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RESEARCH ARTICLE

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Circular RNA_0001742 has potential to predict advanced tumor stage and poor survival profiles in tongue squamous cell carcinoma management

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Abstract

Background: Circular RNA_0001742 (circ_0001742) has been reported to be up-regulated in tongue squamous cell carcinoma (TSCC) tissues/cells and regulate TSCC cell proliferation, migration, and invasion. This study aimed to further investigate the clinical significance of circ_0001742 in TSCC management.

Methods: Totally, 146 TSCC patients underwent surgical treatment were reviewed. Their fresh-frozen tumor tissue and adjacent tissue were acquired for detecting circ_0001742 expression via reverse transcription-quantitative polymerase chain reaction. According to circ_0001742 expression in tumor tissue, all patients were classified as tumor circ_0001742 low (0%-50% percentile) and high (50%-100% percentile) patients, the latter were further divided into the tumor circ_0001742 high+ (50%-75% percentile), high++ (75%-90% percentile), and high+++ (90%-100% percentile) patients, respectively.

Results: Circ_0001742 expression was increased in TSCC tumor tissue compared with adjacent tissue, and it presented good value in discriminating tumor tissue from adjacent tissue (area under the curve (AUC): 0.870, 95% CI: 0.831-0.910). Tumor high circ_0001742 expression was associated with higher T stage, N stage, and TNM stage, but not age, gender, or pathological grade. Furthermore, OS was reduced in tumor circ_0001742 high patients compared with tumor circ_0001742 low patients; moreover, OS was the shortest in tumor circ_0001742 high+++ patients, followed by tumor circ_0001742 high++ patients and tumor circ_0001742 high+ patients, and the longest in tumor circ_0001742 low patients. In addition, multivariate Cox's regression analysis revealed that higher tumor circ_0001742 expression was an independent predictive factor for decreased OS.

Conclusion: Circ_0001742 serves as a potential biomarker for advanced tumor stage and poor survival in TSCC patients.

KEYWORDS

circular RNA_0001742, overall survival, reverse transcription-quantitative polymerase chain reaction, tongue squamous cell carcinoma, tumor stage

1 | INTRODUCTION

Oral cancer is one of the most prevalent cancer globally with approximately 300 000 new cases and 130 000 deaths annually all over the world, and 90% of oral cancers are identified as squamous cell carcinoma.[1,2] Tongue squamous cell carcinoma (TSCC) is a major type of oral cancers, which is characterized by a high incidence of lymph node and distant metastasis.[3] The therapy management, including surgery, chemotherapy, and radiotherapy, has experienced great technological advancement in last decades; however, the survival rates have not improved greatly and still remain below 50%, and 6.9%-37.4% TSCC patients suffer from locoregional recurrence and the mortality in these patients is approximately 90%.[4-6] Therefore, it is essential to discover novel prognostic biomarker which can be applied in the follow-up surveillance and improve the survival rate in TSCC patients.

Circular RNAs (circRNAs) are a type of non-coding regulatory RNA and form closed-loop structures via covalent bonds, which present stably and abundantly in various organisms.[7] Recent studies reveal that endogenous circRNAs have several molecular functions, including being the microRNA (miRNA) sponges, regulating process of splicing and transcription, and modifying gene expression.[7] Furthermore, circRNAs are reported to be closely involved in the pathological process of various cancers.[8-10] In TSCC, a previous high-throughput sequencing revealed that circular RNA_0001742 (circ_0001742) is upregulated in TSCC tissue specimens compared with the adjacent tissue specimens, which is further validated using reverse transcription-quantitative polymerase chain reaction (RT-qPCR).[11] Another study exhibits that circ_0001742 is upregulated in TSCC tissues as well as cells, and its knockdown inhibits cell proliferation, migration, and invasion via regulating microRNA (miR)-431/ATF3 axis in TSCC cells.[12] According to these previous studies and the finding of our preliminary study with small sample size, which indicated that circ_0001742 was upregulated in TSCC tissue compared with adjacent non-cancerous tissue, we hypothesized that circ_0001742 might be of clinical significance in the management of TSCC patients; however, little information is available until now. Thus, we preformed the present study to investigate the association of circ_0001742 with clinicopathological features and survival profile in TSCC patients.

