Expression of the long noncoding RNA RP11-169D4.1-001 in Hypopharyngeal Squamous cell carcinoma tissue and its clinical significance

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Abstract

Background: Increased research efforts have demonstrated that IncRNAs are associated with multiple head and neck tumors and play important roles in cancer. We previously found that RP11-169D4.1-001 plays a tumor-suppressive role in laryngeal cancer, but its function in human hypopharyngeal squamous cell carcinoma (HSCC) remains unknown. Thus, this research aimed to analyze the relationship between RP11-169D4.1-001 expression and HSCC clinicopathological features.

Methods: Real-time quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to detect the expression of RP11-169D4.1-001 in 70 pairs of HSCC and adjacent normal tissues.

Results: The expression level of RP11-169D4.1-001 in HSCC tissues was significantly lower than that in adjacent normal tissues (P = .001). The expression of RP11-169D4.1-001 had no significant relationship with tumor differentiation, stage, smoking, drinking, age, tumor location, or treatment. RP11-169D4.1-001 expression was associated with T category (P = .008) and lymph node metastasis (P = .001). Survival data were assessed by Kaplan-Meier curves. Patients with high RP11-169D4.1-001 expression were found to have a shorter overall survival than patients with low RP11-169D4.1-001 expression. Multivariate analysis also indicated that target RNA was an independent factor for prognosis. The ROC curve was constructed to clarify the diagnostic value of RP11-169D4.1-001.

Conclusions: RP11-169D4.1-001 may serve as a new biomarker and potential drug target and can be used as a new biomarker and a potential drug target for the detection and treatment of hypopharyngeal cancer, respectively. Furthermore, RP11-169D4.1-001 expression may be an independent prognostic factor affecting the survival of hypopharyngeal cancer patients.

Keywords
biomarker, diagnosis, HSCC, noncoding RNA, RP11-169D4.1-001
1 | INTRODUCTION

The long noncoding RNA (lncRNA) described herein, RP11-169D4.1-001, is expressed in eukaryotic cells, exceeds 200 nucleotides in length, and has no protein-coding functions. In recent years, many experiments have proven that lncRNAs play important roles in tumor occurrence and progression. lncRNAs can be used for early tumor diagnosis, prognosis evaluation, and novel treatment, but their specific mechanism of action remains unclear. Hypopharyngeal squamous cell carcinoma (HSCC) accounts for only 3%-5% of all head and neck malignancies, and the annual incidence rates of HSCC are approximately 2 ~ 5/100 000 worldwide and approximately 2 ~ 4/100 000 in China. Among the malignant tumors diagnosed annually worldwide, 2.4% are hypopharyngeal cancer, and the incidence of hypopharyngeal cancer has increased in recent years.

Clinically, squamous cell carcinoma is the main type of hypopharyngeal cancer, which has hidden sites of onset and poor biological characteristics and is prone to cervical lymph node metastasis. Despite advances in surgical and nonsurgical treatment, the overall fraction of hypopharyngeal cancer patients who survive has not been enhanced, and the illness is continually associated with a poor prognosis. In the initial treatment of hypopharyngeal carcinoma, 60%-80% of patients exhibit lymph node metastasis on the same side of the neck, and 40% of patients exhibit contralateral neck lymph node metastasis. The larynx and posterior ring space are easily invadable in the early disease stages, and hypopharyngeal carcinoma is often diagnosed in the late stages, a factor underlying the poor prognosis for head and neck cancer patients. The 5-year survival rate for these patients remains at approximately 40%. Patients with this cancer often exhibit no specific symptoms in the early stages of disease, often reach the late stages of clinical treatment, and thus miss out on valuable treatment opportunities. Furthermore, because the larynx and pharynx play key roles in the functions of pronunciation, breathing, and eating, among other activities, surgical resection treatment will inevitably severely damage the abovementioned functions and greatly reduce the patient's quality of life.

