Clinics in Oncology

Research Article
Published: 28 Aug, 2024

Analysis of lncRNA-miRNA-mRNA and circRNA-miRNA-mRNA ceRNA Network in Oral Squamous Cell Carcinoma

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Abstract

Background: Increasing evidence suggests that long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) function as competitive endogenous RNAs (ceRNAs) by sponging microRNAs (miRNAs), thereby influencing the expression of target genes. The ceRNA networks involving lncRNA-miRNA-mRNA and circRNA-miRNA-mRNA interactions have been implicated in various cancers, yet their specific roles in Oral Squamous Cell Carcinoma (OSCC) remain unclear.

Methods: OSCC and corresponding paracancerous tissue samples were collected from three patients undergoing surgical resection. RNA expression profiles were obtained using an Illumina high-throughput sequencing platform. Differential Expression analysis identified significantly altered lncRNAs (DElncRNAs), miRNAs (DEmiRNAs), and mRNAs (DEmRNAs) between OSCC and paracancerous tissues using the limma and DESeq packages in R. We constructed lncRNA-miRNA-mRNA and circRNA-miRNA-mRNA ceRNA networks based on miRanda-predicted interactions using Cytoscape (version 3.10.0). Functional enrichment analysis including Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed using the ClusterProfiler package in R, with visualization using ggplot2.

Results: We identified 1188 DElncRNAs, 281 circRNAs, 170 DEmiRNAs, and 4663 DEmRNAs in OSCC. The OSCC-specific lncRNA-miRNA-mRNA ceRNA network comprised 299 nodes and 512 edges (219 DElncRNA-DEmiRNA interactions and 293 DEmiRNA-DEmRNA interactions). The OSCC-specific circRNA-miRNA-mRNA ceRNA network consisted of 12 nodes and 11 edges (8 DEcircRNA-DEmiRNA interactions and 3 DEmiRNA-DEmRNA interactions).

Conclusion: Our study provides insights into the regulatory mechanisms underlying OSCC progression. We propose lncRNA-miRNA-mRNA and circRNA-miRNA-mRNA ceRNA networks that may facilitate further exploration of OSCC molecular mechanisms.

OPEN ACCESS

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Received Date: *23 Jul 2024*
Accepted Date: *21 Aug 2024*
Published Date: *28 Aug 2024*

Citation:
Xuying L, Kun Z, Jincun L, Wen D, Chen H, Dongyang L, et al. Analysis of lncRNA-miRNA-mRNA and circRNA-miRNA-mRNA ceRNA Network in Oral Squamous Cell Carcinoma. Clin Oncol. 2024; 9: 2102.

ISSN: 2474-1663

Introduction

OSCC is a type of Head and Neck Cancer (HNC) characterized by a high mortality rate, with a survival rate of approximately 50% [1]. It encompasses a diverse group of malignancies that originate from the mucosal lining of the oral cavity [2]. According to Global Cancer Statistics 2020, approximately 53,260 new cases and 10,750 deaths from oral cancer were reported in the USA, accounting for about 4% of all male cancer cases [3]. Furthermore, recent data indicate that the total number of cases is projected to rise to 510,948 by 2035 [1]. Advancements in treatment strategies, such as drug development and computer-assisted surgery, were expected to improve survival rates among OSCC patients. However, OSCC remains an incurable malignancy, and outcomes have not significantly changed. Additionally, most OSCC patients are diagnosed at advanced stages, and no effective early screening strategy has been established. Therefore, to enhance diagnostic efficiency and achieve better prognoses, comprehensive investigations aimed at elucidating the molecular mechanisms of OSCC and discovering novel diagnostic tools and precision therapeutic approaches are urgently needed.

LncRNAs represent a newly recognized class of RNAs longer than 200 nucleotides that do not encode proteins or peptides [4,5]. Previously dismissed as 'junk transcripts' lacking function, recent advancements including lncRNA microarray and whole-genome transcriptome analyses have identified over 50,000 lncRNAs, some of which exhibit tissue-specific expression patterns and have been functionally characterized [6]. In human cancers, including OSCC, lncRNAs play pivotal

modulatory roles influencing cellular behaviors, immune responses, and oncogenic phenotypes. For instance, the oncogenic lncRNA HOTAIR is frequently overexpressed in solid tumors, including oral cancer, promoting invasion and metastasis [7]. Another example is THRIL, an immunoregulatory lncRNA implicated in regulating TNF-α expression in inflammatory diseases by forming RNA-protein complexes [8]. Studies have also highlighted lncRNA loc100506114's role in driving fibroblast transformation into Cancer-Associated Fibroblasts (CAFs) in OSCC [9]. Moreover, lncRNAs are abundantly present in body fluids such as blood, urine, saliva, and exosomes, making them potential non-invasive biomarkers [10]. Functionally, lncRNAs contribute to identifying cancer characteristics and hallmarks, positioning them as promising therapeutic targets.

CircRNA is a single-stranded, closed-loop RNA that lacks terminal 5′ caps and 3′ poly(A) tails, making it more stable than linear RNA [11]. CircRNA was first discovered in plant-infected viroid's [12] and later observed in eukaryotes, though initially not associated with the back-splicing mechanism [13]. Improvements in high-throughput sequencing techniques and bioinformatics have led to the identification of increasingly diverse differentially expressed circRNAs. Over the years, various biological functions of circRNAs have been uncovered. Numerous studies suggest that circRNAs are involved in various diseases and may serve as biomarkers for cancer diagnosis and therapeutic targets [14]. Notably, circRNAs play essential roles in cell proliferation, apoptosis, migration, and invasion, thereby regulating the progression of OSCC. Therefore, exploring OSCC-related circRNAs could open new avenues for early detection, prognosis, and effective therapy of OSCC.

In this study, we explored the genome-wide expression profile of lncRNAs, circRNAs, miRNAs and mRNAs inpatients with OSCC by total RNA-Sequencing. In addition, the lncRNA-miRNA-mRNA and circRNA-miRNA-mRNA ceRNA network in OSCC were established through comprehensive analysis. These networks will help researchers find new targets and pathways for the treatment of OSCC patients to prolong the survival time of patients.

