Integrated analysis of lncRNA-associated ceRNA network in p16-positive and p16-negative head and neck squamous cell carcinoma

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Abstract

Determination of human papillomavirus (HPV) status has become clinically relevant for head and neck squamous cell carcinoma (HNSCC) patients. p16 immunohistochemistry is one of the recommended methods for classifying HPV status. However, long noncoding RNAs (lncRNAs) and related competing endogenous RNA (ceRNA) networks linked to different p16-status HNSCC are still absent.

In the present study, The Cancer Genome Atlas database provided RNA profiles as well as clinical information from 26 p16-positive HNSCC samples, 71 p16-negative HNSCC samples, and 44 adjacent normal control samples. Differentially expressed RNAs (DERNAs) between HNSCC samples and normal samples were identified by limma package in R. Functional enrichment analysis of differentially expressed mRNAs was performed using Clusterprofiler package in R. Survival analysis of DERNAs was carried out by survival package in R. The ceRNA network was constructed using GDCRNATools package in R.

A total of 102 lncRNAs, 196 microRNAs (miRNAs), and 2282 mRNAs were identified as p16-positive-specific DERNAs. There were 90 lncRNAs, 153 miRNAs, and 2038 mRNAs were identified as p16-negative-specific DERNAs. Functional enrichment analysis revealed that the differentially expressed mRNAs in the p16-positive and the p16-negative group were mainly enriched in the "DNA replication" and "extracellular matrix -receptor interaction" pathway, respectively. Among the top 25 DERNAs, there were 1 key lncRNA, 1 key miRNA, and 1 key messenger RNA in the p16-positive group and 2 key lncRNAs, 1 key miRNA, and 2 key mRNAs in the p16-negative group were significantly related to the overall survival. Then the ceRNA network in the p16-positive and p16-negative group was constructed. There were 5 lncRNAs, 16 miRNAs, and 66 mRNAs included in the p16-positive group ceRNA network and 1 lncRNA, 4 miRNAs, and 28 mRNAs included in the p16-negative group ceRNA network. Among the RNAs in the ceRNA network, 5 mRNAs were significantly related to the overall survival.

Taken together, we revealed the differential RNA expression profiling and the differential ceRNA network in the p16-positive and p16-negative group of HNSCC. Our findings provided a novel insight into this HPV-related cancer and potential biomarkers and therapeutic targets for HNSCC based on p16 status.

Abbreviations: AJCC = American Joint Commission on Cancer, BP = biological process, CC = cellular component, ceRNA = competing endogenous RNA, DElncRNA = differentially expressed lncRNA, DEmiRNA = differentially expressed miRNA, DEmRNA = differentially expressed mRNA, DERNAs = differentially expressed RNAs, FC = fold change, FDR = false discovery rate, GO = Gene Ontology, HNSCC = head and neck squamous cell carcinoma, HPV = human papillomavirus, IHC = immunohistochemistry, KEGG = Kyoto Encyclopedia of Genes and Genomes, lncRNAs = long noncoding RNAs, MF = molecular function, miRNA = microRNA, mRNA = messenger RNA, ncRNAs = noncoding RNAs, OPSCC = oropharyngeal squamous cell carcinoma, OS = overall survival, TCGA = The Cancer Genome Atlas, UICC = Union for International Cancer Control.

Keywords: competing endogenous RNA, head and neck squamous cell carcinoma, long noncoding RNA, p16, prognosis

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1. Introduction

Head and neck squamous cell carcinoma (HNSCC) ranks as the sixth most common cancer worldwide. It has a yearly incidence of 660,000 cases with 40%–50% mortality.[1] The carcinogenesis relates to different etiologies and a large variety of molecular changes. Tobacco use and excessive alcohol consumption are the major classical risk factors for HNSCC.[2] Currently, human papillomaviruses (HPVs) have emerged as a novel risk factor for HNSCCs, especially for oropharyngeal squamous cell carcinoma (OPSCC).[3] In general, patients with HPV-positive OPSCC tend to be a relatively younger age than their HPV-negative counterparts and less likely to be smokers or excessive alcohol consumers.[4] Moreover, they tend to be more frequently male and have a better overall prognosis.[5] Although the effect of HPV in HNSCCs located outside the oropharynx is less well understood, HPV positivity is also associated with more favorable outcomes.[6] p16 is a tumor suppressor protein that is overexpressed in HPV-driven carcinomas.[7] Many research showed that p16 immunohistochemistry (IHC) is a cost-effective method with good sensitivity and negative predictive value. This method is recommended as a surrogate marker of HPV status by the Union for International Cancer Control and the College of American Pathologists.[8,9] However, the precise mechanisms of how p16-positive HNSCC and p-16 negative HNSCC occurs and progresses are still not elucidated.

Tumorigenesis and cancer development has been closely associated with the aberrant expression of RNAs. Less than 2% of the total genome encodes protein-coding genes, suggesting that noncoding RNAs (ncRNAs) represent most of the human transcriptome.[10] Long noncoding RNAs (lncRNAs) are defined as transcripts of more than 200 nucleotides and have been implicated in a diverse range of biological processes (BPs) in cancer.[11] MicroRNAs (miRNAs) are short ncRNAs, with a length of approximately 18–25 nucleotides. They are evolutionarily conserved and are pivotal post-transcriptional mediators of gene regulation.[12] In 2011, Salmena et al[13] hypothesized a novel regulatory mechanism between lncRNAs and messenger RNAs (mRNA), namely competing endogenous RNA (ceRNA) network. In this theory, ceRNAs regulate each other by competing specifically for shared miRNAs. miRNA competition thus extends beyond the noncoding transcriptome to protein-coding mRNAs. Increasing evidence has confirmed that the lncRNA-miRNA-mRNA ceRNA network plays key roles in human cancers, such as esophageal cancer,[14] lung cancer,[15] as well as HNSCC.[16] However, current knowledge for lncRNA-miRNA-mRNA in human cancers is not enough, including p16-positive and p16-negative HNSCC.

