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Differential expression of hsa_circ_0064357 and hsa_circ_0064358 between oral squamous cell carcinoma and oral lichen planus

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Abstract. Background/aims: Reliable biomarkers with high specificity and sensitivity and the potential to discriminate precancerous or early lesions from oral cancer improve scientific assessment and early detection. Dysregulated circRNAs play a critical role in the occurrence and progression of malignant biological behaviors of OSCC. The study of potential diagnostic roles of hsa_circ_0064357 and hsa_circ_0064358 in early diagnostic of precancerous lesions such as OLP to OSCC as the most common type of head-and-neck squamous cell carcinoma (HNSCC) was the focus of present research. **Methods:** The differential expression of hsa_circ_0064357, hsa_circ_0064358, and *RAF1* target gene predicted using CircInteractome and Circbase databases between OSCC (n=30), OLP (n=10) tissues and their adjacent normal tissues were evaluated by qRT-PCR. The potential diagnostic value of circRNAs was identified by receiver operating characteristic (ROC) curve analysis. **Results:** hsa_circ_0064357 and hsa_circ_0064358 were identified to be lowly expressed, while RAF1 was upregulated in OSCC and OLP tissues more than adjacent normal tissues. Low expression of circRNAs was markedly correlated with TNM stages of OSCC patients. ROC analysis revealed AUC of 0.962 and 0.965 for hsa_circ_0064357 and hsa_circ_0064358, respectively, suggesting that circRNAs can serve as novel diagnostic biomarkers for early detection of OSCC. Conclusion: hsa_circ_0064357 and hsa_circ_0064358 might be involved in the progression and metastasis of OSCC and could be used as promising novel biomarkers for early diagnosis and the clinical monitoring of the malignant transformation of OLP into OSCC.

Keywords: hsa_circ_0064357, hsa_circ_0064358, OSCC, OLP, *RAF1* gene

Introduction

Oral squamous cell carcinoma (OSCC) is the most common type of malignant tumor of the oral and maxillofacial region (More than 90% of cancer cases) and the sixth most common neoplasm arising in the oral mucosa worldwide (Lin *et al*., 2021). The Global Cancer Observatory (GCO) estimated the annual incidence of OSCC at 377,713 cases and 177,757 deaths globally in 2020 (Ali, 2022). In Iran, lip and oral cavity cancer exhibit similar incidence to India and Pakistan, with an estimated incidence and age-standardized incidence rates (ASIR) of 1.4 and 1.3 per 100,000 individuals among men and women, respectively, in 2020 (Zendehdel, 2021). Among patients diagnosed with OSCC, approximately 60% present advanced locoregionally disease (stage III or IV), and 30-50% have local or distant metastasis probably due to invasion ability and lymphangio genesis of tumor cells (Biswas *et al*., 2019; Xu *et al*., 2021). Detection of late-stage or fully developed tumors and irregular follow-up in the progression of the malignant transformation of precancerous lesions such as oral lichen planus (OLP), which are amenable to resection, are markedly associated with the poor 5-year survival rate of OSCC patients (60%) (Ferlini *et al*., 2013; Tampa *et al*., 2018). Therefore, identifying biomarkers and molecular alterations in OLP as predictors of OLP malignant transformation is imperative.

Raf1 (C-Raf or c-Raf-1) is the integral serine-threonine kinase and downstream effector of the central signal transduction mediator (Ras) belonging to the ERK/MAPK (Extracellular signal-regulated kinase /mitogen-activated protein kinase) pathway, a critical signaling network responsible for regulating diverse physiological processes, including cell proliferation, differentiation, and apoptosis (Guo *et al*., 2020). Several lines of evidence demonstrated that dysregulated upregulation of wild-type Raf-1 and oncogenic raf-1 mutations, particularly those leading to cellular proliferation and increased cell survival, are associated with carcinogenesis and tumor invasion in various cancer types such as colorectal prostate and thyroid cancer (Gollob *et al*., 2009; Tian *et al*., 2018). Circular RNAs (circRNAs) are a class of endogenous non-coding RNAs that, unlike linear RNAs, have unique circular covalently bonded structures without polarity or a polyadenylated tail, which give them stability and higher tolerance to exonuclease (Meng *et al*., 2017). In recent decades, circRNAs have been widely discovered in human, animal, and plant species through high-throughput sequencing (Huang *et al*., 2022). Abnormal expression of circRNAs is reportedly correlated with the onset and development of various cancers, suggesting their potential role as biomarkers in molecular diagnosis and predicting the development of tumors (Wang *et al*., 2021).