Currently, the role of lncRNAs in hypopharyngeal cancer is largely unknown. While how to diagnose hypopharyngeal cancer early and how to preserve these functions during treatment are concerns, specific early diagnostic markers and new treatments that can be applied clinically are lacking, and studying the occurrence and developmental mechanisms of laryngeal and hypopharyngeal cancer can provide a theoretical basis and a new direction for solving this problem. In this study, we further studied the expression of RP11-169D4.1-001 in hypopharyngeal cancer tissue. We aimed to analyze the relationship between RP11-169D4.1-001 expression and clinicopathological features to determine whether this IncRNA can serve as a biomarker of hypopharyngeal cancer.

2 | MATERIALS AND METHODS

2.1 | Patient sample collection

Seventy HSCC tissue samples and the corresponding matched normal tissues (dissected at >0.5 cm from the margin of the neoplastic lesion) were surgically resected from April 2010 to December 2015. All patients were from Ningbo Medical Center of Lihuili Hospital, and all hypopharyngeal carcinoma samples were independently examined by two or more pathologists to confirm cancerous and paratumoral normal tissues. Tumor stage was determined according to the American Joint Committee on Cancer (AJCC) tumor-node-metastasis (TNM) staging criteria. All specimens were harvested from males aged 42-82 years (median age, 61 years), and there were no females in this study. Before admission, none of the patients underwent biological treatment, radiotherapy, or chemotherapy; had a history of hepatitis B infection; or had a family history of hereditary disease. In addition, B-scan ultrasonography, computed tomography (CT), and other tests were used to confirm that no malignant tumors other than those of primary hypopharyngeal origin were present. Finally, the study adhered to the principle of informed consent and was approved by the Scientific Research Ethics Committee. Tumor specimens and adjacent normal tissues were immediately transferred to liquid nitrogen and stored in a −80°C cryogenic freezer upon dissection.

2.2 | Total RNA extraction

TRIzol reagent (Invitrogen) was used to extract total RNA from the HSCC and adjacent normal tissue samples, and a NanoDrop spectrophotometer and an Agilent 2100 Bioanalyzer (Agilent Technologies) were used to measure the RNA concentration and purity, respectively. The amount of RNA was calculated based on 1 OD$_{260}$ = 40 µg of RNA, and an A260/A280 ratio ranging from 1.8 to 2.1 was used to qualify RNA that could be used in subsequent experiments.

2.3 | Quantitative real-time polymerase chain reaction

Sequences of the IncRNA and the housekeeping gene GAPDH were obtained from the NCBI database (http://www.ncbi.nlm.nih.gov/), and the corresponding PCR primers were prefabricated by Invitrogen. The primer sequences were as follows: GAPDH: sense, 5'-ACCCACTCCTC-CACCTTGTGAC-3'; and antisense, 5'-TGTGCTGGTAC-GAAATTCGTT-3'; and RP11-169D4.1-001: sense, 5'-CTCCTAAAGTGGCTGATGGG-3'; and antisense, 5'-GACTCCTAGGGAAAATGGAAC-3'. Total RNA from the sample was reverse-transcribed into cDNA using the Promega GoScript Reverse Transcription (RT) System Kit, purchased from Invitrogen Corporation, according to the manufacturer's instructions. After reverse transcription, the cDNA was placed on ice and diluted with 80 µL of DEPC-H$_2$O; the sample was inverted and stored at −20°C.
The qPCR parameters for amplifying the lncRNA were as follows: 95°C for 10 minutes, followed by 45 cycles of 95°C for 15 seconds, 56°C for 30 seconds, and 72°C for 30 seconds. Reactions were performed according to standard protocol using GoTaq qPCR Master Mix (Promega) on a Stratagene MX3005P Real-time PCR machine. The threshold cycle (Ct) is used to determine the number of PCR cycles when a particular amplification threshold is reached and is used to reflect the amount of template. We used the housekeeping gene GAPDH to normalize the level of lncRNA. The ΔCt method was used to quantitatively analyze the level of lncRNA by ΔCt = Ct (target lncRNA) - Ct (GAPDH). The ΔCt value is one of the detection results. The 2^{-ΔΔCt} method was used to calculate the relative expression of RP11-169D4.1-001. Each sample was analyzed in biological triplicate.