In the current study, to detect the aberrant expression profile of ncRNAs and mRNAs in p16-positive and negative HNSCC patients, the p16-positive and negative HNSCC tissue and normal tissue expression profiles from The Cancer Genome Atlas (TCGA) database were obtained. Subsequently, a functional enrichment analysis for these aberrantly expressed mRNAs was conducted. Furthermore, p16-positive group and p16-negative group ceRNA networks were constructed including differentially expressed RNAs (DERNAs). Kaplan-Meier analysis was performed to identify key DERNAs and ceRNA-related RNAs that are associated with overall survival (OS). They may also serve as promising diagnostic biomarkers and therapeutic targets for different p16 status HNSCC in the future.

2. Materials and Methods

2.1. Data collection and processing

Gene expression data (RNA sequencing profiles and miRNA profiling) and corresponding clinical data of HNSCC were downloaded from the TCGA database (https://portal.gdc.cancer.gov/). The inclusion criteria were set as follows: the p16 IHC status of HNSCC samples were recorded and patients with complete data including age, gender, OS time, and vital status. The exclusion criteria were set as follows: the p16 IHC status of HNSCC samples were unrecorded and patients with incomplete clinicopathological data. According to the criteria, 97 primary HNSCC samples, including 26 patients with immunohistochemically p16-positive status (p16-positive group) HNSCC and 71 patients with immunohistochemically p16-negative status (p16-negative group), and 44 normal controls were collected in our analysis. The clinicopathological characters of the p16-positive group and p16-negative group HNSCC patients are shown in Table 1.

Among these data, mRNA and lncRNA data were collected from Illumina HiSeqRNASeq platforms, and miRNA data were collected from Illumina HiSeqmiRNASeq platforms. mRNAs and lncRNAs were encoded according to the ENSEMBL database version 89, European Bioinformatics Institute, Cambridge, UK (https://www.ensembl.org), miRNAs were annotated according to miRbase version 22, The University of Manchester, Manchester, UK (http://www.mirbase.org). The ethical approval

Table 1

Clinicopathological characters of the p16 positive and p16 negative HNSCC patients.

Characteristics	Subtype	No. of cases (%)	Characteristics	Subtype	No. of cases (%)
	p16 positive HNSCC patients			**p16 negative HNSCC patients**	
Age	<60	18 (69.2)	Age	<60	35 (49.3)
	≥60	8 (30.8)		≥60	36 (50.7)
Gender	Male	23 (88.5)	Gender	Male	54 (76.1)
	Female	3 (11.5)		Female	17 (23.9)
Pathologic stage	Stage I	1 (3.85)	Pathologic stage	Stage I	5 (7.0)
	Stage II	3 (11.5)		Stage II	5 (7.0)
	Stage III	1 (3.85)		Stage III	9 (12.7)
	Stage IV	10 (38.5)		Stage IV	44 (62.0)
	NA	11 (42.3)		NA	8 (11.3)
Pathologic T	T1–T2	10 (38.5)	Pathologic T	T1–T2	20 (28.2)
	T3–T4	7 (26.9)		T3–T4	43 (60.5)
	Tx	9 (34.6)		Tx	8 (11.3)
Pathologic N	N0	3 (11.5)	Pathologic N	N0	16 (22.5)
	N1–N3	12 (46.2)		N1–N3	48 (67.6)
	Nx	11 (42.3)		Nx	7 (9.9)
Vital status	Alive	22 (84.6)	Vital status	Alive	46 (64.8)
	Dead	4 (15.4)		Dead	25 (35.2)

HNSCC = head and neck squamous cell carcinoma, NA, Nx, Tx = pathologic stage is unknown.

was not necessary. This study conformed with the publication guidelines provided by TCGA and all the data are publicly available.

2.2. Analysis of differentially expressed lncRNAs, mRNAs, and miRNAs

The differentially expressed lncRNA (DElncRNA), mRNA (DEmRNA), and miRNA (DEmiRNA) in p16-positive HNSCC samples and normal controlled samples or p16-negative HNSCC samples and normal controlled samples were identified using "limma" package of R software. *P* value was adjusted to the false discovery rate (FDR). |log$_2$ Fold Change|>1.0 and FDR <0.01 were used as statistically significant. In order to visualize the results of differential genetic analysis, the heatmaps were plotted using "pheatmap" packages of R software.

2.3. Functional enrichment analysis

To understand the potential mechanisms involved in the tumorigenesis of p16-positive and p16-negative HNSCC, Gene Ontology (GO) functional enrichment, and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed on DEmRNAs using "ClusterProfiler" package of R software. A *P* < .01 was considered as enriched gene sets.

2.4. Survival analysis

To investigate the prognostic characteristics of DERNAs in different p16 status groups, the p16-positive group and p16-negative group HNSCC samples were divided into the high expression group or the low expression group (according to the median) in terms of the expression of lncRNAs, mRNAs, and miRNAs, separately. To correlate the DERNAs with the OS, the survival curves were plotted using "survival" package of R software based on the Kaplan-Meier method. A *P* < .05 was considered statistically significant.

2.5. Construction of lncRNA-miRNA-mRNA ceRNA network

Based on the theory that lncRNAs can invoke miRNA sponges to affect the expression of mRNA, we predict the interactions between DElncRNA and DEmiRNA and DEmiRNA. A ceRNA network was constructed by using "GDCRNATools" package of R software.[17] The ceRNA network was visualized using Cytoscape 3.6.1 software, Institute for Systems Biology, Seattle, USA.

3. Results

3.1. Screening of differentially expressed RNAs in the p16-positive group and p16-negative group HNSCC samples

By comparing the expression of RNAs between the p16-positive (or negative) group HNSCC tissue samples with normal tissue samples, we identified some DERNAs. According to the cutoff criteria of |log$_2$ Fold Change|>1.0 and an FDR <0.01, 102 DElncRNAs (56 upregulated and 46 downregulated), 196 DEmiRNAs (89 upregulated and 107 downregulated), and 2282 DEmRNAs (970 upregulated and 1312 downregulated) were identified in the p16-positive group. In the p16-negative group, 90 DElncRNAs (45 upregulated and 45 downregulated), 153 DEmiRNAs (72 upregulated and 81 downregulated), and 2038 DEmRNAs (1012 upregulated and 1026 downregulated) were identified. The variable expression of differentially expressed DElncRNAs, DEmiRNAs, and DEmRNAs between the p16-positive (Fig. 1A) group (or the p16-negative group [Fig. 1B]). HNSCC samples with the normal controls were visualized in the heatmap. The top 25 differentially expressed