However, to the best of our knowledge, the differential expression of hsa_circ_0064357 and hsa_circ_0064358 in OSCC and OLP have not been clarified. Therefore, in this experimental study, we aimed to identify the expression of circRNAs and their predicted target gene (*RAF1*) in OSCC and OLP samples compared to their normal adjacent tissues. Further, we determined whether the circRNAs and *RAF1* level are associated with clinicopathological characteristics in OSCC patients.

Materials and methods

Clinical samples

In this study, a total of 40 samples (30 patients with OSCC and 10 patients with OLP) and their related-adjacent normal oral epithelial tissue specimens were collected from surgical specimens by the Iran National Tumor Bank, Cancer Institute, Imam Khomeini Hospital, Tehran, between October 2020 and January 2021. All patients have a precise histologic diagnosis of OSCC based on diagnostic criteria of the World Health Organization (Pindborg *et al*., 1997). All protocols of the present study were approved by the Research Medical Ethics Committee of Imam Khomeini Hospital (IR.IAU.SRB.REC.1399.093). Every sample is obtained with the patient's informed consent, and none of the patients had previously undergone chemotherapy and any local or systemic treatment before surgery. The tissue samples were confirmed by experienced pathologists, frozen in liquid nitrogen, and stored at −80°C until RNA extraction. Clinicopathological information such as age, gender, tumor stage, and other variables was collected from patients' medical records. The degree of tumor differentiation was classified into well, moderately, and poorly differentiated squamous cell carcinoma, according to World Health Organization (Pindborg *et al*., 1997).

RNA extraction and Quantitative RT-PCR (qRT-PCR)

Total RNA extraction was performed from tissues with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol, and the concentration, quality, and integrity of extracted RNAs were evaluated using 1% agarose gel electrophoresis and NanoDrop spectrophotometer (Thermo scientific-Nanodrop 2000), respectively. The reverse transcription (RT) reaction in 20 µL of reaction mixture containing 1 µg of total RNA was performed with oligo-dT primers using a BioFACT cDNA (complementary DNA) Synthesis kit (Daejeon, South Korea). For circRNAs, Total RNA was incubated with RNase R for 15 min at 37 °C to deplete the linear RNAs and cDNA was synthesized from

2 µg of total RNA with random hexamer primers by a High-Capacity cDNA Reverse Transcription kit (Thermo Fisher Scientific, USA) to determine the hsa_circ_0064357 and hsa_circ_0064358 levels. qRT-PCR analysis of circRNAs and *RAF1* gene was performed on a LightCycler TM 96 (Roche) using a SYBR Green Master Mix (TAKARA, Japan) as per the manufacturer's instructions. PCR was performed using the following program: holding stage at 95°C for 5 min, cycling stage comprising 40 cycles (95°C for 15 s, 60°C for 30 s, 72°C for 20 s), melt curve stage at 60°C for 1 min, and 95°C for 15 s. All the primers were designed using Primer3plus software, and sequences have been shown in Tab. 1. Relative quantification of circRNAs and *RAF1* expression was compared with *ACTB* internal standards and were measured using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Table 1. Primer sequences used for qRT-PCR

Target transcript	Primer type	Sequence (5'→3')
has_circ_0064357	Forward	CCCTTTCTCCAGAGGCAGAA
	Reverse	TCCACTTGCGCATCTACAGA
has_circ_0064358	Forward	ATCATCTTCATGGTGGGCC
	Reverse	CCTCTTCATTGCTTTGGGGC
RAF1	Forward	AGATGGCGGGAGTAAGAGGA
	Reverse	CATCGTAGCAAACGCGCTC
ACTB	Forward	GATCAAGATCATTGCTCCTCCTG
	Reverse	CTAGAAGCATTTGCGGTGGAC

Prediction of circRNAs and target gene

In the preliminary experimental screening, OSSC-associated circRNAs, hsa_circ_0064357 and hsa_circ_0064358, and the potential circRNA target gene, *RAF1*, were selected using the online circRNA bioinformatics databases such as Circinteractome (https://circinteractome.nia.nih.gov/) and Circbase (http://www.circbase.org/). Then, the selected gene for both circRNAs was subjected to analysis.