Materials and Methods

Sample collection

The OSCC samples and the corresponding paracancerous tissue samples were collected from three patients who underwent surgery for oral squamous cell carcinoma in the Department of Oral and Maxillofacial Surgery at the General Hospital of Ningxia Medical University from January to December 2019. All sample collections were conducted with the patients' consent and approved by the Ethics Committee of the General Hospital of Ningxia Medical University. Inclusion criteria were as follows: (1) Pathological diagnosis of OSCC; (2) Patients who had not undergone radiotherapy or chemotherapy before surgery; (3) Primary tumors; (4) First-time resection of OSCC lesions; (5) Absence of other tumors or relevant systemic medical history; (6) Voluntary participation in the study. Exclusion criteria were as follows: (1) Secondary tumors; (2) Receipt of radiotherapy or chemotherapy before surgery; (3) Presence of other tumors or relevant systemic medical history. The information of three included patients was presented in Table 1.

RNA extraction and sequencing

Total RNA is extracted from samples using the Trizol reagent kit, with quality and concentration assessed *via* spectrophotometry or electrophoresis. Subsequently, ribosomal RNA (rRNA) was removed.

Table 1: The information of three OSCC patients.

ID	Gender	Age (years)	T	N	M	Tumor stage
1	Male	62	T3	N1	M0	III
6	Female	68	T1	N0	M0	I
7	Male	74	T1	N0	M0	I

Following rRNA removal, RNA is reverse-transcribed into cDNA using reverse transcriptase, followed by the synthesis of the second strand. The cDNA is then fragmented, and sequencing adapters are ligated to the fragmented cDNA to construct the library. After quality assessment, the library is loaded onto a high-throughput sequencing platform (Illumina), for sequencing. Finally, the sequencing data undergoes quality control, alignment, and gene expression analysis.

Differentially expressed analysis

Differentially expressed lncRNAs (DElncRNAs), Differentially expressed circRNAs (DEcircRNAs), differentially expressed miRNAs (DEmiRNAs), and differentially expressed mRNAs (DEmRNAs) between OSCC samples and corresponding paracancerous tissues were analyzed and normalized by the limma package and DESeq package in R with thresholds of Fold Change (FC) >1.0 and P value <0.01. We used gplots package in R to generate volcano plots and heatmaps.

Functional enrichment analysis

The ClusterProfiler package in R was utilized for conducting functional enrichment analysis. Enriched GO biological processes and KEGG pathways were identified with statistical significance (q-value <0.01).

Construction of ceRNA regulatory network

The corr.test function in R was used to analyze the co-expression of DElncRNAs, DEcircRNA, DEmiRNAs, and DEmRNAs with thresholds of correlation coefficient >0.95 and P value <0.05. MiRNA target genes were predicted on the basis of miRanda. The target of lncRNA and mRNA was conducted by lncTar. Subsequently, we integrated the interaction between DEmiRNAs and DElncRNAs, DEcircRNAs or DEmRNAs to construct a ceRNA regulatory network. Cytoscape (version 3.10.0) was used to visualize the ceRNA network.

Results

Identification of differentially expressed lncRNAs, circRNAs, miRNAs and mRNAs

We analyzed DElncRNAs, DEcircRNA, DEmiRNAs, and DEmRNAs between 3 OSCC samples and the corresponding paracancerous samples. A total of 1188 OSCC-specific lncRNAs (576 upregulated and 621 downregulated; Figure 1), 281 circRNAs (33 upregulated and 248 downregulated; Figure 2), 170 miRNAs (76 upregulated and 94 downregulated; Figure 3) and 4663 mRNAs (2107 upregulated and 2556 downregulated; Figure 4) were identified as DERNAs in OSCC. In these DERNAs, 1051 lncRNAs, 10 circRNAs, and 32 miRNAs has not been defined yet. The top 20 DElncRNAs, DEcircRNA, DEmiRNAs, and DEmRNAs with their names, log2FC values, and adjust P-values were listed in Tables 2-5. All of the top 20 upregulated lncRNAs and 18 lncRNAs of the top20 downregulated lncRNAs has not been found previously. The information of these novel lncRNAs in chromosome was presented in Table 2.

GO and pathway analysis of DEmRNAs

GO analysis of DEmRNAs displayed that the enrichment of

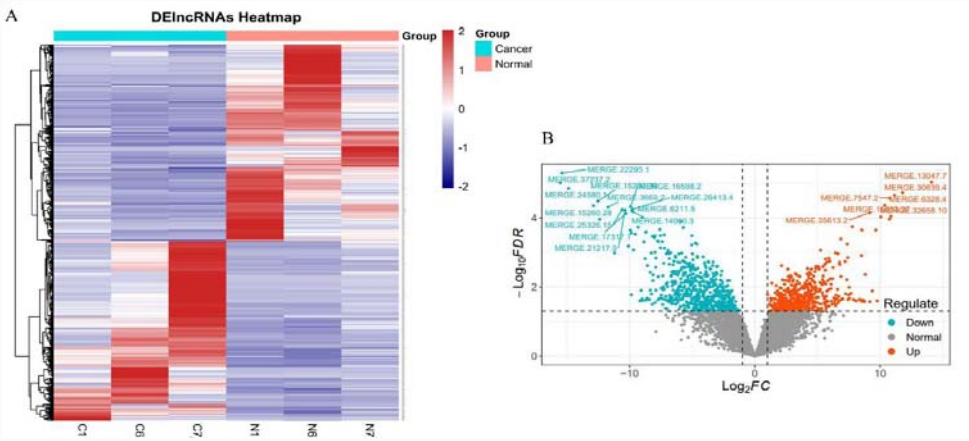

Figure 1: Identification of DElncRNAs in OSCC and normal tissues. (A) The heatmap of genome-wide DElncRNAs. (B) The volcano plot showed that a total of 576 upregulated and 621 downregulated lncRNAs were identified. Blue and red represents downregulated and upregulated lncRNAs respectively.
DElncRNAs: Differentially Expressed lncRNAs; OSCC: Oral Squamous Cell Carcinoma