DElncRNAs/DEmiRNAs/DEmRNAs in the p16-positive group and the p16-negative group HNSCC samples compared with normal controls were listed. See Table S1, Supplemental Digital Content, http://links.lww.com/MD/H39, which illustrates the top 25 differentially expressed DElncRNAs in the p16-positive group. See Table S2, Supplemental Digital Content, http://links.lww.com/MD/H39, which illustrates the top 25 differentially expressed DElncRNAs in the p16- negative group. See Table S3, Supplemental Digital Content, http://links.lww.com/MD/H39, which illustrates the top 25 differentially expressed DEmiRNAs in the p16-positive group. See Table S4, Supplemental Digital Content, http://links.lww.com/MD/H39, which illustrates the top 25 differentially expressed DEmiRNAs in the p16-negative group. See Table S5, Supplemental Digital Content, http://links.lww.com/MD/H39, which illustrates the top 25 differentially expressed DEmRNAs in the p16-positive group. See Table S6, Supplemental Digital Content, http://links.lww.com/MD/H39, which illustrates the top 25 differentially expressed DEmRNAs in the p16-negative group.

3.2. Functional enrichment analysis for DEmRNAs in the p16-positive group and p16-negative group

To further analyze the biological function and corresponding pathways of DEmRNAs in the p16-positive group and the p16-negative group, GO and KEGG analysis was performed. GO annotation consists of cellular component, BP, and molecular function. The top 5 enrichment scores in GO analysis of the p16-positive group and the p16-negative group, DEmRNAs were shown in Figure 2A and B.

Additionally, the top 10 KEGG pathways of the p16-positive and the p16-negative group were shown in Figure 3A and B. The DNA replication pathway was found to have the lowest *P* value in the p16-positive group and the pathview was shown in Figure 3C. The extracellular matrix(ECM)-receptor interaction pathway was found to have the lowest *P* value in the p16-negative group and the pathview was shown in Figure 3D.

3.3. Prognostic OS assessment of key DElncRNAs, DEmiRNAs, and DEmRNAs in the p16-positive and p16-negative group

To evaluate the prognostic signatures of DElncRNAs, DEmiRNAs, and DEmRNAs, the survival analysis was performed and a *P* < .05 was considered as a cutoff. In the p16-positive group, a total of 9 DElncRNAs, 31 DEmiRNAs, and 167 DEmRNAs were significantly related to the OS. In the p16-negative group, a total of 10 DElncRNAs, 9 DEmiRNAs, and 106 DEmRNAs were significantly related to the OS. Combined with the results of survival analysis and expression analysis, we redefined the key DElncRNAs/DEmiRNAs/DEmRNAs as the top 25 upregulated or downregulated DElncRNAs/DEmiRNAs/DEmRNAs, which could also indicate the OS of HNSCC patients significantly. In the p16-positive group, key DElncRNA (U62317.3), key DEmiRNA (hsa-miR-375), and key DEmRNA (KLHDC7B) were found (Fig. 4A). Similarly, there were 2 key DElncRNAs (AL161431.1, ZNF667-AS1), 1 key DEmiRNA (has-miR-424-5p), and 2 key DEmRNAs (FAM3B, PTHLH) (Fig. 4B) in the p16-negative group.

3.4. Construction and analysis of lncRNA-miRNA-mRNA ceRNA network in the p16-positive group and p16-negative group

To further understand the interaction between DElncRNAs, DEmiRNAs, and DEmRNAs in the p16-positive group and the p16-negative group, the ceRNA networks were constructed. In the p16-positive group, 5 lncRNA nodes, 16 miRNA nodes,
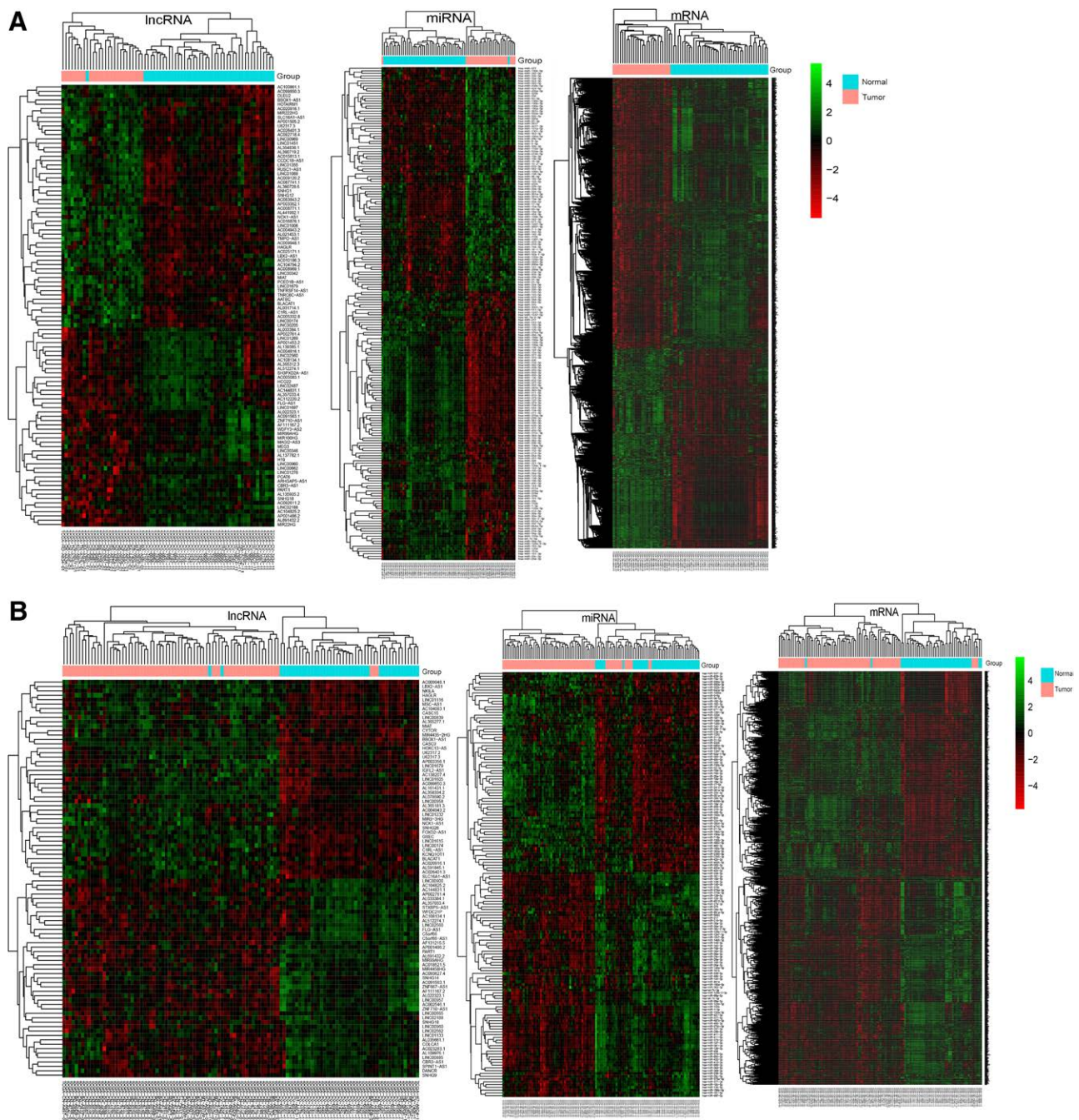
Figure 1. Heatmap of DElncRNAs, DEmiRNAs, and DEmRNAs in p16-positive HNSCC samples and normal control samples. (A); in p16-negative HNSCC samples and normal control samples (B). The pink and light blue colors represent the tumor group and the normal controls group, respectively. The red and green colors indicate higher expression levels and lower expression levels, respectively. The rows represent DERNAs and the columns represent the samples. DElncRNAs = differentially expressed lncRNAs, DEmiRNAs = differentially expressed miRNAs, DEmRNAs = differentially expressed mRNAs, DERNAs = differentially expressed RNAs, HNSCC = head and neck squamous cell carcinoma, lncRNAs = long noncoding RNAs, miRNA = microRNA, mRNA = messenger RNA.

