International Journal of General Medicine

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Tongue Squamous Cell Carcinoma Prognosis Can Be Effectively Predicted by LncRNA LIPH4: A Prospective Study

Hailei He[1], Zhen Wang[2], Cuilin Fang[3], Junfeng Yan[4]

[1]Department of Oral and Maxillofacial Surgery, Ganzhou People's Hospital, Ganzhou, Jiangxi, 341000, People's Republic of China; [2]Department of Stomatology, The Quzhou Affiliated Hospital of Wenzhou Medical University, Quzhou, Zhejiang, 324000, People's Republic of China; [3]Department of Medicine and Equipment, Ganzhou Cancer Hospital, Ganzhou, Jiangxi, 341005, People's Republic of China; [4]Department of Stomatology, The First Affiliated Hospital of Nanchang University, Nanchang, Jiangxi, 330006, People's Republic of China

Correspondence: Junfeng Yan, Department of Stomatology, The First Affiliated Hospital of Nanchang University, No. 17, Yongwaizheng Street, Donghu District, Nanchang, Jiangxi, 330006, People's Republic of China, Email yanjunfengd@163.com

Purpose: LIPH4 has been identified as an oncogenic lncRNA in different malignant diseases. This research aims to elucidate the link between the expression of LIPH4 and its prognostic application in tongue squamous cell carcinoma (TSCC).

Methods: To assess the expression of LIPH4, 142 TSCC and normal cases, respectively, which met the selection parameters, were used for qRT-PCR analysis. Furthermore, the association of LIPH4 expression with TSCC's clinicopathological features was identified via the Chi-square test. Moreover, the Kaplan–Meier test was used for calculating the survival rates, whereas the association of patient survival with prognostic factors was assessed with the help of Cox proportional hazard analysis.

Results: The data indicated upregulated LIPH4 levels in TSCC samples than healthy samples. Furthermore, LIPH4 expression was associated with TSCC differentiation and stage, where increased expression indicated reduced disease-free survival (DFS) and overall survival (OS) rates. Additionally, advanced TSCC individuals with enhanced LIPH4 expression had reduced OS and DFS rates than those with reduced LIPH4 expression. Serum LIPH4 could be a promising diagnostic bio-index for TSCC, with an area under the curve of 0.8920 (95% CI = 0.8540–0.9299). These data revealed that the overexpression of LIPH4 might be a substantial prognostic factor for independently predicting the OS and DFS rates of TSCC patients.

Conclusion: Altogether, this research revealed that the expression of LIPH4 expression is closely associated with TSCC progression and, therefore, can be employed as a biomarker for its prognosis.

Keywords: lncRNA, LIPH4, biomarker, tongue squamous cell carcinoma (TSCC)

Introduction

In the past decades, oral cancer (OC) has become a health concern because of its increased morbidity and death rates.[1] OC is most frequently observed in the tongue. Furthermore, individuals with tongue squamous cell carcinoma (TSCC) have a markedly sub-standard prognosis than those with other type of OC. Moreover, TSCC patients always indicate severe oral dysfunction, including difficulties in speech and swallowing.[2] Despite the development of novel therapeutic technologies and proper TSCC treatment, inevitably, these patients have an increased risk of secondary tumors in the region surrounding the surgical site and emerging tumor recurrence.[3] In TSCC patients, tumor cell migration to the lymph nodes substantially increases the overall mortality rates, with most cases indicating a 5-year overall survival (OS) rate of < 50%.[4] Therefore, more reliable bio-indices for improving the prediction of cancer recurrence and patient survival are urgently required.

The literature has frequently indicated genetic alterations during TSCC development.[5,6] Furthermore, genetic strategies have shown promising potential in TSCC treatment. Moreover, genome-wide gene expression analysis has also revealed that TSCC is correlated with altered expression of many long (>200 nt) non-coding RNAs (lncRNAs),[7]

Received: 12 June 2024
Accepted: 4 September 2024
Published: 16 September 2024

which are essential factors in cancer biology.[8] For instance, LIPH4, a lncRNA, has always indicated overexpression in esophageal squamous cell carcinoma.[9] However, the association of LIPH4 expression with TSCC patient survival and its potential prognostic influence have not been determined. This research includes TSCC and healthy tongue tissue quantitative real-time PCR (qRT-PCR) samples. Furthermore, multivariate analyses were carried out to elucidate the correlation between LIPH4 levels and TSCC's clinicopathological features.