### 2.4 Statistical analysis

The data were analyzed with SPSS software 18.0 (SPSS Inc.). Values of \( P < .05 \) were deemed statistically significant. The paired-sample t test was used to assess differences between two experimental groups. The relationships between clinicopathological characteristics (age, primary location, differentiation type, clinical stage, smoking history, etc) and RP11-169D4.1-001 were analyzed by the chi-square test or Fisher’s exact test, and the data in the study are expressed as the means ± standard deviations to determine high and low expression levels based on the mean of the HSCC tissue expression. The Kaplan-Meier method was used to calculate survival curves for patients with high or low RP11-169D4.1-001 expression and used multivariate regression analysis to define the determining factors for prognosis. Diagnostic value was determined by an evaluation of receiver operating characteristic (ROC) curves.

### 3 RESULTS

#### 3.1 RP11-169D4.1-001 expression levels in hypopharyngeal carcinoma and adjacent normal tissues

In total, 70 male patients were enrolled in the study. None of the 70 patients diagnosed with squamous cell cancer included in this analysis exhibited distant metastasis, but there may have been cervical lymph node metastasis. Furthermore, 13 patients were nonsmokers, while 57 patients had a history of smoking, and all patients were diagnosed for the first time. The expression of RP11-169D4.1-001 was significantly lower in the tumor samples compared to that in adjacent noncancerous mucosal samples (\( P = .001 \); Figure 1).

#### 3.2 Relationship between the expression level of RP11-169D4.1-001 and clinicopathological factors in patients with hypopharyngeal carcinoma

As shown in Table 1, the expression of RP11-169D4.1-001 had no significant relationship with tumor differentiation, stage, smoking, drinking, age, tumor location, or treatment. RP11-169D4.1-001 was expressed at significantly lower levels in tumor tissues than in matched normal mucosal tissues (\( n = 70, P = .001 \)).

![FIGURE 1](image)

**FIGURE 1** RP11-169D4.1-001 was expressed at significantly lower levels in tumor tissues than in matched normal mucosal tissues (\( n = 70, P = .001 \)).

**TABLE 1** Associations between RP11-169D4.1-001 expression and HSCC patient clinicopathological characteristics

| Characteristics          | Cases | Low (%) | High (%) | \( P \) value |
|--------------------------|-------|---------|----------|---------------|
| Age (y)                  |       |         |          |               |
| <60                       | 43    | 36      | 7        | .534          |
| ≥60                       | 27    | 21      | 6        |               |
| Primary location          |       |         |          |               |
| Pyriform sinuses          | 51    | 42      | 9        | .739          |
| Else                      | 19    | 15      | 4        |               |
| Differentiation           |       |         |          |               |
| Well and moderate         | 46    | 36      | 10       | .520          |
| Poor                      | 24    | 21      | 3        |               |
| Clinical stage            |       |         |          |               |
| I-II                      | 25    | 20      | 5        | .819          |
| III-IV                    | 45    | 37      | 8        |               |
| Smoking history           |       |         |          |               |
| Yes                       | 57    | 45      | 12       | .437          |
| No                        | 13    | 12      | 1        |               |
| Drinking history          |       |         |          |               |
| Yes                       | 52    | 42      | 10       | 1.000         |
| No                        | 18    | 15      | 3        |               |
| T category                |       |         |          |               |
| T1-T2                     | 45    | 37      | 8        | .008          |
| T3-T4                     | 25    | 20      | 5        |               |
| Lymph node metastasis     |       |         |          |               |
| N0                        | 18    | 10      | 8        | .001          |
| N+                        | 52    | 47      | 5        |               |
| Treatment                 |       |         |          |               |
| Only surgery              | 8     | 5       | 3        | .161          |
| Other                     | 62    | 52      | 10       |               |
expression was associated with T category \( (P = .008) \) and lymph node metastasis \( (P = .001) \).