2 | METHODS

2.1 | Patients

A total of 146 TSCC patients underwent surgical treatment in our hospital were reviewed in this retrospective study between January 2015 and December 2019. The main screening criteria included the following: (1) histological diagnosis of primary TSCC; (2) aged 18-80 years; (3) without distant metastasis; (4) received surgical resection; (5) complete preoperative tumor features; (6) complete survival data; and (7) the fresh-frozen tumor tissue and adjacent tissue excised from surgery were available. Besides, the patients with relapsed or secondary TSCC, history of malignancies, history of neoadjuvant therapy or severe abnormities in hepatorenal function/hemogram indexes were excluded from this study. The Ethics Committee in our hospital approved this study. All patients or their family members provided the written informed consents.

2.2 | Data and sample collection

Demographics, preoperative tumor features, adjuvant treatment records, and follow-up data were collected from electrical database. The fresh-frozen tumor tissue and adjacent tissue were acquired from the storeroom in Pathology Department.

2.3 | Circ_0001742 detection

The relative expressions of circ_0001742 in tumor tissue and adjacent tissue were detected by RT-qPCR. Total RNA was extracted from tumor tissue and adjacent tissue using TRIzol™ Reagent (Invitrogen™), and linear RNA was removed using RNase R (Epicentre). Following that, RNA was reversely transcribed using iScript™ cDNA Synthesis Kit (Bio-Rad). Then, qPCR was performed using QuantiNova SYBR Green PCR Kit (Qiagen) to quantify circ_0001742 and GAPDH Ct value. In addition, the expression of circ_0001742 was calculated using $2^{-\triangle\triangle Ct}$ method with GAPDH as internal reference. The detailed description of circ_0001742 expression normalization was as follows: (1) qPCR was performed in triplicate, and the average of circ_0001742 Ct and GAPDH Ct in every sample were determined, respectively. (2) Calculations of $\triangle$Ct (Ct avg. circ_0001742– Ct avg. GAPDH) were presented in every sample, which was shown as $\triangle$Ct (sample). (3) The median of $\triangle$Ct in healthy controls was referred as the calibrator, which was shown as $\triangle$Ct (calibrator). (4) $\triangle\triangle$Ct =$\triangle$Ct (sample) - $\triangle$Ct (calibrator). (5) The relative expression of circ_0001742 was proceeded via calculating $2^{-\triangle\triangle Ct}$.[13] The primers used in this study were designed referring to the study published previously,[12] and the primers were as follow: circ_0001742 forward primer: GGGATTTGTTTTGTGGGCTA, circ_0001742 reverse primer: CACTGGCCTGAACTGTTGAA; GAPDH forward primer: CGGAGTCAACGGATTTGGTCGTAT, GAPDH reverse primer: AGCCTTCTCCATGGTGGTGAAGAC. All patients were classified as tumor circ_0001742 low group (n = 73) and tumor circ_0001742 high group (n = 73) based on the median value of circ_0001742 in tumor tissue, and the patients in tumor circ_0001742 high group were further divided into tumor circ_0001742 high+ (50%-75% percentile, n = 37) cases, tumor circ_0001742 high++ (75%-90% percentile, n = 22) cases, and tumor circ_0001742 high+++ (90%-100% percentile, n = 14) cases.[14,15]

2.4 | Follow-up

Patients were followed up by clinic visit or telephone calls regularly. Furthermore, Patients' survival status was extracted from follow-up

data, and overall survival (OS) was calculated from the date of surgery to the date of death with the last follow-up data of December 31, 2019.

2.5 | Statistical analyses

All statistical analyses were performed using SPSS (version 22.0 IBM), and all figures were plotted using GraphPad Prism (version 7.00 GraphPad Software). Comparison of circ_0001742 between tumor tissue and adjacent tissue was determined by the Wilcoxon signed-rank test. The ability of circ_0001742 in discriminating tumor tissue from adjacent tissue was displayed by receiver operating characteristic (ROC) curve and area under the curve (AUC) with 95% confidence interval (CI). Comparison of clinicopathological features between tumor circ_0001742 high group and tumor circ_0001742 low group was determined by the chi-square test or Wilcoxon rank-sum test. Kaplan-Meier curve was used to illuminate OS, and comparison of OS between tumor circ_0001742 high group and tumor circ_0001742 low group was determined by log-rank test. For further analyzing the correlation of tumor circ_0001742 with OS, comparisons of OS among tumor circ_0001742 low patients, tumor circ_0001742 high+ patients, tumor circ_0001742 high++ patients, and tumor circ_0001742 high+++ patients were determined by log-rank test as well. Factors affecting OS were analyzed by univariate and multivariate Cox's proportional hazard regression model. *P* value <.05 was considered as significant.