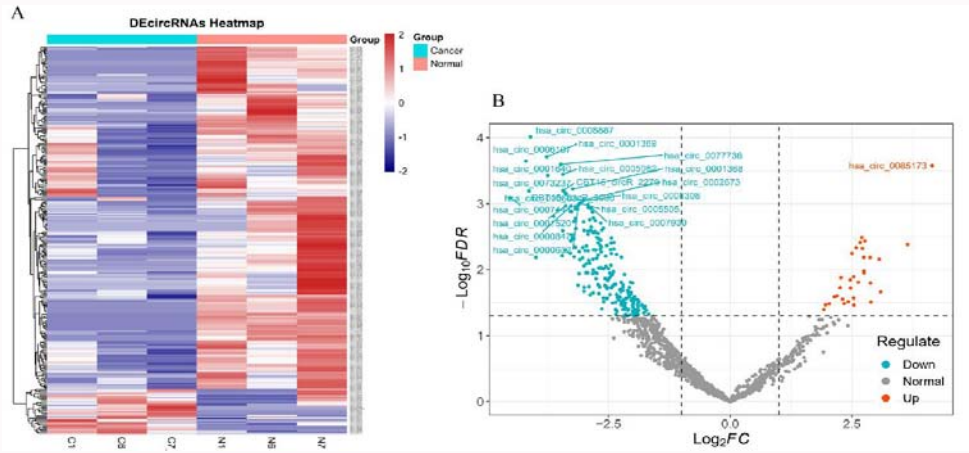

Figure 2: Identification of DEcircRNAs in OSCC and normal tissues. (A) The heatmap of genome-wide DEcircRNAs. (B) The volcano plot showed that a total of 33 upregulated and 248 downregulated circRNAs were identified. Blue and red represents downregulated and upregulated circRNAs respectively.
DEcircRNAs: Differentially Expressed circRNAs; OSCC: Oral Squamous Cell Carcinoma

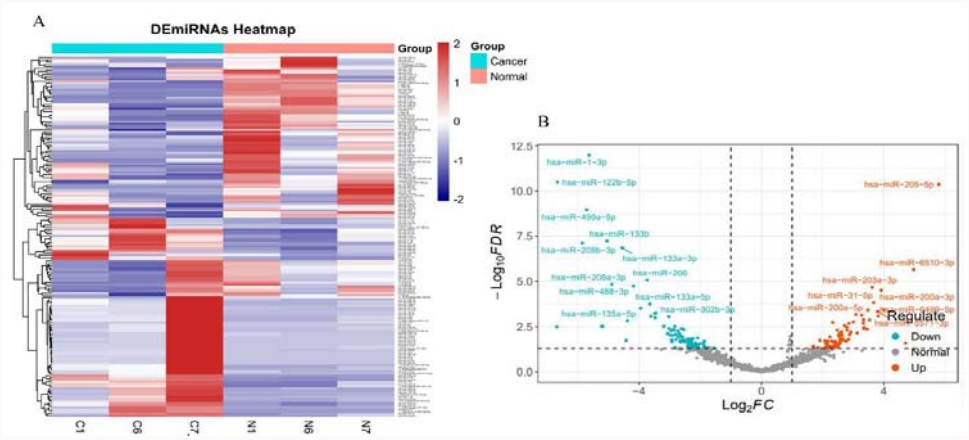

Figure 3: Identification of DEmiRNAs in OSCC and normal tissues. (A) The heatmap of genome-wide DEmiRNAs. (B) The volcano plot showed that a total of 76 upregulated and 94 downregulated miRNAs were identified. Blue and red represents downregulated and upregulated miRNAs respectively.
DEmiRNAs: Differentially Expressed miRNAs; OSCC: Oral Squamous Cell Carcinoma

Biological Processes (BP) is mainly related to purine ribonucleotide metabolic process, ribonucleotide metabolic process, ribose phosphate metabolic process, muscle system process, purine ribonucleotide biosynthetic process, ribonucleotide biosynthetic process, ribose phosphate biosynthetic process, purine nucleotide biosynthetic process, thioester biosynthetic process, acyl-CoA

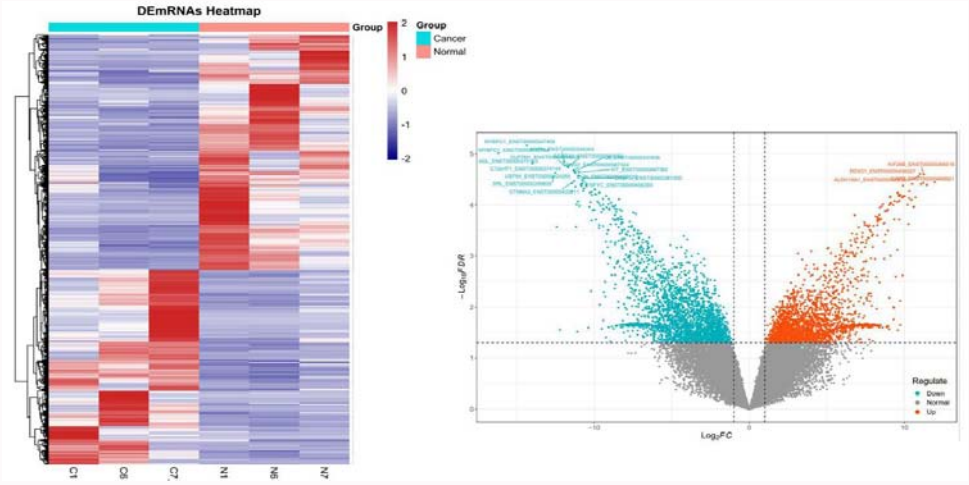

Figure 4: Identification of DEmRNAs in OSCC and normal tissues. (A) The heatmap of genome-wide DEmRNAs. (B) The volcano plot showed that a total of 2107 upregulated and 2556 downregulated mRNAs were identified. Blue and red represents downregulated and upregulated mRNAs respectively.
DEmRNAs: Differentially Expressed mRNAs; OSCC: Oral Squamous Cell Carcinoma