3.3 | Relationship between RP11-169D4.1-001 expression level and survival time

Kaplan-Meier analysis demonstrated that patients with high RP11-169D4.1-001 expression had a shorter overall survival than patients with low RP11-169D4.1-001 expression \( (n = 57) \). A log-rank test confirmed that the results were statistically significant \( (P < .05) \). Importantly, multivariate analysis also indicated that RP11-169D4.1-001 was an independent factor for prognosis (Table 2). The results indicate that the expression of RP11-169D4.1-001 may be involved in the development of HSCC and may affect patient survival.

3.4 | Diagnostic efficiency of the target gene

Receiver operating characteristic curves were constructed using paracancerous tissues as controls (Figure 3). The area under the RP11-169D4.1-001 curve was 0.66 (95% CI = 0.568 – 0.749, \( P < .05 \)), the cutoff point was \( \Delta Ct = 13.82 \), and the sensitivity and specificity were 0.54 and 0.7, respectively.

4 | DISCUSSION

This research aimed to analyze the relationship between RP11-169D4.1-001 expression and HSCC clinicopathological features. We used q-RTPCR to detect the expression of RP11-169D4.1-001 in 70 pairs of HSCC and adjacent normal tissues. We found that the expression level of RP11-169D4.1-001 in HSCC tissues was significantly lower than that in adjacent normal tissues. A variety of treatments for hypopharyngeal or laryngeal tumors have been developed and compared, including surgery, radiotherapy (CRT), radiation therapy (RT), or a combination of these various treatments.\(^{11}\) In this study, the patients underwent the most appropriate surgical procedure, 8 patients underwent only surgery, and 62 patients were supplemented with radiotherapy and/or radiotherapy. The expression of RP11-169D4.1-001 had no significant relationship with tumor differentiation, stage, smoking, drinking, age, tumor location, and treatment. RP11-169D4.1-001 expression was associated with T category and lymph node metastasis. The survival time of 70 patients was based on the time of first diagnosis. The end of the follow-up period was June 30, 2018. Kaplan-Meier analysis showed that patients with high RP11-169D4.1-001 expression had shorter survival rates than patients with low RP11-169D4.1-001 expression. Sensitivity reflected the true-positive rate, while specificity reflected the true-negative rate. The closer AUC was to 1, the better the diagnostic effect; AUC had a lower accuracy at 0.5 – 0.7; a certain accuracy at 0.7 – 0.9; and a higher accuracy above 0.9.\(^{12}\)

Through ROC curve analysis, we found that RP11-169D4.1-001 had a lower diagnostic value and lower sensitivity and specificity in HSCC. Of course, this finding may be due to the limited number of samples studied, and we are not completely sure that RP11-169D4.1-001 has diagnostic value in HSCC. Studies have shown that combined

| Variables | Hazard ratio | 95% CI | \( P \) value |
|-----------|--------------|--------|-------------|
| Age       | 0.991        | 0.926-1.061 | .804        |
| Primary location (pyriform sinuses/other) | 0.879 | 0.280-2.757 | .825 |
| Differentiation (poor/well & moderate) | 0.900 | 0.237-3.424 | .878 |
| Clinical stage (I-II/III-IV) | 1.032 | 0.357-2.980 | .954 |
| Smoking history (no/yes) | 1.650 | 0.346-7.873 | .530 |
| Drinking history (no/yes) | 0.989 | 0.250-3.918 | .988 |
| T category (T1-T2/ T3-T4) | 0.695 | 0.232-2.082 | .516 |
| Lymph node metastasis (no/yes) | 1.805 | 0.482-6.765 | .381 |
| Treatment (only surgery/else) | 1.265 | 0.238-6.732 | .783 |
| RP11-169D4.1-001 levels (high/low) | 0.258 | 0.071-0.946 | .41 |