Materials and Methods

Clinical Samples

A total of 142 TSCC patients who underwent surgery at the First Affiliated Hospital of Nanchang University and met the rigid follow-up criteria were selected for sampling. Inclusion criteria: Patients with primary TSCC who did not receive radiotherapy or chemotherapy before surgery underwent primary lesion enlargement resection and neck lymph node dissection. The primary lesion was confirmed to be TSCC by routine pathology after surgery. Furthermore, their data, including tumor size, gender, differentiation, disease-free survival (DFS), age, OS, and TNM stage, were analyzed. The patients underwent radiotherapy and chemotherapy prior to the surgery. This investigation was authorized by the Ethics Committee of First Affiliated Hospital of Nanchang University (No.20231205), and this study complied with the Declaration of Helsinki. All the included patients agreed to participate in a 5-year follow-up and provided the signed informed consent. Additionally, 142 healthy tissue samples were also acquired as control.

Real-Time qPCR

From the tissue sample, the whole RNA was acquired with the help of TRIzol™ Plus RNA purification kit (Invitrogen, CA, USA), per the kit's recommendations. cDNA was produced using the acquired RNA (1 μg) by reversed transcription using a ReverTra Ace-α kit (Toyobo, Shanghai, China). Subsequently, RT-PCR was performed using Powered SYBRMT Green PCR Master Mix (Applied Biosystems, CA, USA).

Statistical Measurements

For all the statistical measurements, GraphPad Prism 8 was employed. With the help of Fisher's exact tests and $\chi 2$ tests, the link between LIPH4 levels and TSCC patients' clinicopathological characteristics was assessed. The post-surgical DFS and OS rates were assessed via the Kaplan–Meier analysis, whereas the survival curve differences were elucidated by Log rank tests. Furthermore, the study employed the Cox proportional hazard regression model in conjunction with univariate survival analysis to conduct various comparisons of notable features. The primary objective was to determine the correlation between patient survival and prognostic. The statistically significant p-value was ≤ 0.05.

Results

Profiling of lncRNA from the TSCC Patients

Three GEO datasets were used to assess markedly dysregulated genes in NSCLC, which indicated 2448 differentially expressed genes in the GSE215767 datasets (Figure 1A–C). Additionally, each dataset's highly expressed genes that could differentiate TSCC from the normal tissues were identified via hierarchical clustering (Figure 1D). LIPH4 revealed the highest expression in TSCC tissues than normal tissues (Figure 1E) and was, therefore, selected for subsequent analyses.

LIPH4 Was Upregulated in TSCC

Using the RT-qPCR, LIPH4 expression was elucidated in 142 TSCC and normal tissues, respectively. The data indicated increased LIPH4 expression levels in TSCC samples than in healthy tissue samples ($p < 0.05$, Figure 2).

Association of LIPH4 Expression with Clinicopathological Factors

The median value of the LIPH4 level was set as a cutoff point to categorize 142 patients into two cohorts (low and high). Table 1 summarizes the link between LIPH4 levels and TSCC patients' clinicopathological characteristics. It was