3 | RESULTS

3.1 | Clinicopathological features of TSCC patients

The mean age of TSCC patients was 57.9 ± 11.9 years (Table 1). There were 41 (28.1%) females and 105 (71.9%) males. As for pathological grade, the number of patients with G1, G2, and G3 was 21 (14.4%), 101 (69.2%), and 24 (16.4%), respectively. Regarding T stage, the number of patients with T1, T2, and T3 was 30 (20.5%), 75 (51.4%), and 41 (28.1%), respectively. There were 94 (64.4%), 47 (32.2%), and 5 (3.4%) patients with N0, N1, and N2, respectively, and there were 25 (17.1%) patients with TNM stage I, 59 (40.4%) patients with TNM stage II, 57 (39.1%) patients with TNM stage III, and 5 (3.4%) patients with TNM stage IV. More detailed information of clinicopathological features of TSCC patients was shown in Table 1.

TABLE 1 Clinicopathological features of TSCC patients

Items	TSCC patients (N = 146)
Age (y), mean ± SD	57.9 ± 11.9
Gender, No. (%)	
Female	41 (28.1)
Male	105 (71.9)
Pathological grade, No. (%)	
G1	21 (14.4)
G2	101 (69.2)
G3	24 (16.4)
T stage, No. (%)	
T1	30 (20.5)
T2	75 (51.4)
T3	41 (28.1)
N stage, No. (%)	
N0	94 (64.4)
N1	47 (32.2)
N2	5 (3.4)
TNM stage, No. (%)	
I	25 (17.1)
II	59 (40.4)
III	57 (39.1)
IV	5 (3.4)
Adjuvant radiotherapy, No. (%)	
No	46 (31.5)
Yes	100 (68.5)

Abbreviations: SD, standard deviation; TSCC, tongue squamous cell carcinoma.

3.2 | Circ_0001742 expression in TSCC tumor tissue and adjacent tissue

Circ_0001742 expression was increased in TSCC tumor tissue (2.624 [1.972-3.574]) compared with adjacent tissue (0.997 (0.717-1.748)) (*P* < .001) (Figure 1A). ROC curve analysis exhibited that circ_0001742 presented good value in discriminating tumor tissue from adjacent tissue (AUC: 0.870, 95% CI: 0.831-0.910) (Figure 1B).

3.3 | Correlation of tumor circ_0001742 expression with clinicopathological features in TSCC patients

All patients were classified as tumor circ_0001742 low group (n = 73) and tumor circ_0001742 high group (n = 73) according to the median value of circ_0001742 in tumor tissue. Tumor high circ_0001742 expression was associated with higher T stage (*P* = .023) (Figure 2D), N stage (*P* < .001) (Figure 2E), and TNM stage (*P* < .001) (Figure 2F); however, there was no correlation of tumor circ_0001742 expression with age (*P* = .618) (Figure 2A), gender (*P* = .854) (Figure 2B), or pathological grade (*P* = .467) (Figure 2C) in TSCC patients.

3.4 | Correlation of tumor circ_0001742 expression with OS in TSCC patients

OS was reduced in patients with tumor circ_0001742 high expression compared with those with tumor circ_0001742 low expression (*P* = .010) (Figure 3A). Furthermore, the patients in tumor circ_0001742 high group were further divided into tumor circ_0001742 high+ (50%-75% percentile, n = 37) cases, tumor circ_0001742 high++ (75%-90%