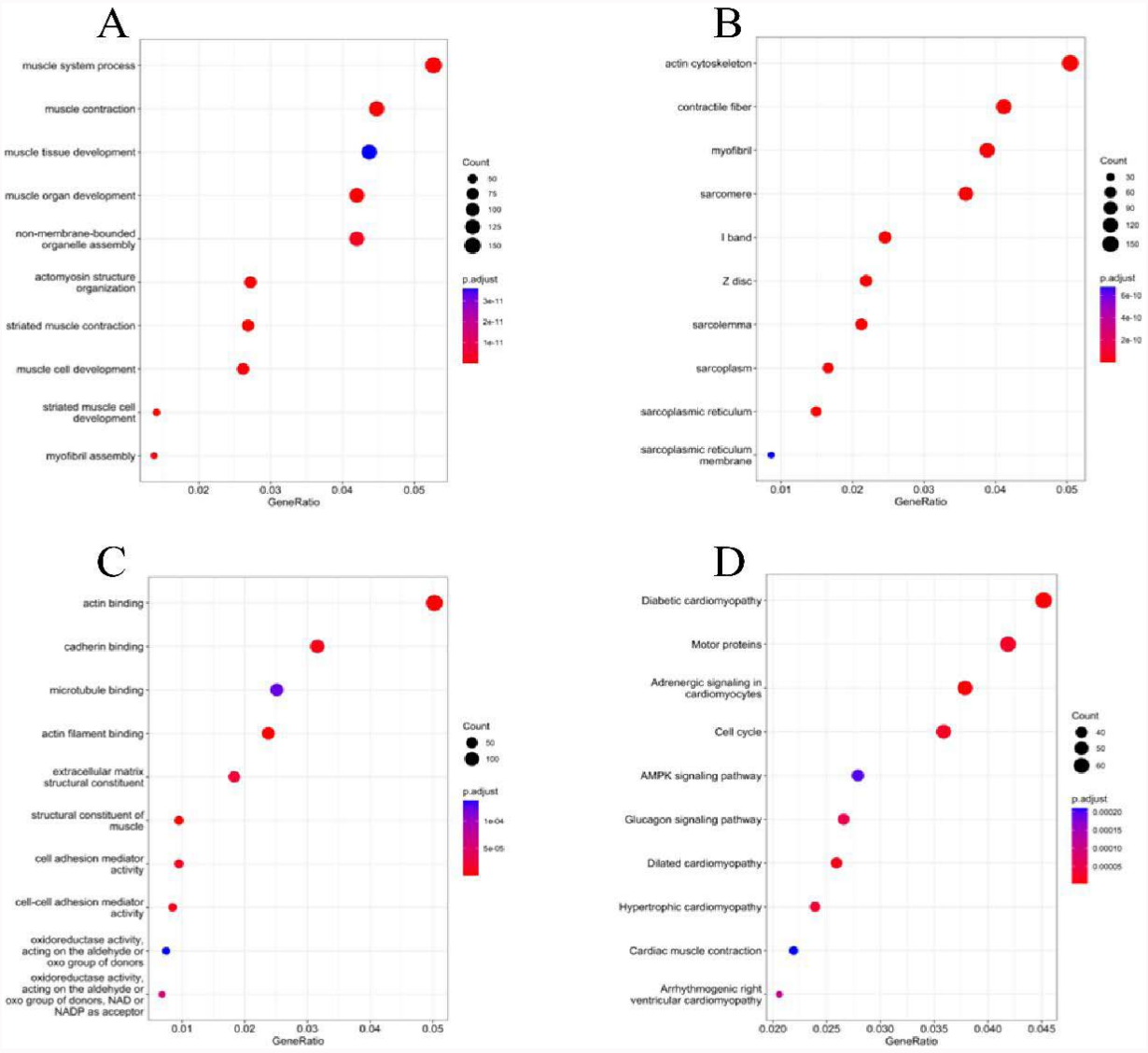

Figure 5: GO and KEEG enrichment analysis of DEmRNAs in OSCC. (A) Bubble plot of BP. (B) Bubble plot of CC. (C) Bubble plot of MF. (D) Bubble plot of KEGG.
GO: Gene Ontology; BP: Biological Processes; CC: Cell Component; MF: Molecular Function

Table 2: Top 20 upregulated and downregulated lncRNAs in OSCC.

Regulation	LncRNAs	Log2FC	Adjust P value	Chromosome	Strand	Start	End
Up	MERGE.13047.7	14.095	0.0000092	15	+	98798271	98891619
	MERGE.30639.4	11.7948	0.00001844	6	-	17759254	17794697
	MERGE.6328.4	11.1453	0.00002186	11	-	63759899	63768671
	MERGE.32658.10	10.8542	0.00009011	7	-	23505320	23531980
	MERGE.7547.2	10.77	0.00002671	12	-	3614082	3753097
	MERGE.11507.10	10.7453	0.00010782	14	-	105624197	105771405
	MERGE.15653.27	10.366	0.00004258	17	-	29121030	29178320
	MERGE.28068.1	10.056	0.00009361	4	-	113900284	113979714
	MERGE.8373.1	9.7656	0.02504146	12	-	52768155	52831615
	MERGE.31848.2	9.6594	0.00022282	6	-	116119026	116158747
	MERGE.16851.21	9.3668	0.01291326	17	-	72556578	72592796
	MERGE.37604.7	9.169	0.0263871	X	-	45792754	45851490
	MERGE.35613.2	9.1575	0.00007103	8	-	142784882	142786529
	MERGE.11507.12	8.8145	0.00434595	14	-	105624341	105626066
	MERGE.19254.12	8.7527	0.02602513	19	-	39834458	39846365
	MERGE.11507.32	8.6702	0.02505874	14	-	105741338	105771405
	MERGE.11192.1	8.6351	0.02366205	14	-	91232511	91265690
	MERGE.25936.11	8.6072	0.02363606	3	-	78647636	79019447
	MERGE.6101.1	8.5604	0.0257316	11	-	46743048	46846278
	MERGE.14828.2	8.5386	0.00022298	16	+	89711866	89718164
Down	MERGE.3222.3	-9.9428	0.00022541	1	-	201408826	201430240
	MERGE.16598.2	-9.9248	0.00004739	17	-	59109971	59155184
	MERGE.14348.6	-9.8607	0.00027307	16	-	67230314	67247464
	MERGE.4150.9	-9.8588	0.01670591	10	-	29484687	29634972
	MERGE.6211.5	-9.8187	0.00006122	11	-	60172020	60184666
	MERGE.26413.4	-9.7811	0.00005631	3	-	134603282	134651018
	MERGE.14060.3	-9.7246	0.00006711	16	-	46707668	46763246
	MERGE.18246.4	-9.6898	0.00084681	19	-	4502192	4520231
	MERGE.15260.7	-9.5519	0.00029354	17	-	10362413	10549797
	MERGE.8822.15	-9.3184	0.00018328	12	-	79775935	79934931
	MERGE.15260.20	-9.1657	0.02457664	17	-	10445248	10537546
	MERGE.10365.14	-9.0748	0.01245439	14	-	23400256	23435724
	MERGE.10365.5	-9.0744	0.02409233	14	-	23382019	23419304
	XIST	-9.0096	0.02447825	X	-	73826978	73850624
	MERGE.19893.8	-9.0011	0.02203544	19	-	55132828	55149375
	MERGE.3101.4	-8.9676	0.00009765	1	-	183555725	183592717
	LINC01405	-8.85	0.00049356	12	+	110936602	110937451
	MERGE.15260.6	-8.7631	0.00150583	17	-	10362413	10518628
	MERGE.6432.3	-8.7433	0.02389366	11	+	65497762	65503609
	MERGE.31171.7	-8.7174	0.00025725	6	-	35869589	35921101