66 mRNA nodes, and 162 edges were identified. In the p16-negative group, 1 lncRNA node, 4 miRNA nodes, 28 mRNA nodes, and 59 edges were identified. Cytoscape was used to visualize the p16-positive group (Fig. 5A) and the p16-negative group (Fig. 5B) ceRNA network.

3.5. Identify the RNAs in the p16-positive and p16-negative group ceRNA network associated with HNSCC patient OS

To identify critical RNAs that play important roles in both the BP and prognosis of the ceRNA network, Kaplan-Meier estimate and log-rank test were used to determine the relationship between OS and RNAs in the ceRNA network. In the p16-positive group, 3 mRNAs (PDLIM5, USP25, SLMAP) were observed to be significantly related to OS ($P < .05$) (Fig. 6A). In the p16-negative group, 2 mRNAs (ITGA5, ADA) were observed to be significantly related to OS ($P < .05$) (Fig. 6B).

4. Discussion

HNSCCs affect more than 660,000 patients per year worldwide. Despite surgery, radiation, and chemotherapy, approximately half of patients will die of the disease.[18] Smoking is implicated in the rise of HNSCC in developing countries, and
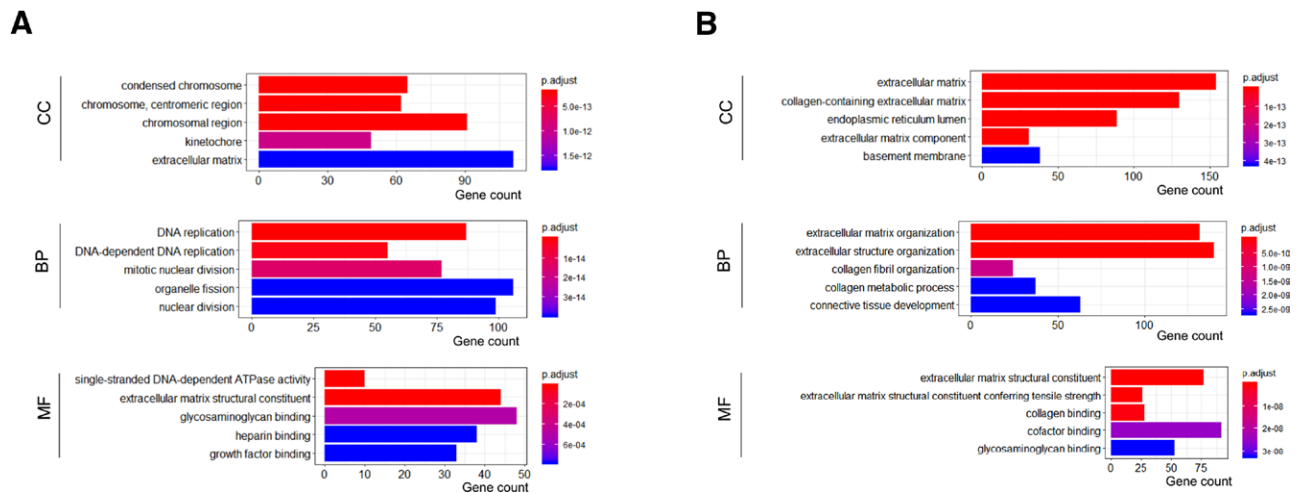
Figure 2. The top 5 enrichment scores in GO enrichment analysis of DEmRNAs. (A) CC, BP, MF of DEmRNAs in the p16-positive group; (B) CC, BP, MF of DEmRNAs in the p16-negative group. ATPase = adenosine-triphosphate synthase, BP = biological process, CC = cellular component, DEmRNAs = differentially expressed mRNAs, GO = Gene Ontology, MF = molecular function.
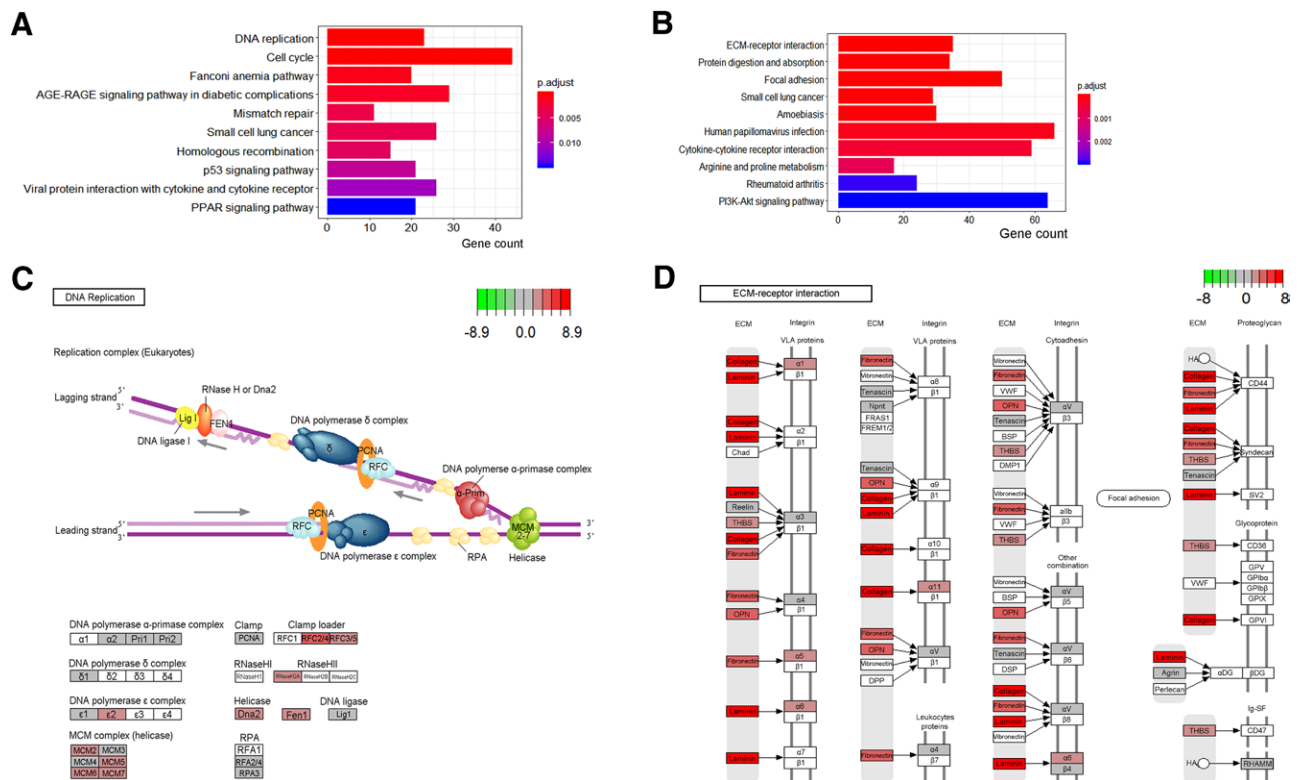