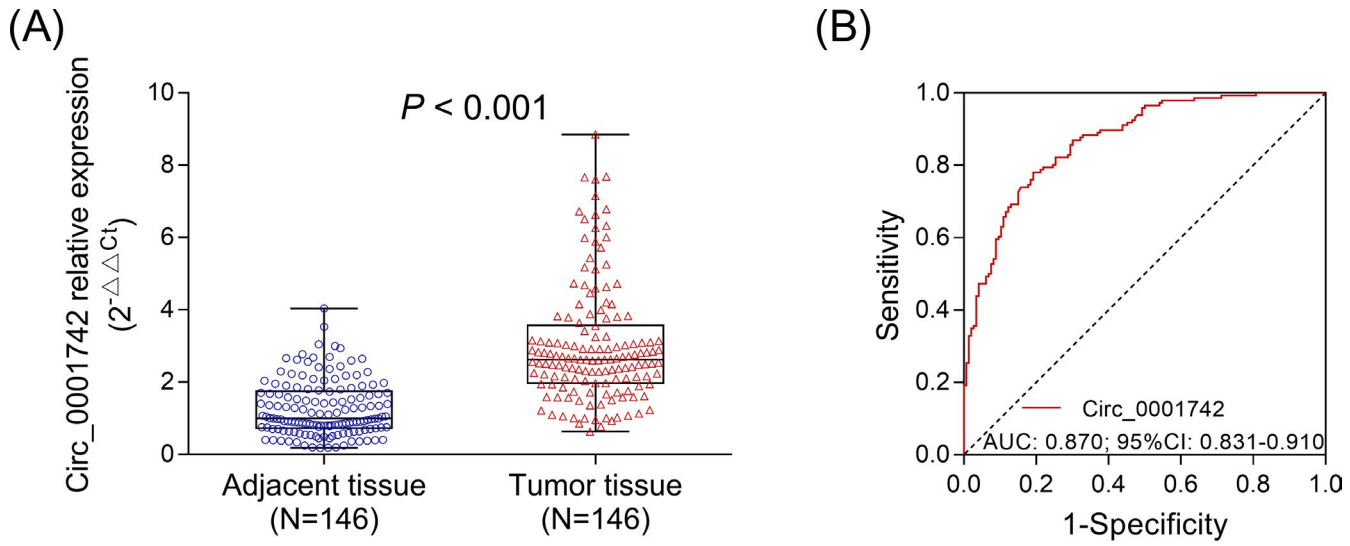

FIGURE 1 Circ_0001742 expression in TSCC. Comparison of circ_0001742 expression between tumor tissue and adjacent tissue (A). The ability of circ_0001742 in discriminating tumor tissue from adjacent tissue (B). Circ_0001742, circular RNA_0001742; TSCC, tongue squamous cell carcinoma; AUC, area under the curve; CI, confidence interval; ROC, receiver operating characteristic; TSCC, tongue squamous cell carcinoma

percentile, n = 22) cases, and tumor circ_0001742 high+++ (90%-100% percentile, n = 14) cases. OS was the shortest in patients with tumor circ_0001742 high+++ expression, followed by patients with tumor circ_0001742 high++ expression and those with tumor circ_0001742 high+ expression, and the longest in patients with tumor circ_0001742 low expression (P = .005) (Figure 3B).

3.5 | Factors affecting OS in TSCC patients

Univariate Cox's regression analysis indicated that higher tumor circ_0001742 (HR = 1.689, P = .001), higher pathological grade (HR = 2.826, P = .001), higher T stage (HR = 2.491, P = .001), and higher N stage (HR = 4.221, P < .001) were negatively associated with OS in TSCC patients (Table 2). Further multivariate Cox's regression analysis revealed that higher tumor circ_0001742 expression (HR = 1.559, P = .013), higher pathological grade (HR = 2.186, P = .039), higher T stage (HR = 2.014, P = .047), and higher N stage (HR = 2.270, P = .014) were independent predictive factors for decreased OS in TSCC patients.

4 | DISCUSSION

In the present study, we found that (a) circ_0001742 expression was increased in TSCC tumor tissue compared with adjacent tissue and presented good value in discriminating tumor tissue from adjacent tissue. (b2) Tumor high circ_0001742 expression was associated with higher T stage, N stage, and TNM stage in TSCC patients. (c) Tumor circ_0001742 high expression was correlated with decreased OS and was an independent predictive factor for unfavorable OS in TSCC patients.