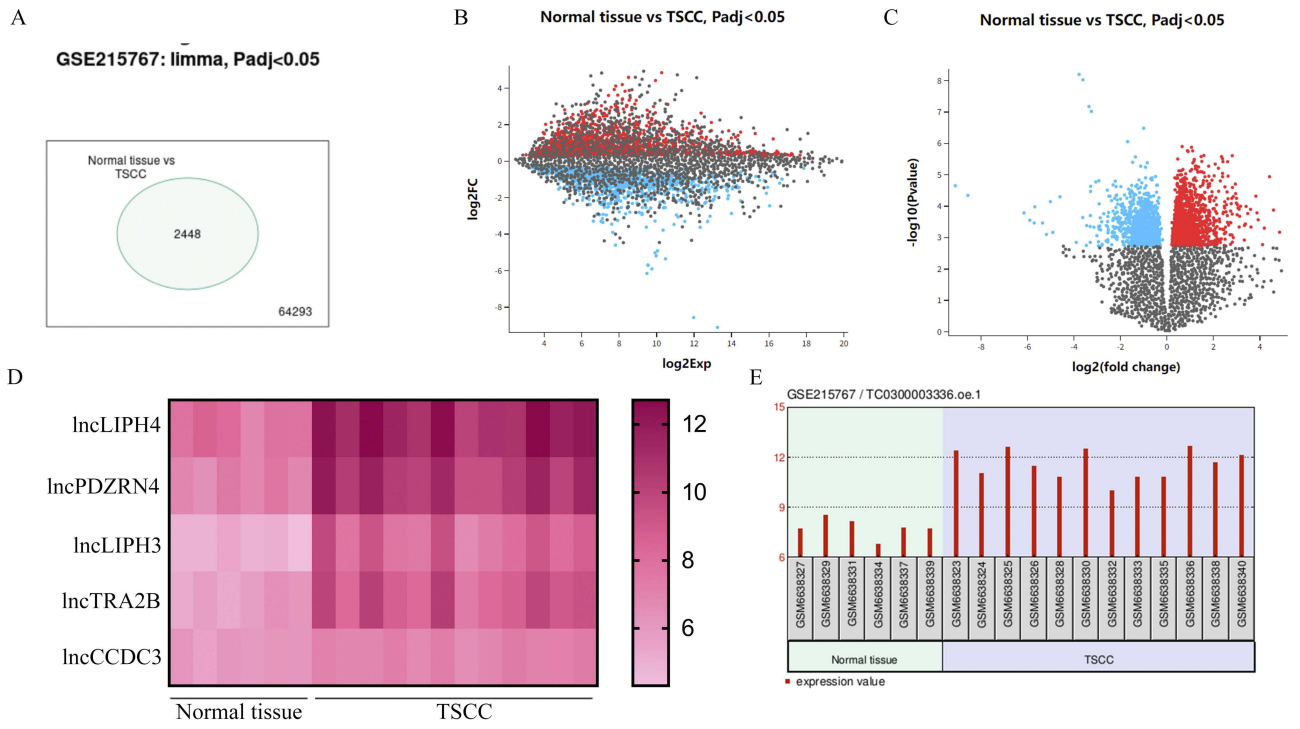

Figure 1 Data of lncRNA from the TSCC patients. (**A**) Venn diagram analysis of differentially expressed genes. (**B**) Mean-difference plot, (**C**) Volcano plot of differentially expressed gene, and (**D**) Cluster analysis of upregulated genes in GEO datasets GSE215767 were performed. (**E**) LIPH4 is upregulated in TSCC compared to normal tissues.

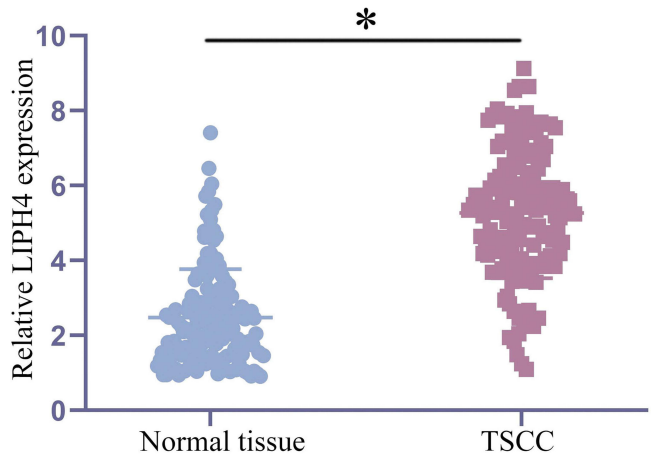

Figure 2 LIPH4 was upregulated in TSCC. The mRNA expression of LIPH4 was markedly enhanced in TSCC than in healthy tissues. *p value ≤ 0.05.

observed that increased LIPH4 expression was substantially linked with the tumor size, TNM stage, and differentiation of TSCC. However, no marked differences were identified in other characteristics between the two cohorts.

LIPH4 Upregulation Indicated a Poor TSCC Prognosis

The Kaplan–Meier and Log rank tests revealed that the duration of OS and DFS were notably shorter in patients with increased LIPH4 expression than those with reduced LIPH4 levels (Figure 3A and B). Furthermore, advanced TSCC patients in the high LIPH4 cohort had reduced DFS and OS rates than those in the low LIPH4 cohort (Figure 3C and D), as evidenced by combined analysis. The survival rates in early-stage patients are not shown.

In addition, a multivariate survival test was performed using the Cox proportional hazards model of significant parameters, which revealed that LIPH4 might independently predict DFS and OS of TSCC (Table 2).