Statistical analysis

All experiments were repeated three times, and numerical data were expressed as means ± standard deviation (SD). The data obtained were all statistically analyzed using GraphPad Prism software 5.0 (GraphPad Software, Inc., San Diego, CA, USA) and SPSS (GraphPad Prism 5 software (version 18.0; SPSS, Inc., Chicago, IL, USA). Expression data were controlled for normal

distribution by one-sample Kolmogorov-Smirnov (K–S test). A one-way ANOVA was used to determine statistical differences in *RAF1* gene expression levels. The associations between hsa_circ_0064357 and hsa_circ_0064358 and *RAF1* levels and clinic-pathological parameters of OSCC patients were assessed using independent sample test and independent-sample Kruskal-Wallis test. The correlation between variables was performed by Pearson correlation analysis. The diagnostic value, sensitivity and specificity of circRNAs were determined by ROC curve analysis. A p-value less than 0.05 (≤0.05) was considered statistically significant.

Results

Hsa_circ_0064357 and hsa_circ_0064358 was downregulated in OSCC and OLP

Hsa_circ_0064357 and hsa_circ_0064358 expression in OSCC and OLP tissues was measured using RT-qPCR. As shown in figures, hsa_circ_0064357 (11.7-fold), and hsa_circ_0064358 (8.5-fold) were expressed at significantly lower levels in OSCC tissues than in the corresponding non-tumorous tissues (n=30, p <0.0001)(Fig. 1a, b). Moreover, we found that the expression levels of hsa_circ_0064357 (12.8-fold) and hsa_circ_0064358 (4.4-fold) were lowly expressed in OLP tissues in comparison to that in normal tissues (n=10, p <0.05, Fig. 1 c, d). Specifically, compared to OLP, OSCC tissues showed 52.1 and 704.6-fold decreases in mRNA expression of hsa_circ_0064357 and hsa_circ_0064358, respectively (p < 0.001).

Correlation between clinicopathological features and RAF1, hsa_circ_0064357 and hsa_circ_0064358 expression levels

RAF1 was predicted as a potential target gene of studied circRNAs via Circinteractome and Circbase online bioinformatics databases. By qRT-PCR analyses, we determined the expression level of *RAF1* gene in each pair of OSCC, OLP tissues, and related adjacent normal tissue; *RAF1* was expressed at significantly higher and lower levels in OSCC (3.09-fold, n=30, p<0.001) and OLP (0.48-fold, n=10, p<0.001) tissues than in the corresponding non-tumorous tissues (Fig. 2 a, b). Pearson's correlation analysis revealed weak, mainly non-significant negative correlations between hsa_circ_0064357 (r = – 0.0897; p = 0.6371) and hsa_circ_0064358 (r = – 0.0683; p = 0.7195) expression levels and *RAF1* overexpression in OSCC tissues (Fig. 3 a, b).