CircRNAs are common across all eukaryotes and are ubiquitously expressed in the transcriptional process of human.[16] Recently, emerging researches have demonstrated that considering the developmental regulation, localization, and tissue-specific expression, circRNAs are of diverse molecular functions and have potential role in various cancers.[16,17] For example, cirRNA TP63 is upregulated in lung squamous cell carcinoma tissue compared with paired non-cancerous tissue, and mechanically, it facilitates cell cycle progression via downregulating miR-873 targeted FOXM1 in lung squamous cell carcinoma.[18] In another study, circRNA-CCND1 is increased in laryngeal squamous cell carcinoma tissue compared with adjacent tissue, and in vitro, its depletion inhibits cell proliferation in laryngeal squamous cell carcinoma.[10] As for circ_0001742, one prior study indicates that circ_0001742 is upregulated in TSCC tissues compared with adjacent tissue via high-throughput sequencing, and further RT-PCR is performed for validation of circ_0001742 expression profile.[11] Meanwhile, several studies reveal tumorigenic implication of circ_0001742 in the TSCC development.[12,19] Silencing circ_0001742 is reported to inhibit cell proliferation, invasion and epithelial-mesenchymal transition (EMT) processes of TSCC cells via targeting miR-634/RAB1A pathway.[19] Based on the previous evidence, we proposed that circ_0001742 might be associated with clinicopathological features and survival in TSCC patients. We enrolled 146 TSCC patients underwent surgical treatment, and collected their fresh-frozen tumor tissue and adjacent tissue excised from surgery for detection of circ_0001742 expression. We observed that circ_0001742 was upregulated in TSCC tumor tissue compared with adjacent tissue, and the further ROC analysis exhibited that circ_0001742 was of good value in distinguishing tumor tissue from adjacent tissue, which was consistent with the previous studies that circ_0001742 expression was increased in TSCC tumor tissue compared with adjacent tissue.[12,19]