biosynthetic process (Figure 5A). Changes in Cell Component (CC) of DEmRNAs were mainly enriched in actin cytoskeleton, myofibril, contractile fiber, sarcomere, I band, Z disc, actomyosin, actin filament, stress fiber, contractile actin filament bundle (Figure 5B). Changes in Molecular Function (MF) of DEmRNAs were mainly enriched in actin binding, muscle alpha-actin binding, alpha-actin binding, actinin binding, structural constituent of muscle, 3',5'-cyclic-AMP phosphodiesterase activity, semaphoring receptor binding, 3',5'-cycle-nucleotide phosphodiesterase activity, cycle-nucleotide phosphodiesterase activity, spectrin binding (Figure 5C). KEGG pathway enrichment analysis showed that DEmRNAs were mainly enriched in thermogenesis, adrenergic signaling in cardiomyocytes, diabetic cardiomyopathy, glucagon signaling pathway, apelin signaling pathway, cardiac muscle contraction, morphine addiction,

Table 3: Top 20 upregulated and downregulated CircRNAs in OSCC.

Regulation	CircRNAs	Log2FC	Adjust P value
Up	hsa_circ_0085173	4.1479	0.00026559
	hsa_circ_0067492	3.6454	0.00416888
	hsa_circ_0069399	3.0888	0.02164703
	hsa_circ_0000374	3.0604	0.00695924
	hsa_circ_0044177	2.8898	0.03104445
	hsa-circRNA11459-21	2.8798	0.01599042
	hsa_circ_0007788	2.8735	0.00653149
	hsa_circ_0001667	2.7663	0.00367209
	hsa_circ_0001307	2.7489	0.01113309
	hsa_circ_0003567	2.7488	0.01056833
	hsa_circ_0022601	2.7452	0.00651823
	hsa_circ_0000119	2.7176	0.00476718
	hsa_circ_0007542	2.7018	0.00325638
	hsa_circ_0008153	2.6683	0.0038717
	hsa_circ_0000825	2.6282	0.01549318
	hsa_circ_0003507	2.5878	0.00465741
	hsa_circ_0033144	2.542	0.03433947
	hsa_circ_0001821	2.536	0.01283435
	hsa_circ_0005399	2.5358	0.02688422
	hsa_circ_0000893	2.5027	0.00566723
Down	hsa_circ_0007444	-4.5204	0.00081518
	hsa_circ_0006107	-4.1958	0.0002266
	hsa_circ_0073237	-4.1346	0.00064014
	hsa_circ_0008887	-4.1029	0.00009695
	hsa_circ_0086414	-3.9903	0.00651756
	hsa_circ_0001369	-3.7692	0.00019658
	hsa_circ_0001640	-3.7511	0.00037473
	hsa_circ_0105377	-3.6275	0.00156014
	hsa_circ_0056019	-3.5433	0.00126897
	hsa_circ_0077736	-3.4804	0.00025354
	hsa_circ_0006773	-3.4506	0.0061606
	hsa_circ_0000982	-3.4399	0.00256209
	CBT15_circR_2279	-3.4365	0.00063334
	hsa_circ_0005982	-3.4361	0.00036014
	hsa_circ_0006834	-3.3982	0.00067668
	hsa_circ_0085362	-3.3948	0.00503578
	hsa_circ_0001520	-3.3808	0.00108342
	CBT15_circR_5080	-3.3667	0.00071607
	hsa_circ_0004365	-3.2871	0.00561896
	hsa_circ_0005660	-3.2761	0.00364999

Table 4: Top 20 upregulated and downregulated miRNAs in OSCC.

Regulation	miRNAs	Log2FC	Adjust P value
Up	hsa-miR-205-5p	5.7907	0.00000000
	hsa-miR-6510-3p	4.9708	0.00000224
	17_33102-3p	4.7083	0.02554269
	22_37613-3p (rno-miR-764-3p)	4.5021	0.02200338
	hsa-miR-6499-5p	3.9956	0.00037735
	hsa-miR-200a-3p	3.9119	0.00003059
	hsa-miR-200c-5p	3.8179	0.00083304
	hsa-miR-5571-3p	3.7975	0.00045941
	hsa-miR-31-5p	3.6616	0.00015073
	hsa-miR-203a-3p	3.6134	0.00002108
	hsa-miR-934	3.5311	0.0013666
	hsa-miR-200a-5p	3.4769	0.00038177
	1_3611-5p (mdo-miR-12402-5p)	3.454	0.00436684
	hsa-miR-205-3p	3.3515	0.00344131
	hsa-miR-1293	3.3444	0.00405698
	hsa-miR-141-5p	3.3333	0.00134482
	hsa-miR-6854-5p	3.3244	0.00206298
	hsa-miR-141-3p	3.2704	0.00074343
	hsa-miR-7974	3.1214	0.00728134
	hsa-miR-200c-3p	3.0857	0.00067167
Down	2_7793-5p	-6.6856	0.00321657
	hsa-miR-122b-5p	-6.6656	0.00000000
	hsa-miR-208b-3p	-5.8513	0.00000007
	hsa-miR-499a-5p	-5.7117	0.00000000
	hsa-miR-1-3p	-5.6313	0.00000000
	4_11277-5p	-5.2159	0.00301945
	6_14981-5p	-5.1877	0.00303279
	hsa-miR-133b	-5.0462	0.00000006
	hsa-miR-208a-3p	-4.8885	0.00001447
	hsa-miR-133a-3p	-4.5522	0.00000014
	2_7915-5p(rno-miR-6329)	-4.4196	0.01825318
	18_34372-3p(oan-miR-1400-3p)	-4.378	0.0014846
	hsa-miR-488-3p	-4.1793	0.00001793
	hsa-miR-135a-5p	-3.9498	0.0003016
	hsa-miR-206	-3.734	0.00000844
	hsa-miR-133a-5p	-3.648	0.00017747
	hsa-miR-337-3p	-3.6078	0.00085593
	hsa-miR-578	-3.48	0.00058574
	hsa-miR-499a-3p	-3.4709	0.00100788
	hsa-miR-302b-3p	-3.448	0.00055267

GnRH signaling pathway, dilated cardiomyopathy, melanogenesis (Figure 5D).