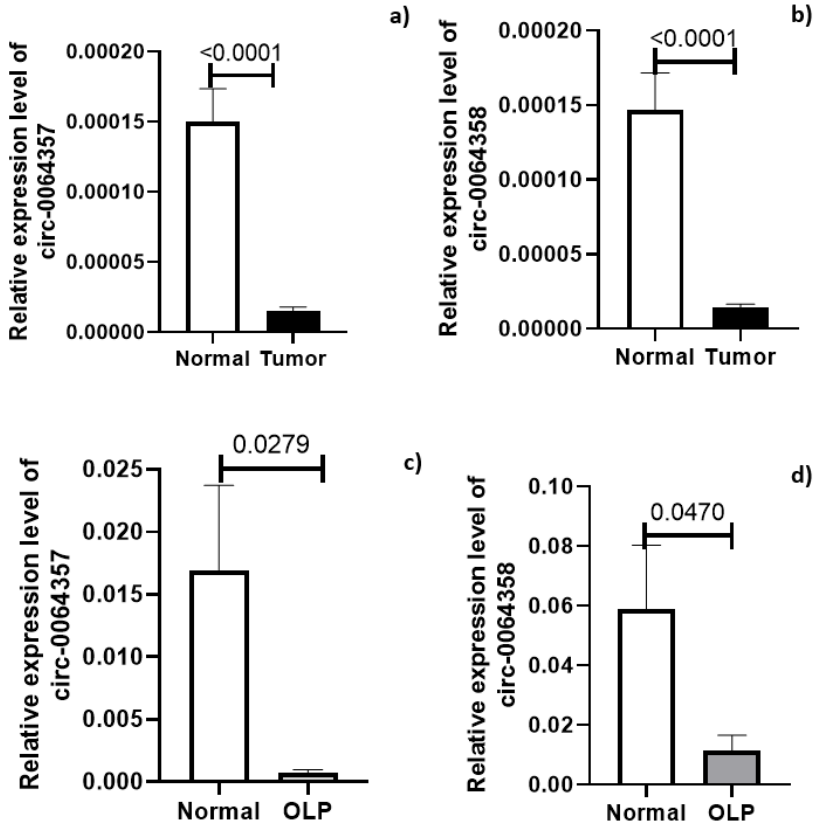

Figure 1. Quantitative RT-PCR analysis of hsa_circ_0064357 and hsa_circ_0064358 expressions in OSCC tissues and adjacent normal tissues (n=30) (a and c), OLP (Oral lichen planus), and adjacent normal tissues (n=10) (b and d). Transcript levels were normalized to *ACTB* expression. Data are presented as means ± SD. *** indicates a statistically significant difference ($p < 0.0001$).

The association of hsa_circ_0064357 and hsa_circ_0064358 and *RAF1* expression with several clinicopathological characteristics of OSCC patients were analyzed (Tab. 2). We found that *RAF1* level does not correlate with any of the clinicopathological factors, possibly due to insufficient sample sizes ($p>0.05$). hsa_circ_0064357 expression levels were positively correlated with age (P=0.002), gender (P=0.042), vascular invasion (P=0.043), clinical stage (P=0.005), histologic grade (P=0.000) and metastasis (P=0.000). However, we did not find significant differences in the tumor size, necrosis, and perineural and lymphatic invasion of hsa_circ_0064357 expression. Moreover, hsa_circ_0064358 levels were related to

age (P=0.020), tumor size (P=0.018), lymphatic and vascular invasion (p=0.000 and p=0.031, respectively). We also found a significant association with the presence of clinical stage (P=0.016), histologic grade (P=0.000), and metastasis (P=0.005). In contrast, no significant differences were observed concerning other clinicopathological factors (gender, lymphatic invasion, and necrosis (P>0.05) (Tab. 2).

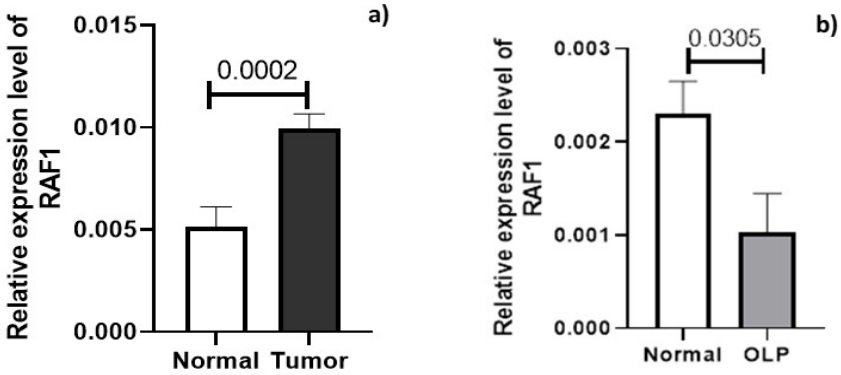

Figure 2. Quantitative RT-PCR analysis of *RAF1* expression in OSCC tissues and adjacent normal tissues (n=30) (a), OLP (Oral lichen planus), and adjacent normal tissues (n=10) (b). Transcript levels were normalized to *ACTB* expression. Data are presented as means ± SD. ***indicates a statistically significant difference ($p < 0.0001$).