Table 1 The Association of High LIPH4 Expression with Clinicopathological Features in 142 TSCC Patients

Characteristic	LIPH4 Expression		P value
	Low	High	
Gender			0.519
Male	45	49	
Female	26	22	
Age (years)			0.341
≥59	51	48	
< 59	20	23	
Tumor size (cm)			< 0.05
T1–T2	51	25	
T3–T4	20	46	
Differentiation			< 0.05
High	29	48	
Low	42	23	
Stage			< 0.05
I–IIA	44	25	
IIB-IV	27	46	

Diagnostic Significance of LIPH4 in TSCC

To elucidate the diagnostic application of LIPH4 in TSCC, an ROC curve was generated. The curve indicated that serum LIPH4 could be a promising diagnostic bio-index for TSCC, with an area under the curve of 0.8920 (95% CI = 0.8540–0.9299, Figure 4). The corresponding optimal cutoff point was 3.447, providing a specificity and sensitivity of 80.99 and 86.62%, respectively. The data suggests that LIPH4 could distinguish TSCC from healthy controls.

Discussion

The TSCC is highly malignant because of its infinite cellular reproduction cycle and its substantial impact on cervical lymph node metastasis and local invasion.[10] Because of the local invasion, TSCC rapidly develops in the tongue within a few weeks or months. Furthermore, the TSCC cell's migratory ability allows its metastasis to the cervical lymph nodes, which is why TSCC patients indicate increased recurrence rates, even after successful resection.[11] Therefore, for improved prediction of patient survival and cancer recurrence, more efficient markers are urgently required.

LncRNAs are transcripts with > 200 nt and very low protein-coding ability.[12,13] LncRNAs are believed to be a kind of transcriptional noise, which is produced by RNA polymerase II transcription and has no biological activity. However, recently, it was identified that lncRNAs essentially mediate gene expression and nuclear chromatin structure during the developmental process and are also frequently associated with disease incidence and development,[14,15] especially in tumors.[16–18] The biological functions of lncRNAs include epigenetic regulation of gene expression, virus–host interactions modulation, chromatin remodeling, and cancer cell migration and proliferation control.[12,19–21] Currently, they have become a hotspot in research for their potential as biomarkers for various malignancies because of their high tissue-specific expression.[22–24]

LIPH4 is a lncRNA that has been linked with the progression of esophageal squamous cell carcinoma.[9] This research investigated the association of LIPH4 expression with TSCC progression. It was indicated that LIPH4 was overexpressed