Figure 3. The top 10 enrichment scores in KEGG pathway analysis of the DEmRNAs in the p16-positive group. (A) and in the p16-negative group (B). (C) DNA replication map from KEGG analysis; (D) ECM-receptor interaction map from KEGG analysis, the red colors represent the upregulated DEmRNAs green colors represent the downregulated DEmRNAs. AGE-RAGE = advanced glycosylation end-product specific receptor, BSP = binding sialoprotein, regulatoryD-EmRNAs = differentially expressed mRNAs, DMP1 = dentin matrix acidic phosphoprotein 1, Dna2 = DNA replication helicase/nuclease 2, DPP = dipeptidyl peptidase, DSP = desmoplakin, ECM = extracellular matrix, FEN1 = flap structure-specific endonuclease 1, FRAS1 = fraser extracellular matrix complex subunit 1, FREM1/2 = FRAS1 related extracellular matrix ½, GPIbα = glycoprotein Ib platelet subunit alpha, GPIbβ = glycoprotein Ib platelet subunit beta, GPIX = glycoprotein IX platelet, GPV = glycoprotein V platelet, HA = glucose-6-phosphate isomerise, Ig-SF = junctophilin 4, Lig I = tumor protein P53, KEGG = Kyoto Encyclopedia of Genes and Genomes, MCM = minichromosome maintenance, Npnt = nephronectin, OPN = secreted phosphoprotein 1, PFNA = peroxisome proliferator activated receptor alpha, PI3K-Akt = phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha-AKT serine/threonine kinase, PPAR = peroxisome proliferator activated receptor, RFC = solute carrier family 19 member 1, RHAMM = hyaluronan mediated motility receptor, RNase = ribonuclease, RPA, RPA interacting protein, SV2 = SV2 related protein, THBS = thrombospondin 1, VLA = integrin subunit alpha 4, VWF = von willebrand factor, αDG = dystroglycan 1, α-Prim = DNA polymerase alpha 1, catalytic subunit, βDG = dolichyl-phosphate mannosyltransferase subunit 3, regulatory.