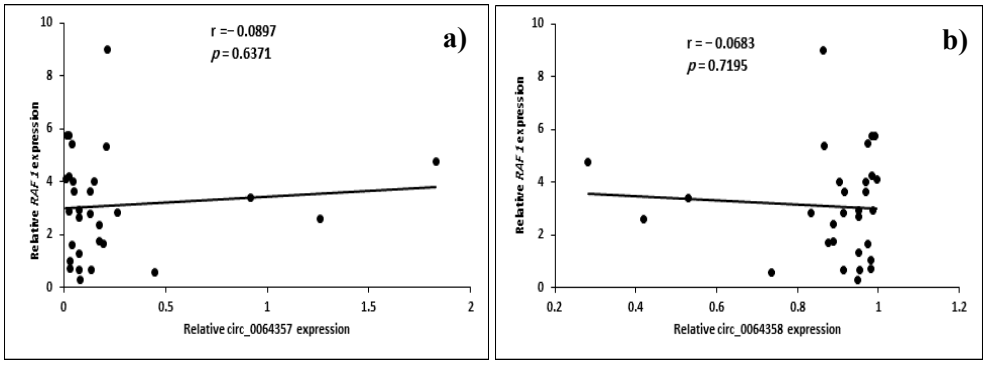

Figure 3. Correlations between hsa_circ_0064357, hsa_circ_0064358 and *RAF1* expression were measured using Pearson's correlation coefficient (r), and the significance levels are reported as follows: *p < 0.05; ***p < 0.001. Non-significant and very weak negative correlation was found between the expression of *RAF1* and circRNAs in OSCC patients' tissues.

Table 2. Association of hsa_circ_0064357, hsa_circ_0064358 and *RAF1* target gene expression with clinicopathological characteristics in OSCC

Clinical features	Case No. (%)	hsa_circ_0064357		hsa_circ_0064358		*RAF1*	
		Mean±SD	*P* value	Mean±SD	*P* value	Mean±SD	*P* value
Gender			**0.042**		**0.020**		0.443
Female	8	0.06±0.05		0.05±0.04		1.75±1.43	
Male	22	0.28±0.45		0.28±0.45		3.58±1.93	
Age (Years)			**0.002**		0.134		0.883
≥ 40	26	0.18±0.28		0.25±0.45		3.08±2.01	
< 40	4	0.52±0.86		0.44±0.77		3.18±1.96	
Size (cm)			0.095		**0.018**		0.105
≥ 5	9	0.12±0.13		0.08±0.06		3.30±2.80	
< 5	21	0.27±0.47		0.36±0.57		3.00±1.56	
Perineural invasion			0.631		0.076		0.878
Present	12	0.27±0.39		0.43±0.63		2.84±2.29	
Absent	18	0.19±0.42		0.17±0.37		3.26±1.77	
Vascular invasion			**0.043**		**0.000**		0.335
Present	6	0.39±0.54		0.61±0.87		3.18±1.62	
Absent	24	0.18±0.36		0.19±0.33		3.07 ±2.07	
Lymphatic invasion			0.308		**0.031**		0.159
Present	5	0.32±0.52		0.49±0.88		4.78±2.65	
Absent	25	0.20±0.38		0.23±0.39		2.75±1.68	
Necrosis Presence			0.636		0.157		0.244
Present	7	0.24±0.45		0.42±0.74		3.09±4.76	
Absent	23	0.22±0.40		0.23±0.40		3.09±2.13	
Stage			**0.005**		**0.016**		0.250
Low (I and II)	13	0.09±0.08		0.16±0.18		3.35±1.94	
High (III and IV)	17	0.32±0.51		0.37±0.63		2.89±2.03	
Grade			**0.000**		**0.000**		0.737
I	16	0.08±0.07		0.16±0.17		3.08±2.13	
II	13	0.41±0.56		0.44±0.71		3.31±1.72	
Status unknown	1						
Clinical Metastasis			**0.000**		**0.005**		0.396
M0	29	0.17±0.27		0.23±0.43		3.03±1.98	
M1	1	1.82		1.60		4.77	

