroRNA Protocols. 2013:313-24.
11. Bam M, Yang X, Busbee BP, Aiello AE, Uddin M, Ginsberg JP, et al. Increased H3K4me3 methylation and decreased miR-7113-5p expression lead to enhanced Wnt/β-catenin signaling in immune cells from PTSD patients leading to inflammatory phenotype. Molecular Medicine. 2020;26(1):1-15.
12. Santoro G, Lapucci C, Giannoccaro M, Caporilli S, Rusin M, Seidenari A, et al. Abnormal Circulating Maternal miRNA Expression Is Associated with a Low (< 4%) Cell-Free DNA Fetal Fraction. Diagnostics. 2021;11(11):2108.
13. Capote-Moreno A, Brabyn P, Muñoz-Guerra M, Sastre-Pérez J, Escorial-Hernandez V, Rodríguez-Campo F, et al. Oral squamous cell carcinoma: epidemiological study and risk factor assessment based on a 39-year series. International Journal of Oral and Maxillofacial Surgery. 2020;49(12):1525-34.
14. Rivera C. Essentials of oral cancer. International journal of clinical and experimental pathology. 2015;8(9):11884.
15. Irimie AI, Ciocan C, Gulei D, Mehterov N, Atanasov AG, Dudea D, et al. Current insights into oral cancer epigenetics. International journal of molecular sciences. 2018;19(3):670.

16. Rapado-González Ó, López-López R, López-Cedrún JL, Triana-Martínez G, Muinelo-Romay L, Suárez-Cunqueiro MM. Cell-free microRNAs as potential oral cancer biomarkers: from diagnosis to therapy. Cells. 2019;8(12):1653.
17. Barbato S, Solaini G, Fabbri M. MicroRNAs in oncogenesis and tumor suppression. International Review of Cell and Molecular Biology. 2017;333:229-68.
18. D'Souza W, Kumar A. microRNAs in oral cancer: Moving from bench to bed as next generation medicine. Oral Oncology. 2020;111:104916.
19. Fang C, Li Y. Prospective applications of microRNAs in oral cancer. Oncology Letters. 2019;18(4):3974-84.
20. Manasa V, Kannan S. Impact of microRNA dynamics on cancer hallmarks: an oral cancer scenario. Tumor Biology. 2017;39(3):1010428317695920.
21. Schultz DJ, Muluhngwi P, Alizadeh-Rad N, Green MA, Rouchka EC, Waigel SJ, et al. Genome-wide miRNA response to anacardic acid in breast cancer cells. PloS one. 2017;12(9):e0184471.
22. Guo Q, Li D, Luo X, Yuan Y, Li T, Liu H, et al. The Regulatory Network and Potential Role of LINC00973-miRNA-mRNA ceRNA in the Progression of Non-Small-Cell Lung Cancer. Frontiers in immunology. 2021;12.
23. Xia Q, Ding T, Zhang G, Li Z, Zeng L, Zhu Y, et al. Circular RNA expression profiling identifies prostate cancer-specific circRNAs in prostate cancer. Cellular Physiology and Biochemistry. 2018;50(5):1903-15.
24. Jeong S, Kim S-A, Ahn S-G. HOXC6-Mediated miR-188-5p Expression Induces Cell Migration through the Inhibition of the Tumor Suppressor FOXN2. International journal of molecular sciences. 2021;23(1):9.
25. Jin Z, Jia B, Tan L, Liu Y. miR-330-3p suppresses liver cancer cell migration by targeting MAP2K1. Oncology Letters. 2019;18(1):314-20.
26. You J, Zhang Y, Li Y, Fang N, Liu B, Zu L, et al. MiR-449a suppresses cell invasion by inhibiting MAP2K1 in non-small cell lung cancer. American journal of cancer research. 2015;5(9):2730.
27. Jain AP, Patel K, Pinto S, Radhakrishnan A, Nanjappa V, Kumar M, et al. MAP2K1 is a potential therapeutic target in erlotinib resistant head and neck squamous cell carcinoma. Scientific reports. 2019;9(1):1-12.
28. Lien M-Y, Chang A-C, Tsai H-C, Tsai M-H, Hua C-H, Cheng S-P, et al. Monocyte chemoattractant protein 1 promotes VEGF-A expression in OSCC by activating ILK and MEK1/2 signaling and downregulating miR-29c. Frontiers in Oncology. 2020;10:592415.
29. Judd NP, Winkler AE, Murillo-Sauca O, Brotman JJ, Law JH, Lewis JS, et al. ERK1/2 Regulation of CD44 Modulates Oral Cancer Aggressiveness-sERK1/2 Controls Mouse Oral Cancer Growth via CD44. Cancer research. 2012;72(1):365-74.
30. Lin C, Tu C, Ma Y, Ye P, Shao X, Yang Z, et al. Curcumin analog EF24 induces apoptosis and downregulates the mitogen activated protein kinase/extracellular signal-regulated signaling pathway in oral squamous cell carcinoma. Molecular Medicine Reports. 2017;16(4):4927-33.
31. Zhang M, Zhu Z-L, Gao X-L, Wu J-S, Liang X-H, Tang Y-L. Functions of chemokines in the perineural invasion of tumors. International Journal of Oncology. 2018;52(5):1369-79.
32. Matsushita Y, Yanamoto S, Takahashi H, Yamada S-i, Naruse T, Sakamoto Y, et al. A clinicopathological study of perineural invasion and vascular invasion in oral tongue squamous cell carcinoma. International journal of oral and maxillofacial surgery. 2015;44(5):543-8.