Construction and analysis of the lncRNA-miRNA-mRNA ceRNA network

We built the lncRNA-miRNA-mRNA ceRNA network on the basis of the miRNA, lncRNA, and mRNA expression profiles and interaction in patients with OSCC. A total of 97 DElncRNAs and 36 DEmiRNAs were paired into 219 DElncRNAs-DEmiRNAs interactions, whereas 36 DEmiRNAs and 166 DEmRNAs were matched to 293 pairs of DEmiRNAs-DEmRNAs interactions. Finally, the OSCC-specific lncRNA–miRNA–mRNA ceRNA regulatory network, which contained 299 nodes and 512 edges, was constructed (Figure 6). The top 10 DElncRNAs and their matching DEmiRNAs in the ceRNA network was listed in Table 6. The top 10 DEmiRNAs and their matching DEmRNAs in the ceRNA network was listed in

Table 5: Top 20 upregulated and downregulated mRNAs in OSCC.

Regulation	mRNAs	Log2FC	Adjust P value
Up	CAPG	11.9579	0.00003593
	MMP7	11.4516	0.00004064
	KIF26B	11.2462	0.00002539
	RNF121	11.2073	0.00003712
	C17orf62	11.068	0.00003946
	DDX31	11.0011	0.00002473
	ALDH16A1	10.8969	0.00002803
	LY6E	10.7124	0.00007123
	PMM2	10.5981	0.00010884
	MST1R	10.5018	0.00003916
	POLQ	10.4588	0.00007971
	SKA2	10.3137	0.00005405
	SRP72	10.2993	0.00007422
	MDFI	10.2567	0.00023845
	SLC9A3	10.2086	0.00010028
	CDCA7	10.0117	0.00006822
	CSDE1	9.912	0.00004221
	BTF3	9.908	0.00006741
	FDCSP	9.8809	0.00242374
	SLC12A8	9.8656	0.00007241
Down	SMCO1	-9.9742	0.0001256
	PTPN1	-9.9664	0.00004967
	SPIN3	-9.9315	0.00005053
	RRAS2	-9.9306	0.00006203
	ESRRG	-9.8855	0.00019482
	PPARG	-9.8785	0.00006262
	SRPK3	-9.7822	0.00008647
	CALML6	-9.6888	0.00028386
	ENPP1	-9.6799	0.00009307
	TP63	-9.6704	0.00005806
	INTS2	-9.6656	0.0002489
	PNPLA4	-9.6523	0.00005723
	ADIPOQ	-9.6329	0.00907969
	MLF1	-9.5969	0.00010408
	PPDPFL	-9.5266	0.00013868
	PFKM	-9.5026	0.02660297
	HDAC6	-9.4233	0.00011743
	NOS1	-9.4206	0.00085127
	SIX4	-9.4202	0.00007626
	LRRC39	-9.4178	0.00026352

Table 7.

Functional enrichment analysis of DEmRNAs in the ceRNA network

We also analyzed the GO and KEEG enrichment of DEmRNAs in ceRNA network. The enrichment of BPs revealed that DEmRNAs involved in the ceRNA network were remarkably associated with purine ribonucleotide metabolic process, ribonucleotide metabolic process, ribose phosphate metabolic process, muscle system process, purine ribonucleotide biosynthetic process, ribonucleotide biosynthetic process, ribose phosphate biosynthetic process, purine nucleotide biosynthetic process, thioester biosynthetic process, acyl-CoA biosynthetic process (Figure 7A). The enrichment of CCs is mainly related to actin cytoskeleton, myofibril, contractile fiber, sarcomere, I band, Z disc, actomyosin, actin filament, stress fiber, contractile actin filament bundle (Figure 7B). The enrichment of MFs is mainly related to actin binding, muscle alpha-actin binding, alpha-actin binding, actinin binding, structural constituent of muscle, 3',5'-cycle-AMP phosphodiesterase activity, semaphoring receptor binding, 3',5'-cycle-nucleotide phosphodiesterase activity, cycle-nucleotide phosphodiesterase activity, spectrin binding (Figure 7C). The KEEG enrichment revealed that OSCC-specific ceRNA might be involved in the tumor process by regulating thermogenesis, adrenergic signaling in cardiomyocytes, diabetic cardiomyopathy, glucagon signaling pathway, apelin signaling pathway, cardiac muscle contraction, morphine addiction, GnRH signaling pathway, dilated cardiomyopathy, melanogenesis (Figure 7D).

Construction and analysis of the circRNA-miRNA-mRNA ceRNA network

We also built the circRNA-miRNA-mRNA ceRNA network on the basis of the miRNA, circRNA, and mRNA the expression profiles in patients with OSCC. A total of 7 DEcircRNAs and 2 DEmiRNAs were paired into 8 DEcircRNAs-DEmiRNAs interactions, whereas 2 DEmiRNAs and 3 DEmRNAs were matched to form 3 pairs of DEmiRNAs-DEmRNAs interactions. Finally, the OSCC-specific circRNA-miRNA-mRNA ceRNA regulatory network, which contained 12 nodes and 11 edges, was constructed (Figure 8).