Potential Diagnostic Values of hsa_circ_0064357 and hsa_circ_0064358 in OSCC

ROC curve analysis was performed to estimate the potential diagnostic value of hsa_circ_0064357 and hsa_circ_0064358 in OSCC for distinguishing OSCC tissues from paired adjacent normal tissues. The area under the ROC curve (AUC) for hsa_circ_0064357 expression in OSCC tissues was 0.962 (95% confidence interval (CI), 0.923- 1.000; $P<0.0001$), the cut-off value was 15.15, and the sensitivity and specificity were 83.33% and 96.67%, respectively (Fig. 4a, b). As shown in figure 4c, d-D, AUC for hsa_circ_0064358 was found to be 0.965 (95% CI, 0.926- 1.000; $P<0.0001$), and at the cut-off value of 15.18, the optimal sensitivity and specificity were 93.33% and 94.57%, respectively. A higher AUC indicates a higher accuracy of the diagnostic value of the tested variable. These results indicated that hsa_circ_0064357 and hsa_circ_0064358 have high diagnostic values for discriminating OSCC patients from healthy controls.

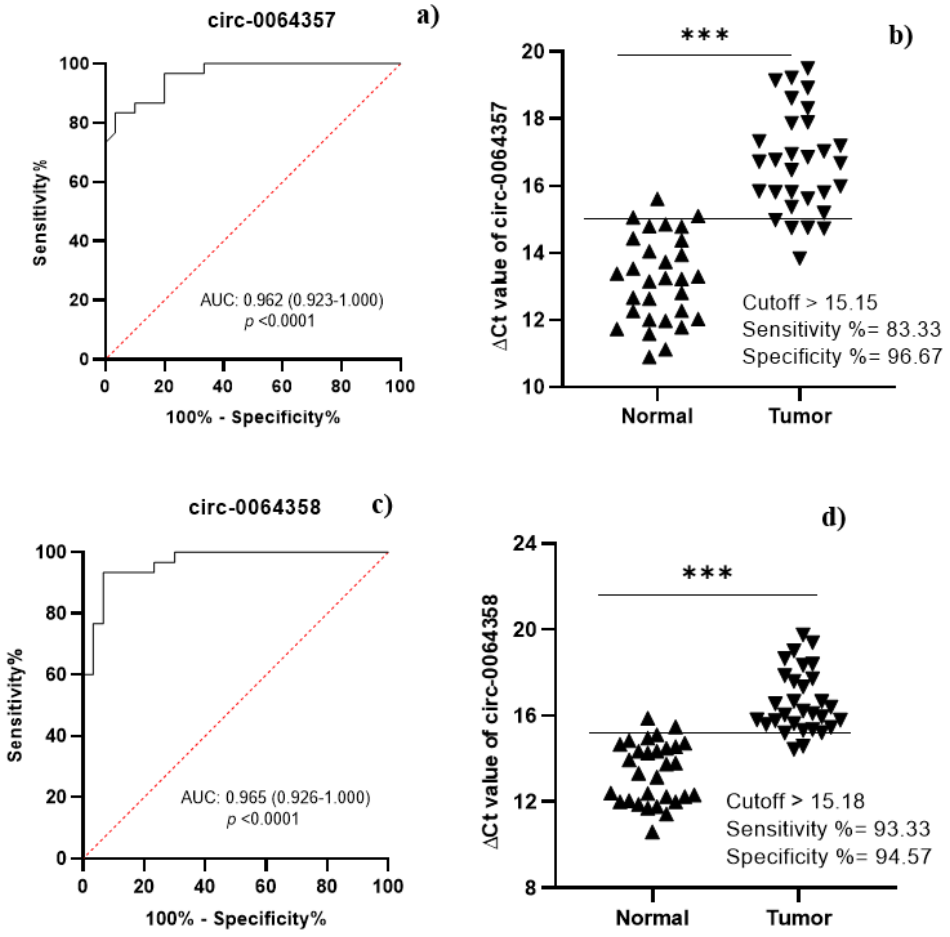

Figure 4. ROC curve analysis for the diagnostic value of hsa_circ_0064357 and hsa_circ_0064358 in OSCC. (A) The AUC for hsa_circ_0064357 and hsa_circ_0064358 were 0.962 (95% CI =0.923–1.000; P<0.0001) (A), and 0.965 (95% CI, 0.926- 1.000; P<0.0001) (C), respectively. The cut-off of circ-0082737(B) and circ-0082738 (D) was 15.15 and 15.18. Data are presented as means ± SD; ***P<0.0001.