the infection of HPV is emerging as an important factor in the rise of HNSCCs affecting nonsmokers in developed countries.[19] HPV-positive HNSCC has unique epidemiological and clinicopathological features.[20] In HPV-driven tumorigenesis, the HPV E6 and E7 oncoproteins decrease the level of p53 and pRb by post-translational regulation, leading to aberrant
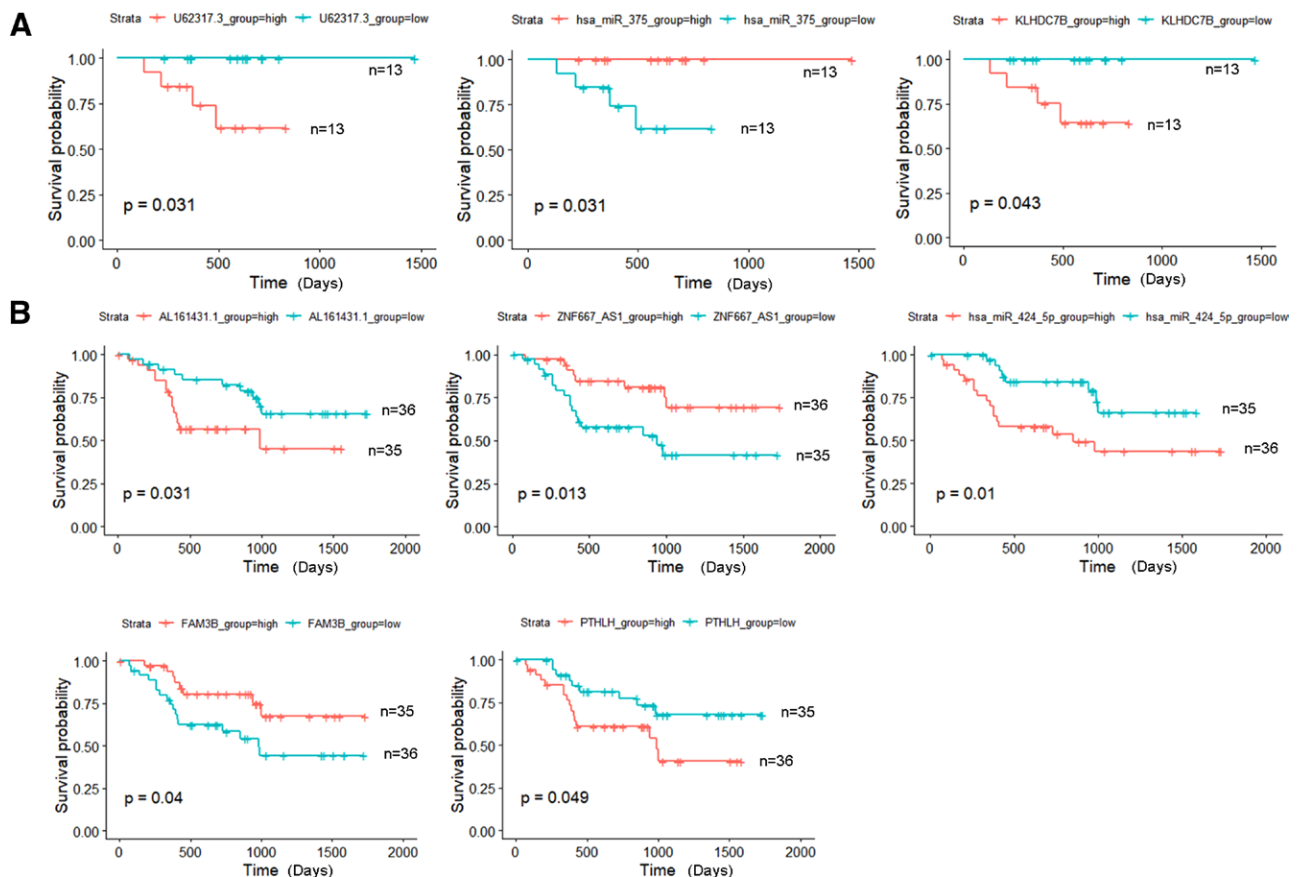
Figure 4. Kaplan-Meier analysis of key DElncRNAs, DEmiRNAs, DEmRNAs, and OS rate in the p16-positive group. (A) and p16-negative group (B) HNSCC patients. The median value of expression was set as cutoff point. The P values are shown on the diagrams (P < .05). DElncRNAs = differentially expressed lncRNA, DEmiRNAs = differentially expressed miRNAs, DEmRNAs = differentially expressed mRNAs, HNSCC = head and neck squamous cell carcinoma, OS = overall survival.

overexpression of the cell-cycle protein p16 (CDKN2A).[21–24] HPV infection can be detected by various mechanisms. p16 IHC is a commonly used and well-established surrogate marker for detection HPV in HNSCC and has become the recommended standalone prognostic test for OPSCC patients. As underlined in the 8th edition American Joint Commission on Cancer staging guidelines, p16 IHC is required to stage OPSCC patients.[25] Some studies demonstrated that p16 IHC positivity is also an independent and positive prognostic factor in non-OPSCC.[26,27] Although some other studies are contradictory to the results,[28] the HPV status classifies 2 distinct entities of HNSCC and p16-positive and p16-negative HNSCC have significantly different disease profiles. We hope that the study of the molecular difference between p16-positive and p16-negative HNSCC will help with the exploration of HNSCC heterogeneity. However, integrated and comprehensive analysis of lncRNA, miRNA, mRNA, and associated ceRNA network in p16-positive and p16-positive HNSCC is still not enough. To the best of our knowledge, this is the first study to investigate the specific ceRNA network in HNSCC by p16-status. Inspiringly, novel lncRNA-miRNA-mRNA ceRNA networks were constructed and RNAs in those networks possessed significant prognostic value were highlighted.

In this present study, using the TCGA database, we identified a total of 102 DElncRNAs, 196 DEmiRNAs, and 2282 mRNAs as p16-positive-specific DERNAs. We also identified 90 DElncRNAs, 153 DEmiRNAs, and 2038 DEmRNAs as p16-negative-specific DERNAs based on the differential expression between tumor tissues and adjacent normal tissues. GO analysis is widely used as a functional enrichment analysis for

a large number of genes.[29] The results of GO analysis demonstrated that the p16-positive-specific and p16-negative-specific DEmRNAs were significantly enriched in some different GO terms that were associated with cancer biological behaviors. In the p16-positive group, the DEmRNAs were significantly enriched in the condensed chromosome, DNA replication, and single-strand DNA-dependent ATPase activity. In the p16-negative group, the DEmRNAs were significantly enriched in the extracellular matrix, extracellular matrix organization, and extracellular matrix structural constituent. KEGG pathway enrichment analysis revealed that multiple different enriched pathways were obtained according to the p16-status. In the p16-positive group, the pathways involving in cancer mainly include DNA replication, cell cycle, and p53 signaling pathway. In the p16-negative group, the upregulated pathways related to cancer mainly enriched in ECM-receptor interaction, focal adhesion, and PI3k-Akt signaling pathway. Thus, these significant DEmRNAs may be involved in different modulation of HNSCC carcinogenesis and progression based on disparate p16-status.