Discussion

In the past few decades, due to the emergence of high-throughput sequencing technologies, there has been an exponential growth in the identification of lncRNAs with aberrant expression in various cancers, confirmed by RNA-Seq and lncRNA-microarray profiling [15]. Fang et al. performed RNA-Seq to profile lncRNA expression in five pairs of OSCC tissues and adjacent normal tissues, identifying 2,915 significantly differentially expressed lncRNAs, 11 of which were associated with OSCC metastasis [16]. Based on The Cancer Genome Atlas (TCGA) database, RNA sequencing analysis of 523 oral cancer samples in India by Ganesan Arunkumar identified 11 dysregulated lncRNAs in OSCC closely related to tobacco chewing/smoking history [17]. Evidence also shows that various functional studies have revealed the roles of lncRNAs in oncogenesis, tumor suppression, and chemoresistance, as well as in governing virtually every physiological cell process. Abnormal lncRNAs are involved in many aspects of cancer cell processes, including cell proliferation, apoptosis, invasion and metastasis [18], Epithelial-Mesenchymal Transition (EMT), and drug resistance [19]. Moreover, lncRNAs even affect patient outcomes, such as lymph node metastasis, distant metastasis, and postoperative recurrence [20]. Recent research has indicated the potential involvement of lncRNAs in cancer tumorigenesis, and uncovering the molecular mechanisms of lncRNAs within OSCC remains a challenge. Numerous studies have confirmed that lncRNAs exert regulatory functions primarily through epigenetic regulation, transcriptional regulation, and post-transcriptional regulation [21,22]. In our study, we also found 576 upregulated and 621 downregulated lncRNAs. Besides, most of these lncRNAs has not been defined yet. The characteristic and function of

Table 6: Top 10 lncRNAs and their target miRNAs in the lncRNA-miRNA-mRNA ceRNA network.

LncRNAs	Log2FC	Target miRNAs
MERGE.2576.18	-6.9301	hsa-miR-224-5p, hsa-miR-708-5p
MERGE.9272.5	-6.8476	hsa-miR-138-5p
MERGE.21974.1	6.4787	hsa-miR-378a-3p, hsa-miR-486-3p, hsa-miR-378i, hsa-miR-378g, hsa-miR-378h, hsa-miR-378c, hsa-miR-378a-5p
MERGE.9275.1	-6.1408	hsa-miR-138-5p, hsa-miR-224-5p, hsa-miR-708-5p, 9_20769-5p (gga-miR-1458), hsa-miR-222-3p
LINC00472	-5.9898	hsa-miR-224-5p
MERGE.15972.8	-5.8227	hsa-miR-224-5p
MERGE.32496.3	-5.7144	hsa-miR-671-5p, hsa-miR-25-5p, hsa-miR-138-5p, hsa-miR-320a-3p
MERGE.26413.2	-5.5495	hsa-miR-21-3p
MERGE.8751.3	-5.3348	hsa-miR-21-3p, hsa-miR-138-5p, hsa-miR-125a-3p
MERGE.33373.1	-5.1343	hsa-miR-138-5p

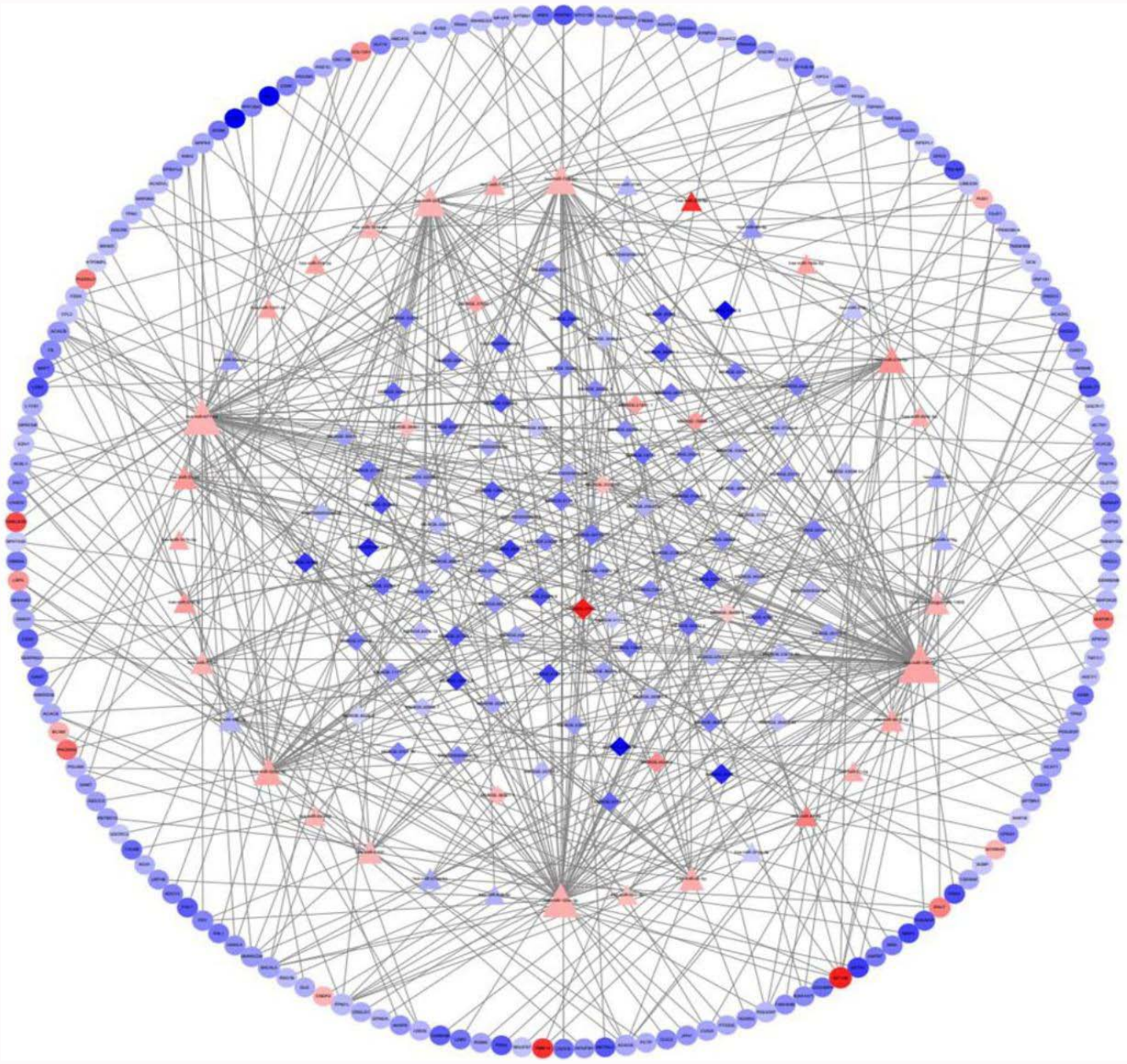

Figure 6: LncRNAs-miRNAs-mRNAs ceRNA network. Red nodes and blue node indicate upregulated and downregulated RNAs respectively. The depth of color is directly proportional to the absolute value of log2FC. Diamonds, triangles, and circles represent lncRNAs, miRNAs and mRNAs respectively.