Discussion

OSCC is often characterized at the late stages (III/IV) due to the inherent ability of lymph node metastasis of the oral cavity (Biswas *et al*., 2019). Accurate and reliable detection of oral precancerous or early lesions such as OLP may

help prevent their potential for malignant transformation and onset of symptoms of severe dysplasia and even squamous cell carcinoma, where treatment is less screening methods based on measuring the expression of cancer-associated molecular biomarkers improve the accuracy, performance sensitivity, and specificity of oral cancer screening over the conventional ocular inspection of the oral cavity and histopathological assessment of biopsy tissue (Yardimci *et al*., 2014; Tampa *et al*., 2018). Cumulative research has demonstrated that the expression level of circRNAs differs substantially between tumor and normal tissues and can be involved in the regulation of tumorigenesis. Therefore, the research of the circRNAs renders favorable as molecular biomarkers to enhance the efficacy of cancer diagnosis (Su *et al*., 2019).

In OSCC, abnormal expression of circRNAs has been implicated in cancer development and progression (Zhou *et al*., 2020). For instance, using high-throughput microarray analysis, Deng *et al*. (2019) found that 213 circRNAs were differentially expressed in 3 pairs of OSCC and matched normal tissues, including 124 up-regulated and 89 down-regulated. In another study, over expression of circRNA_100290 regulate the function of cyclin-dependent kinase 6 (CDK6) by sponging miR-26 in OSCC tissues (Chen *et al*., 2017). circDOCK1 (hsa_circ_100721) is up-regulated in OSCC tissues and can inhibit miR-196a-5p by competing with BIRC3, thereby suppressing apoptosis (Wang *et al*., 2018). Li *et al*. (2018) identified that hsa_circ_0008309 is significantly downregulated in OSCC and could increase ATXN1 (Ataxin 1; components of the Notch signaling pathway) expression through inhibition of miR-136-5P and miR-382-5P expression in the OSCC cell lines. Wang and his colleagues demonstrated that circ_000334, circ_006371, and circ_006740 were significantly downregulated in OSCC and could act as ceRNA (competing endogenous RNA), affecting the development of OSCC (Wang *et al*., 2018). Similarly, other circRNA, such as hsa_circRNA_100533 and hsa_circ_0003829, have also been reported to exhibit low expression levels in OSCC, and their overexpression can effectively inhibit OSCC proliferation, migration and extend cell apoptosis (Zhu *et al*., 2018; Zhang *et al*., 2020). Su *et al*. proved hsa_circ_0055538 was significantly downregulated in OSCC tissue. Also, knockdown of hsa_circ_0055538 correlated with the malignant biological behavior of OSCC by regulation of the p53/Bcl-2/caspase signaling pathway (Su *et al*., 2019).

Most recent studies have focused on the potential prognostic significance of cancer-related circRNAs (Wang *et al*., 2021; Kristensen *et al*., 2022). In contrast, accurate recognition and monitoring of malignant transformation in oral potentially malignant disorders (OPMD) such as OLP with malignant transformation rates ranging from 0.44 to 1.4% through differential expression patterns of circRNAs is a challenging issue that has not been addressed (Tsushima *et al*., 2021). Therefore, in the present study, we focused on the differential expression

of hsa_circ_0064357 and hsa_circ_0064358 in OLP and OSCC patients. The circRNAs hsa_circ_0064357 and hsa_circ_0064358 are encoded by *RAF1* gene and located on chromosome 3 at chr3:12625099-12626752 (spliced sequence length of 1324 bp) and chr3:12626345-12626480 (spliced sequence length of 1354 bp) respectively from UCSC and circBank database; however, the function of these circRNAs in OSCC progression has remained unknown. Here, we carried out qRT-PCR analyses to compare circRNAs expression levels in OSCC (n=30) and OLP (n=10) tissues with those in normal adjacent tissues. We found that hsa_circ_0064357 and hsa_circ_0064358 were significantly downregulated in OSCC and OLP relative to their matched normal tissues (p <0.001). A notable observation was the significantly lower expression of circRNAs in OSCC compared to OLP tissue (p <0.001). Consistent with our previous report, the expression of circ_0045638 and circ_0045639 was significantly downregulated in OSCC and OLP tissues relative to their matched-adjacent normal tissues, and Low expression of circRNAs was also found in OSCC compared with OLP tissues (Jahangiri *et al*., 2022). Thus, this discrepancy may help the precise detection of precancerous lesions at screening programs and follow-up on their malignancy potential and down-staging the disease.