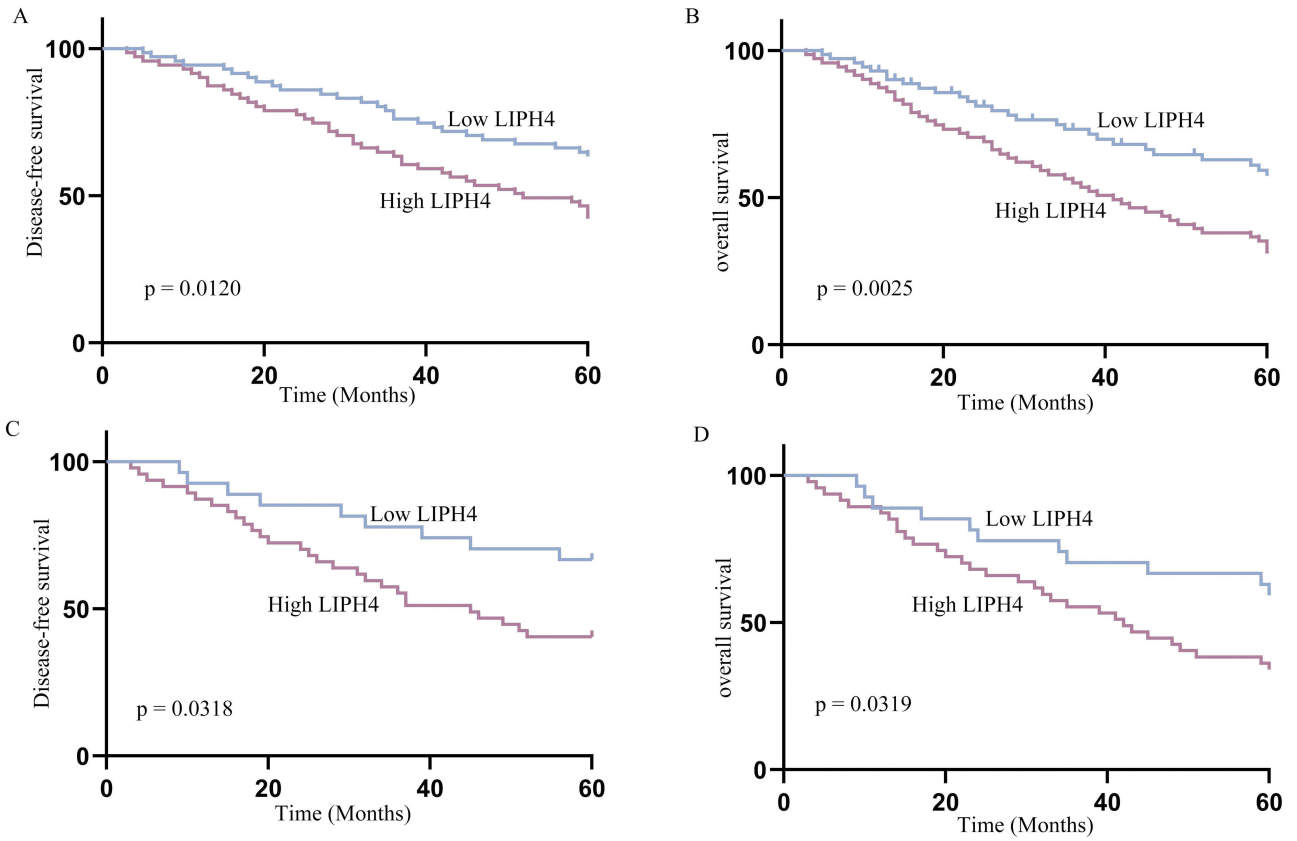

Figure 3 LIPH4 overexpression indicated a substandard prognosis in TSCC. Compared to subjects with low LIPH4 expression, high LIPH4 TSCC, and high LIPH4 advanced TSCC subjects demonstrated diminished disease-free and overall survival rates (**A, B, C**, and **D**, respectively).

in 142 TSCC tissues than normal tissues. Moreover, the LIPH4 levels were markedly linked with TSCC clinical stage and differentiation. Furthermore, the combined analysis indicated that the TSCC and advanced TSCC patients in the high LIPH4 cohort indicated reduced DFS and OS rates than those in the low LIPH4 cohort. Additionally, multivariate survival analysis revealed that increased LIPH4 level was a significant independent hazard factor for DFS and OS in TSCC, as well as the clinical stage. The ROC curve was also generated to assess a diagnostic value for serum LIPH4 in TSCC. However, how LIPH4 modulates the TSCC migration and invasion needs comprehensive research to fully understand LIPH4 functions.

Table 2 Clinicopathological-Related Uni- and Multivariate Analysis for the Disease-Free and Overall Survival of 142 TSCC Subjects

Factors	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Disease-free survival				
Gender	0.512 (0.214–1.025)	0.541	-	-
Age	0.418 (0.195–0.954)	0.348	-	-
Tumor size	1.845 (0.841–2.051)	0.022	1.685 (0.748–1.942)	0.019
Differentiation	1.952 (1.152–2.159)	0.019	1.818 (0.954–2.412)	0.013

(*Continued*)

Table 2 (Continued).

Factors	Univariate		Multivariate	
	HR (95% CI)	P value	HR (95% CI)	P value
Stage	1.754 (1.243–2.418)	0.023	1.524 (0.855–1.987)	0.021
LIPH4	2.125 (1.142–2.543)	0.009	2.018 (1.025–2.418)	0.011
Overall survival				
Gender	0.652 (0.352–1.125)	0.254	-	-
Age	0.519 (0.254–1.248)	0.355	-	-
Tumor size	1.954 (1.254–2.485)	0.025	1.842 (0.912–2.158)	0.026
Differentiation	2.149 (1.021–2.842)	0.021	2.018 (1.154–2.748)	0.022
Stage	2.211 (1.152–2.784)	0.014	2.141 (1.025–2.841)	0.017
LIPH4	2.418 (1.029–2.954)	0.005	2.310 (1.158–2.852)	0.008

This study still has some limitations. 1) The sample size is not very large, and the application of LIPH4 in clinical diagnosis may require an increase in sample size. 2) It has not been further confirmed whether LIPH4 can also serve as a biomarker for the efficacy of chemotherapy or radiotherapy. 3) This study only confirmed that LIPH4 can serve as a biomarker for the diagnosis of TSCC, but rapid detection method has been not developed and applied to patients.