For further identifying key DERNAs in p16-positive and p16-negative HNSCC, the top 25 upregulated and downregulated DERNAs were selected for further expression and survival analyses. The analytic results demonstrated that 2 upregulated DERNAs (lncRNA U62317.3, KLHDC7B) and 1 downregulated DERNA (has-miR-375) may act as the key p16-positive-specific RNAs. There were 3 upregulated DERNAs (lncRNA AL161431.1, hsa-miR-424-5p, PTHLH) and 2 downregulated DERNAs (lncRNA ZNF667-AS1, FAM3B) may act as the key p16-negative-specific RNAs. Intriguingly, most of these
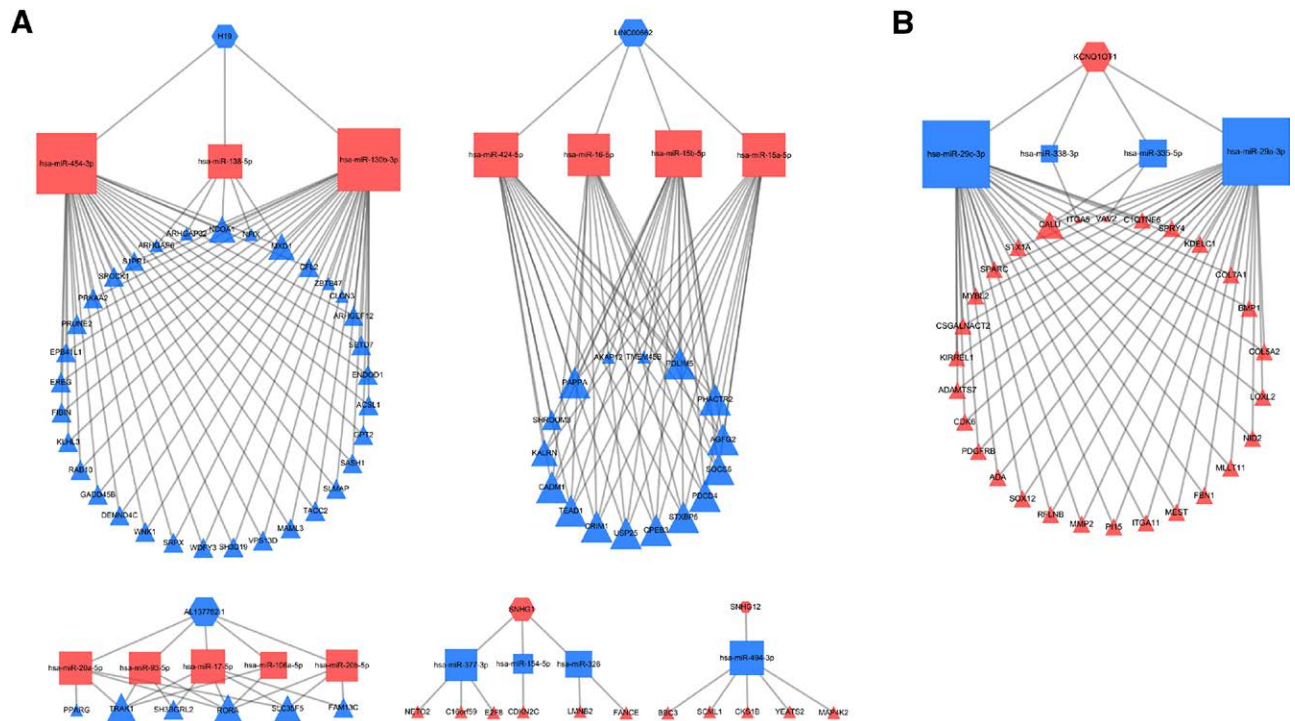
Figure 5. DElncRNAs/DEmiRNAs/DEmRNAs mediated ceRNA network in the p16-positive group. (A) and p16-negative group (B). The red hexagons represent upregulated lncRNAs, red rectangles represent upregulated miRNAs, red triangles represent upregulated mRNAs. The blue hexagons represent downregulated lncRNAs, blue rectangles represent downregulated miRNAs, blue triangles represent downregulated mRNAs. ceRNA = competing endogenous RNA, DElncRNAs = differentially expressed lncRNAs, DEmiRNAs = differentially expressed miRNA, DEmRNAs = differentially expressed mRNAs, lncRNAs = long noncoding RNAs, miRNA = microRNA, mRNA = messenger RNA.
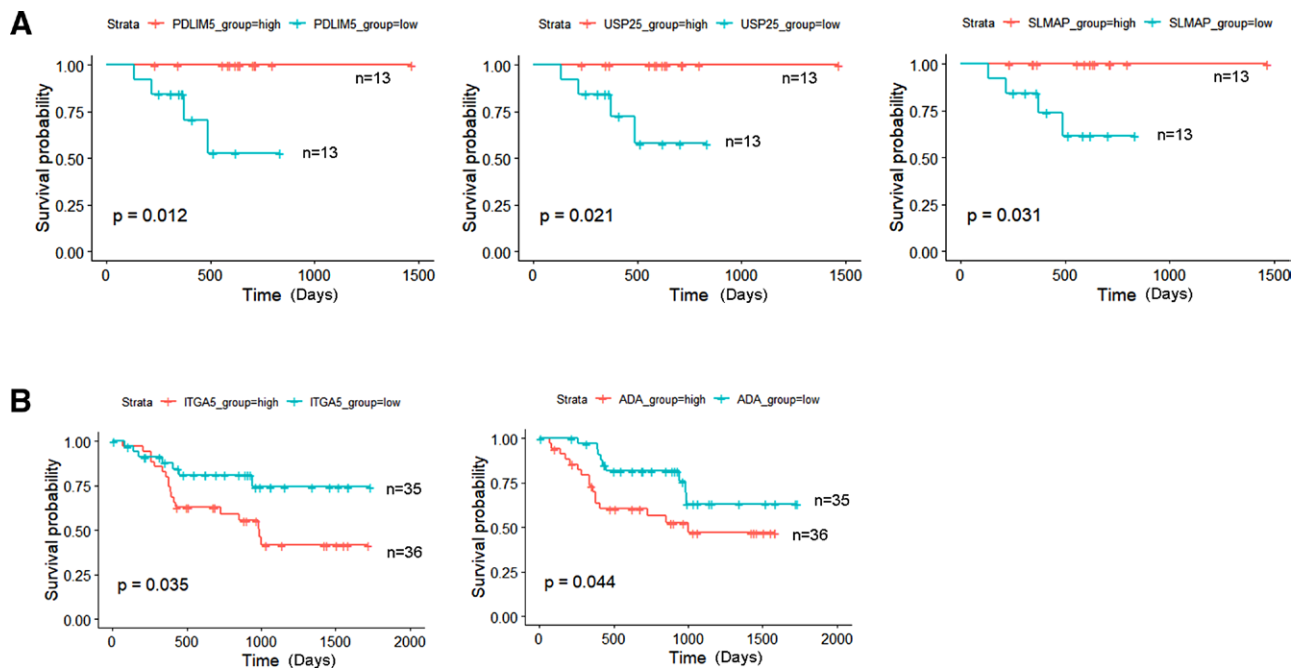