TABLE 2 Multivariate analysis of prognostic factors for overall survival in HSCC patients.
Cancers are among the most common head and neck cancers.17,18 While hypopharyngeal carcinoma, the tumor is prone to cervical lymph nodes metastasis. Due to the unique lymphatic and vascular anatomy of hypopharyngeal tumors in the head and neck are prone to cervical lymph node metastasis and mainly invades the cervical lymph nodes. However, whether this lncRNA can be used as an important marker for the follow-up monitoring of patients with hypopharyngeal cancer requires further study. In addition, further exploring the specific role and mechanism of RP11-169D4.1-001 in the development of hypopharyngeal carcinogenesis is still necessary to provide novel diagnostic and treatment strategies for hypopharyngeal cancer.

5 | CONCLUSION

High RP11-169D4.1-001 expression is associated with the risk and prognosis of HSCC; therefore, this lncRNA may be a biomarker for assessing HSCC.

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ETHICAL APPROVAL

Experimental procedures were reviewed and approved by the Ethics Committee of Ningbo Lihuili Hospital. All participants signed written informed consent documents.

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REFERENCES

1. Mo X, Wu Y, Chen L, et al. Global expression profiling of metabolic pathway-related IncRNAs in human gastric cancer and the identification of RP11-555H23.1 as a new diagnostic biomarker. J Clin Lab Anal. 2019;33(2):e22692.
2. Mo X, Li T, Xie Y, et al. Identification and functional annotation of metabolism-associated IncRNAs and their related protein-coding genes in gastric cancer. Mol Genet Genomic Med. 2018;6(5):728-738.
3. Nie ZL, Wang YS, Mei YP, et al. Prognostic significance of long non-coding RNA Z38 as a candidate biomarker in breast cancer. J Clin Lab Anal. 2018;32(1):e22193.
4. Cooper JS, Porter K, Mallin K, et al. National cancer database report on cancer of the head and neck: 10-year update. Head Neck. 2009;31:748-758.
5. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin. 2011;61:69-90.
6. Gourin CG, Terris DJ. Carcinoma of the hypopharynx. Surg Oncol Clin N Am. 2004;13:81-98.
7. Buckley JG, Macleannan K. Cervical node metastases in laryngeal and hypopharyngeal cancer: a prospective analysis of prevalence and distribution. Head Neck. 2000;22:380-385.
8. Song J, Chang I, Chen Z, Kang M, Wang CY. Characterization of side populations in HNSCC: highly invasive, chemoresistant and abnormal Wnt signaling. PLoS ONE. 2010;5:e11456.
9. Belcher R, Hayes K, Fedewa S, Chen AY. Current treatment of head and neck squamous cell cancer. J Surg Oncol. 2014;110:551-574.
10. Zhu L, Li T, Shen Y, Yu X, Xiao B, Guo J. Using tRNA halves as novel biomarkers for the diagnosis of gastric cancer. Cancer Biomark. 2019;25(2):169-176.
11. Garneau JC, Bakst RL, Miles BA. Hypopharyngeal cancer: a state of the art review. Oral Oncol. 2018;86:244-250.
12. Jones CM, Athanasiou T. Summary receiver operating characteristic curve analysis techniques in the evaluation of diagnostic tests. Ann Thorac Surg. 2005;79:16-20.
13. Sun L, Tu H, Chen T, et al. Three-dimensional combined biomarkers assay could improve diagnostic accuracy for gastric cancer. Sci Rep. 2017;7(1):11621.