Increasing reports have shown that circRNAs play multiple regulatory roles in various cellular events critical in cancer development and progression, such as the abnormal expression of important downstream components of cancer-related signaling pathways, including MAPK/ERK and Wnt/β-catenin signaling and PTEN/PIK3/AKT pathways (Garlapati *et al*., 2021; Xue *et al*., 2021). Dysregulation of Raf1 as a core regulatory signaling molecule in the ERK/MAPK pathway due to aberrant circRNA expression has been reported to activate this pathway in various cancer types (Cheng *et al*., 2022). For example, circ_CDR1 depletion suppresses hepatocellular carcinoma cell (HCC) proliferation and metastasis *in vivo* via regulating miR-1287/Raf1 pathway (Zhang *et al*., 2020). ciRS-7 can interact directly with miR-7, resulting in the upregulation of RAF-1/PIK3CD expression and enhancing the metastatic progression of OSCC (Dou *et al*., 2020).

In the present study, using the online circular RNA databases (CircInteractome and Circbase) for target gene prediction, *RAF1* was identified as the potential targeted gene of hsa_circ_0064357 and hsa_circ_0064358 and the level of *RAF1* in 30 pairs of OSCC and 10 pairs of OLP tissues are detected. Our present findings are consistent with those of Kordi-Tamandani *et al*. (2014) in that compared with healthy samples, the expression level of *RAF1* was significantly up and down-regulated in OSCC and OLP tissues, respectively. In addition, we found a very weak negative and non-significant correlation between *RAF1* overexpression and circRNAs levels in OSCC (Figures 3a, b), and whether

those circRNAs are associated with elevated levels of *RAF1* should be the focus of the further investigation. Recent studies have revealed that abnormal expression of Raf1 are closely correlated with tumor invasion, lymph node metastasis, and T stage in thyroid cancer (Wang *et al*., 2015), non-small cell lung cancer (NSCLC)(Tian *et al*., 2018), prostate (Ren *et al*., 2012), colorectal (Slattery *et al*., 2012; Borovski *et al*., 2017). A study by Li *et al*. (Li *et al*., 2022) showed that up-regulation of *RAF1* can promote tumor growth and lymphatic metastasis by targeting LAGE1 in hypopharyngeal carcinoma.

Our present findings were not in line with previous reports in that we did not find any significant correlations between the up-regulation of *RAF1* expression and the clinicopathological characteristics of OSCC. More importantly, a comparison of hsa_circ_0064357 and hsa_circ_0064358 expression levels with clinicopathological features of OSCC patients revealed that decreased expression of the circRNAs was significantly associated with the TNM stage, an important factor in evaluating the prognosis of OSCC. These results indicate that hsa_circ_0064357 and hsa_circ_0064358 might be involved in the progression and metastasis of OSCC and could be used as promising novel biomarkers for early diagnosis and new therapeutic targets for OSCC. In addition, the ROC analysis indicated that the hsa_circ_0064357 and hsa_circ_0064358 expression levels with high sensitivity and specificity exhibited a potential diagnostic value in distinguishing OSCC tissues from healthy samples.

Conclusion

In summary, the findings of the present study provided the first evidence that hsa_circ_0064357 and hsa_circ_0064358 were downregulated in both OSCC and OLP tissues and were significantly associated with TNM stage in OSCC patients, suggesting that the studied circRNAs may serve as prognostic markers for early detection and new target treatment in OSCC. hsa_circ_0064357 and hsa_circ_0064358 expression levels of OSCC and OLP tissues exhibited significant differences that may facilitate accurate and early discrimination of OSCC from suspicious OLP. However, further studies with larger sample sizes are needed to elucidate the functions and mechanisms of hsa_circ_0064357 and hsa_circ_0064358 in OSCC tumorigenesis and metastasis.

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