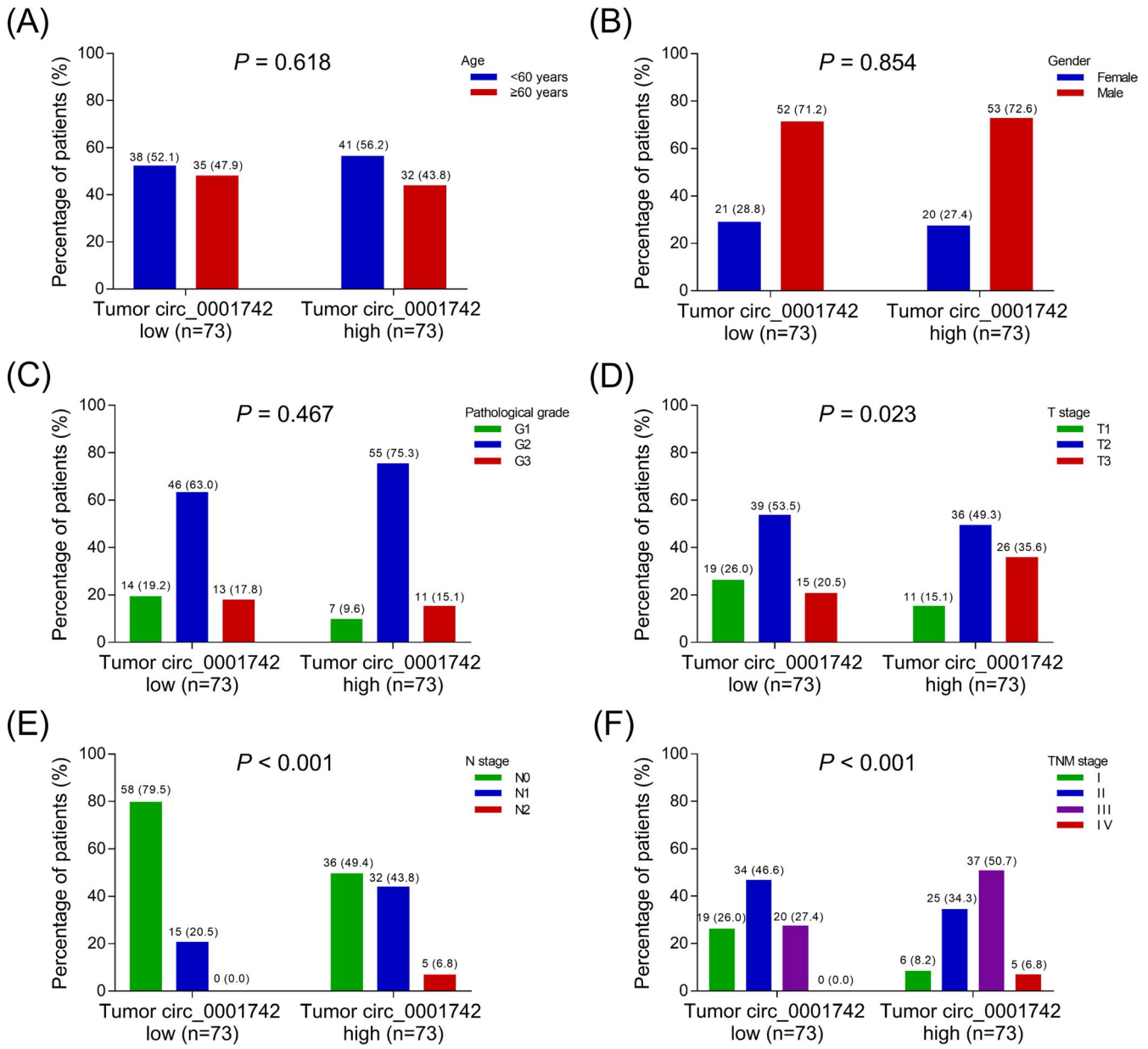

FIGURE 2 Comparison of clinicopathological features between tumor circ_0001742 high patients and tumor circ_0001742 low patients. Comparison of age (A), gender (B), pathological grade (C), T stage (D), N stage (E), and TNM stage (F) between tumor circ_0001742 high patients and tumor circ_0001742 low patients. Circ_0001742, circular RNA_0001742; TSCC, tongue squamous cell carcinoma

We subsequently explored the correlation of tumor circ_0001742 expression with clinicopathological features in TSCC patients and found that tumor circ_0001742 expression was positively associated with T stage, N stage, and TNM stage of TSCC patients. The possible reasons might include that (a) given the existing study that EMT was an essential biological process which was implicated in tumor invasion and metastasis, and considering the prior evidence that circ_0001742 enhances the EMT progress of TSCC cells, therefore, tumor high circ_0001742 expression was associated with advanced tumor stage in TSCC patients.[12,20] (b) Based on the existing evidence, circ_0001742 might promote cell proliferation, invasion, and migration but inhibit cell apoptosis, which further resulting in TSCC progression. Hence, TSCC patients with high circ_0001742 expression presented increased tumor stage.[12] (c) According to the prior study, circ_0001742 might exhibit pro-tumor activities via serving as the sponge of miR-634, and miR-634 exerts anti-tumor function via targeting Rab1A and DHX33; therefore, circ_0001742 was positively correlated with advanced T, N, and TNM stages.[19,21]

Regarding the correlation of tumor circ_0001742 expression with survival profiles in cancers, there is no study reported yet. In order to explore the predictive value of circ_0001742 in prognosis in TSCC patients, we compared the survival profiles in patients with different circ_0001742 expression and observed that tumor circ_0001742 expression was negatively associated with OS, and more importantly, higher tumor circ_0001742 expression independently predicted decreased OS in TSCC patients. The possible