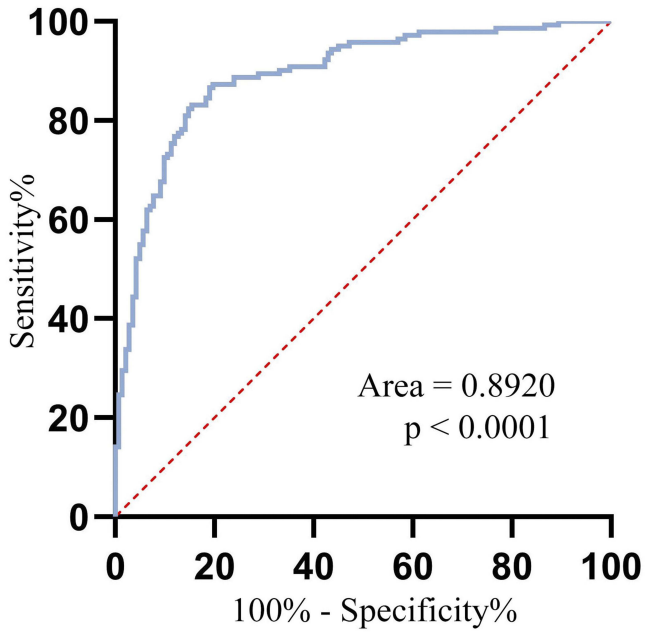

Figure 4 LIPH4 overexpression indicated a substandard prognosis in TSCC. The ROC curve revealed that LIPH4 has the potential to distinguish between TSCC patients and healthy individuals.

Conclusion

Overall, this is the first study to report that LIPH4 is upregulated in TSCC tissues and was linked with its clinical stage and differentiation. Moreover, the overexpression of LIPH4 might be a substantial prognostic factor for independently predicting the OS and DFS rates of TSCC patients. Understanding the association of LIPH4 with TSCC might help develop a novel diagnostic index for this cancer.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding author upon request.

Funding

This study was supported by the Science and Technology Planning Project of Jiangxi Provincial Health and Health Commission (SKJP220226433) and Ganzhou Science and Technology Bureau subject (208170552020).

Disclosure

The authors declare that they have no competing interests.