14. Zhang J, Song Y, Zhang C, et al. Circulating MiR-16-5p and MiR-19b-3p as two novel potential biomarkers to indicate progression of gastric cancer. Theranostics. 2015;5(7):733-745.
15. Zhou C, Chen Z, Dong J, et al. Combination of serum miRNAs with Cyfra21-1 for the diagnosis of non-small cell lung cancer. Cancer Lett. 2015;367(2):138-146.
16. Riaz N, Morris LG, Lee W, Chan TA. Unraveling the molecular genetics of head and neck cancer through genome-wide approaches. Genes Dis. 2014;1:75-86.
17. Zhou C, Li J, Li Q, et al. The clinical significance of HOXA9 promoter hypermethylation in head and neck squamous cell carcinoma. J Clin Lab Anal. 2019;33(5):e22873.
18. Ye D, Zhou C, Wang S, Deng H, Shen Z. Tumor suppression effect of targeting periostin with siRNA in a nude mouse model of human laryngeal squamous cell carcinoma. J Clin Lab Anal. 2019;33(1):e22622.
19. Shen Z, Hao W, Zhou C, et al. Long non-coding RNA AC026166.2-001 inhibits cell proliferation and migration in laryngeal squamous cell carcinoma by regulating the miR-24-3p/p27 axis. Sci Rep. 2018;8(1):3375.
20. Zhou J, Li M, Yu W, et al. AB209630, a long non-coding RNA decreased expression in hypopharyngeal squamous cell carcinoma, influences proliferation, invasion, metastasis, and survival. Oncotarget. 2016;7:14628-14638.
21. Shen Z, Li Q, Deng H, Lu D, Song H, Guo J. Long non-coding RNA profiling in laryngeal squamous cell carcinoma and its clinical significance: potential biomarkers for LSCC. PLoS ONE. 2014;9:e108237.
22. Wang P, Wu T, Zhou H, et al. Long noncoding RNA NEAT1 promotes laryngeal squamous cell cancer through regulating miR-107/CDK6 pathway. J Exp Clin Cancer Res. 2016;35:22.
23. Zhou J, Li M, Yu W, et al. AB209630, a long non-coding RNA decreased expression in hypopharyngeal squamous cell carcinoma, influences proliferation, invasion, metastasis, and survival. Oncotarget. 2016;7:14628-14638.
24. Eun-Jae C, Sang-Hyo L, So-Hye B, Park I, Cho SJ, Rho YS. Pattern of cervical lymph node metastasis in medial wall pyriform sinus carcinoma. Laryngoscope. 2014;124:882-887.
25. Kim SY, Rho YS, Choi EC, et al. Clinicopathological factors influencing the outcomes of surgical treatment in patients with T4a hypopharyngeal cancer. BMC Cancer. 2017;17:904.
26. Kotwall C, Sako K, Razack MS, Rao U, Bakamjian V, Shedd DP. Metastatic patterns in squamous cell cancer of the head and neck. Am J Surg. 1987;154:439-442.
27. Koo BS, Lim YC, Lee JS, Kim YH, Kim SH, Choi EC. Management of contralateral N0 neck in pyriform sinus carcinoma. Laryngoscope. 2006;116:1268-1272.
28. Xing Y, Zhang J, Lin H, et al. Relation between the level of lymph node metastasis and survival in locally advanced head and neck squamous cell carcinoma. Cancer. 2016;122:534-545.
29. Joo YH, Cho KJ, Kim SY, Kim MS. Prognostic significance of lymph node density in patients with hypopharyngeal squamous cell carcinoma. Ann Surg Oncol. 2015;22(Suppl 3):S1014-1019.
30. Roberts TJ, Colevas AD, Hara W, Holsinger FC, Oakley-Girvan I, Divi V. Number of positive nodes is superior to the lymph node ratio for predicting the outcomes of surgical treatment in patients with T4a hypopharyngeal cancer. Ann Surg Oncol. 2016;23:1025-1032.
31. Bosetti C, Gallus S, Peto R, et al. Tobacco smoking, smoking cessation, and cumulative risk of upper aerodigestive tract cancers. Am J Epidemiol. 2008;167:468-473.

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