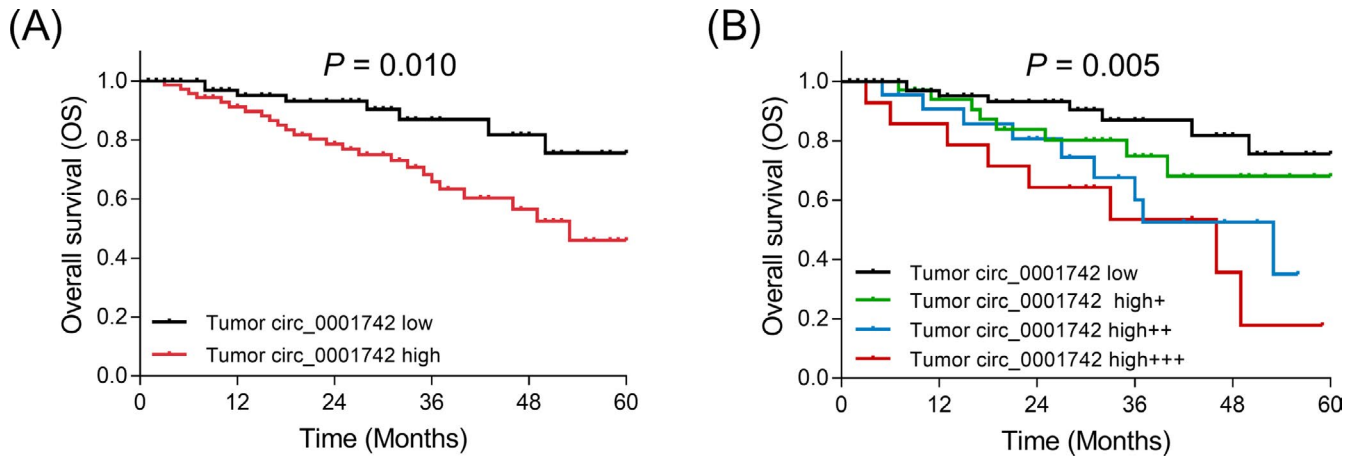

FIGURE 3 Comparison of OS among TSCC patients with different tumor circ_0001742 expressions. Comparison of OS between patients with tumor circ_0001742 high expression and patients with tumor circ_0001742 low expression (A). Comparison of OS among patients with tumor circ_0001742 high+++ expression, patients with tumor circ_0001742 high++ expression, patients with tumor circ_0001742 high+ expression, and those with tumor circ_0001742 low expression (B). Circ_0001742, circular RNA_0001742; OS, overall survival; TSCC, tongue squamous cell carcinoma

TABLE 2 Cox's proportional hazard regression model analysis of factors affecting OS

	Univariate Cox's regression		Multivariate Cox's regression	
Items	***P* value**	**HR (95% CI)**	***P* value**	**HR (95% CI)**
Higher tumor circ_0001742[a]	.001	1.689 (1.246-2.291)	.013	1.559 (1.097-2.216)
Age ≥60 y	.378	1.361 (0.685-2.705)	.495	1.293 (0.618-2.708)
Male	.213	1.702 (0.737-3.928)	.156	1.949 (0.774-4.904)
Higher pathological grade	.001	2.826 (1.539-5.190)	.039	2.186 (1.041-4.588)
Higher T stage	.001	2.491 (1.441-4.304)	.047	2.014 (1.009-4.019)
Higher N stage	<.001	4.221 (2.345-7.596)	.014	2.270 (1.183-4.358)
Adjuvant radiotherapy	.894	0.953 (0.468-1.940)	.146	0.527 (0.223-1.250)

Abbreviations: CI, confidence interval; HR, hazard ratio; OS, overall survival.

[a]Tumor circ_0001742 was categorized as low = 0, high+ = 1, high++ = 2, and high+++ = 3 in the Cox's regression model.

reasons might include that (a) considering the previous founding that higher tumor circ_0001742 expression was associated with increased T stage and N stage, and the observation that higher T stage and N stage were independent predictive factors for reduced OS in TSCC patients, high tumor circ_0001742 expression might be correlated with poor prognosis via interacting with T and N stages. (b) Circ_0001742 might promote TSCC cell proliferation, invasion, and migration via targeting miR-634, which enhanced tumor growth and metastasis of TSCC, and thereby led to tumor recurrence and lymph node metastasis; therefore, higher tumor circ_0001742 expression independently predicted decreased OS in TSCC patients.

There existed some limitations in our study. (a) As our study was a single-center retrospective study, some selection bias might exist, and further prospective study was needed for validating the clinical value of circ_0001742 in TSCC management. (b) As we excluded the patients with relapsed or secondary TSCC or history of neoadjuvant therapy to avoid confounding factors, therefore, the role of circ_0001742 in those patients needed further exploration. (c) Given that our study was a retrospective study, and considering the fact that majority of the patients included were not local patients, it was difficult to obtain accurate disease-free survival data, and further study detecting the association of tumor circ_0001742 expression with disease-free survival was needed to investigate circ_0001742 role as a potential prognostic marker. (d) Considering that circ_0001742 is involved in the TSCC progression via targeting miR-634, it was meaningful to study the role of circ_0001742/miR-634 axis in the TSCC management. (e) The underlying molecular mechanism of circ_0001742 in regulating TSCC tumor progression was not involved in the present study, which needed further cellular experiments to validate.

In conclusion, circ_0001742 is upregulated, associates with advanced TNM stage, and predicts unfavorable OS independently in TSCC patients, providing evidence that circ_0001742 has potential to be a biomarker, which could bring benefit to the follow-up surveillance and the survival prognosis in TSCC management.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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