References

1. Kerawala C, Roques T, Jeannon JP, Bisase B. Oral cavity and lip cancer: United Kingdom National Multidisciplinary Guidelines. *J Laryngol Otol*. 2016;130(S2):S83–S89. doi:10.1017/S0022215116000499
2. Mitsudo K, Hayashi Y, Minamiyama S, et al. Chemoradiotherapy using retrograde superselective intra-arterial infusion for tongue cancer: analysis of therapeutic results in 118 cases. *Oral Oncol*. 2018;79:71–77. doi:10.1016/j.oraloncology.2018.02.002
3. Steens SCA, Bekers EM, Weijs WLJ, et al. Evaluation of tongue squamous cell carcinoma resection margins using ex-vivo MR. *Int J Comput Assist Radiol Surg*. 2017;12(5):821–828. doi:10.1007/s11548-017-1524-6
4. Cannon TL, Lai DW, Hirsch D, et al. Squamous cell carcinoma of the oral cavity in nonsmoking women: a new and unusual complication of chemotherapy for recurrent ovarian cancer? *Oncologist*. 2012;17(12):1541–1546. doi:10.1634/theoncologist.2011-0216
5. Rothenberg SM, Ellisen LW. The molecular pathogenesis of head and neck squamous cell carcinoma. *J Clin Invest*. 2012;122(6):1951–1957. doi:10.1172/JCI59889
6. Lai CC, Lin PM, Lin SF, et al. Altered expression of SIRT gene family in head and neck squamous cell carcinoma. *Tumour Biol*. 2013;34(3):1847–1854. doi:10.1007/s13277-013-0726-y
7. Gao W, Chan JY, Wong TS. Long non-coding RNA deregulation in tongue squamous cell carcinoma. *Biomed Res Int*. 2014;2014:405860. doi:10.1155/2014/405860
8. Bhan A, Soleimani M, Mandal SS. Long Noncoding RNA and Cancer: a New Paradigm. *Cancer Res*. 2017;77(15):3965–3981. doi:10.1158/0008-5472.CAN-16-2634
9. Xiao Y, Tang J, Yang D, et al. Long noncoding RNA LIPH-4 promotes esophageal squamous cell carcinoma progression by regulating the miR-216b/IGF2BP2 axis. *Biomark Res*. 2022;10(1):60. doi:10.1186/s40364-022-00408-x
10. Kato K, Miyazawa H, Kobayashi H, et al. The pattern and spread of invasion can predict late cervical lymph node metastasis in early tongue squamous cell carcinoma. *Diagn Pathol*. 2023;18(1):87. doi:10.1186/s13000-023-01371-3
11. Sun Y, Han J, Lu Y, Yang X, Fan M. Biological characteristics of a cell subpopulation in tongue squamous cell carcinoma. *Oral Dis*. 2012;18(2):169–177. doi:10.1111/j.1601-0825.2011.01860.x
12. Bridges MC, Daulagala AC, Kourtidis A. LNCcation: lncRNA localization and function. *J Cell Biol*. 2021;220(2). doi:10.1083/jcb.202009045
13. Ali T, Grote P. Beyond the RNA-dependent function of LncRNA genes. *Elife*. 2020;9. doi:10.7554/eLife.60583
14. Li D, Yang C, Yin C, et al. LncRNA, Important Player in Bone Development and Disease. *Endocr Metab Immune Disord Drug Targets*. 2020;20(1):50–66. doi:10.2174/1871530319666190904161707
15. Schmitz SU, Grote P, Herrmann BG. Mechanisms of long noncoding RNA function in development and disease. *Cell Mol Life Sci*. 2016;73(13):2491–2509. doi:10.1007/s00018-016-2174-5
16. Tang Y, Tian W, Zheng S, et al. Dissection of FOXO1-Induced LYPLAL1-DT Impeding Triple-Negative Breast Cancer Progression via Mediating hnRNPK/beta-Catenin Complex. *Research*. 2023;6:0289. doi:10.34133/research.0289
17. Ghasemian A, Omear HA, Mansoori Y, et al. Long non-coding RNAs and JAK/STAT signaling pathway regulation in colorectal cancer development. *Front Genet*. 2023;14:1297093. doi:10.3389/fgene.2023.1297093
18. Yu Z, Tang H, Chen S, et al. Exosomal LOC85009 inhibits docetaxel resistance in lung adenocarcinoma through regulating ATG5-induced autophagy. *Drug Resist Updat*. 2023;67:100915. doi:10.1016/j.drup.2022.100915
19. Wang J, Su Z, Lu S, et al. LncRNA HOXA-AS2 and its molecular mechanisms in human cancer. *Clin Chim Acta*. 2018;485:229–233. doi:10.1016/j.cca.2018.07.004
20. Cao HL, Liu ZJ, Huang PL, Yue YL, Xi JN. lncRNA-RMRP promotes proliferation, migration and invasion of bladder cancer via miR-206. *Eur Rev Med Pharmacol Sci*. 2019;23(3):1012–1021. doi:10.26355/eurrev_201902_16988
21. Zong S, Dai W, Guo X, Wang K. LncRNA-SNHG1 promotes macrophage M2-like polarization and contributes to breast cancer growth and metastasis. *Aging*. 2021;13(19):23169–23181. doi:10.18632/aging.203609
22. Jiang N, Meng X, Mi H, et al. Circulating lncRNA XLOC_009167 serves as a diagnostic biomarker to predict lung cancer. *Clin Chim Acta*. 2018;486:26–33. doi:10.1016/j.cca.2018.07.026

23. Liu S, Cao Q, An G, Yan B, Lei L. Identification of the 3-lncRNA Signature as a Prognostic Biomarker for Colorectal Cancer. *Int J Mol Sci*. 2020;21(24):9359. doi:10.3390/ijms21249359
24. Goyal B, Yadav SRM, Awasthee N, Gupta S, Kunnumakkara AB, Gupta SC. Diagnostic, prognostic, and therapeutic significance of long non-coding RNA MALAT1 in cancer. *Biochim Biophys Acta Rev Cancer*. 2021;1875(2):188502. doi:10.1016/j.bbcan.2021.188502