Figure 6. Kaplan-Meier analysis of ceRNA-related DElncRNAs, DEmiRNAs, DEmRNAs and OS rate in the p16-positive group. (A) and p16-negative group (B). The median value of expression was set as cutoff point. The P values are shown on the diagrams (P < .05). ceRNA = competing endogenous RNA, DElncRNAs = differentially expressed lncRNAs, DEmiRNAs = differentially expressed miRNA, DEmRNAs = differentially expressed mRNAs, OS = overall survival.

key RNAs have been well investigated in cancer. For example, downregulated lncRNA ZNF667-AS1 promoted the malignant progression and poor prognosis of cervical cancer, esophageal cancer, and laryngeal squamous cell carcinoma (LSCC)[30–32]; miR-375 was downregulated in LSCC and decreased miR-375 indicated a poor outcome[33]; miR-424-5p enhanced the aggressive progression of LSCC[34]; KLHDC7B participated the breast cancer cell proliferation[35]; PTHLH was a poor prognostic marker of HNSCC.[36] These publications partially support the accuracy of our informatics analyses.

LncRNAs and miRNAs are involved in the regulation of gene expression via the ceRNA mechanism as previously described. By constructing the ceRNA network, our research focused on the potentially different regulatory mechanisms of p16-positive and p16-negative HNSCCs. Several previous studies have already reported the RNAs and the interactions between RNAs in the 2 ceRNA networks. For example, SNHG1, an upregulated lncRNA in the p16-positive ceRNA network, was reported to regulate colorectal cancer cell growth through interactions with miR-154-5p and EZH2.[37] SNHG1 was also reported to regulate NOB1 expression by sponging miR-326 and promotes tumorigenesis in osteosarcoma.[38] These previous studies strongly demonstrated that the regulatory relationship in our ceRNA network was reliable. SNHG12 was another upregulated lncRNA in the p16-positive ceRNA network and was reported to promote the progression of clear cell renal cell carcinoma, hepatocellular carcinoma, and osteosarcoma by sponging miRNAs.[39–41] KCNQ1OT1 was an upregulated lncRNA in the p16-negative ceRNA network and was reported to act as the ceRNA to promote the progression of colorectal cancer and bladder cancer.[42,43] In tongue cancer, it was reported that KCNQ1OT1 not only promoted proliferation but also induced cisplatin resistance.[44] One of the reasons that the prognosis of p16-negative HNSCC patients is significantly worse than p16-positive patients were regarded as chemotherapy resistance.[45] Based on the analyses of our study, KCNQ1OT1 and the ceRNA network in the p16-negative HNSCC were the potential biomarkers and treatment targets of chemoresistance. Several reports suggested that H19, a downregulated lncRNA in the p16-positive ceRNA network, is linked to the poor prognosis of cancer patients. H19 was reported to interact with miR-138-5p and therefore promote tumor proliferation in cervical cancer. H19 could also regulate keratinocyte differentiation by targeting miR-130-3p.[46] Furthermore, H19 expression was reported strongly correlated with miR-675-5p in colorectal cancer. The binding between miR-675-5p and the 3′UTR in the TP53 mRNA resulted in the loss of the p53 protein and metastasis.[47] Another downregulated lncRNA in the p16-positive ceRNA network, LINC00662, was reported to promote hepatocellular carcinoma progression via competitively binding miR-15a and miR-16.[48] According to our study, the downregulation of H19 and LINC00662 in the p16-positive group and the corresponding ceRNA networks might be the potential reason why the p16-positive group HNSCC patients usually have a better prognosis than the patients in the p16-negative group. In the p16-positive ceRNA network, 3 key mRNAs (PDLIM5, USP25, SLMAP) were confirmed to be correlated with OS. PDLIM5 has been reported to be a potential biomarker of papillary thyroid carcinoma[49]; USP25 cancer-associated mutations led to activation in vitro and in vivo[50]; SLMAP was a target gene of miR-224 and regulated giant tumor-derived neoplastic stromal cells.[51] In the p16-negative group ceRNA network, 2 key mRNAs (ITGA5, ADA) were identified to be associated with OS. ITGA5 was reported to correlate with poor OS in colorectal adenocarcinoma,[52] pancreatic cancer, and inhibited the efficacy of chemotherapy in pancreatic cancer.[53] ADA exosomes in HNSCC plasma was regarded as a potential marker of tumor progression and immune competence.[54] These reports further imply the accuracy of our current analytic results. However, more researches and large-scale clinical trials are needed to explore the molecular mechanisms and the prognostic value of these key RNAs in p16-positive and p16-negative HNSCC.

5. Conclusions

In conclusion, HNSCC patients with p16-positive and p16-negative status have differential RNA expression profiling and differential ceRNA network. The findings of our analyses introduce new insights into HPV-related HNSCC and suggest that

the key RNAs in the p16-positive and p16-negative group may be potential biomarkers and therapeutic targets for different p16-status patients. Further studies with multicenter data, larger clinical samples, and more lab experiments are needed to verify these findings.

Author contributions

Conceptualization: Yifan Yang. Data curation: Ling Feng, Ru Wang. Methodology, Software: Yifan Yang, Ling Feng. Project administration and Supervision: Jugao Fang. Validation: Hongzhi Ma, Shizhi He. Writing—original draft: Yifan Yang. Writing—review and editing: Yifan Yang, Ling Feng, Ru Wang, Jugao Fang.

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