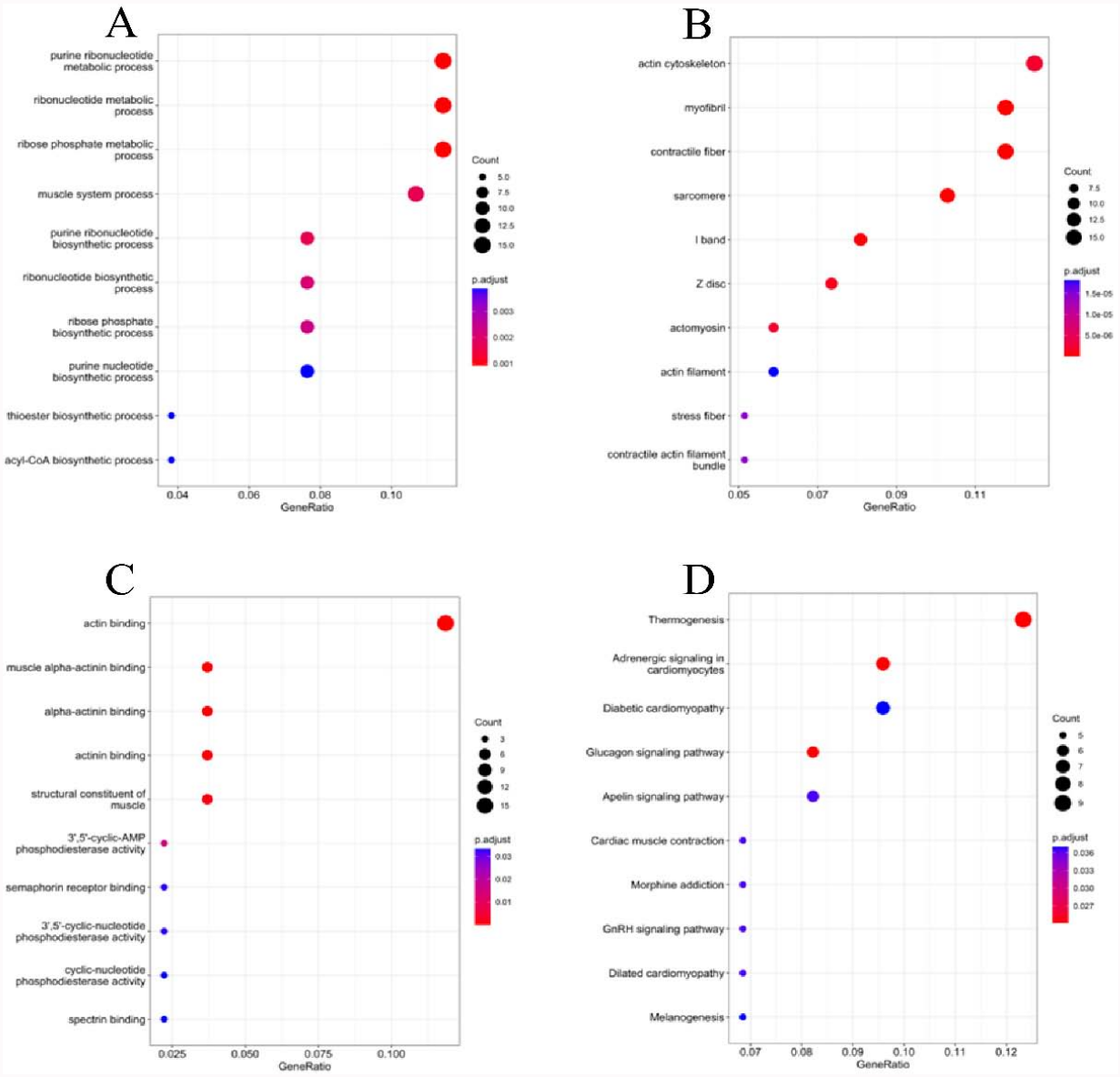

Figure 7: GO and KEEG enrichment analysis of DEmRNAs in lncRNA-miRNA-mRNA ceRNA network. (A) Bubble plot of BP. (B) Bubble plot of CC. (C) Bubble plot of MF. (D) Bubble plot of KEGG.
GO: Gene Ontology; BP: Biological Processes; CC: Cell Component; MF: Molecular Function

these novel lncRNAs is unclear. We provided detailed information of top 20 upregulated and downregulated lncRNAs in chromosome. The role of these novel lncRNAs in OSCC and other diseases needs further exploration.

Research indicates that circRNAs are notably dysregulated in Oral Squamous Cell Carcinoma (OSCC) and contribute to its progression. According to Wang et al., 10,021 novel circRNA molecules were identified, with eight being upregulated and eight downregulated in OSCC patients [23]. Similarly, Deng et al. [24] identified 213 differentially expressed circRNAs, of which 124 were upregulated and 89 were downregulated. Using high-throughput sequencing, Wang et al. [25] identified the expression profiles of circRNAs and confirmed that hsa_circ_009755 is significantly downregulated in OSCC and is associated with T stage I-II in patients with OSCC. Shao et al. [26] found 122 differentially expressed circRNAs, among which 109 were upregulated and 13 were downregulated in OSCC. Additionally, research has reported 12 circRNAs identified for the first time as novel circRNAs [26]. These studies indicate that circRNAs participate in the progression of OSCC, making them potential therapeutic targets. In these researches, most DEcircRNAs were upregulated. Besides, the majority of identified circRNAs function as oncogenes, promoting tumor formation upon dysregulation. Several studies demonstrate that circRNAs play critical roles in various biological processes such as cell invasion, growth, apoptosis, and drug resistance [27]. They exert regulatory effects on several pathways involved in OSCC cell invasion and proliferation, including Hippo, AKT/mTOR, and PD-1/PD-L1 pathways [27]. However, the precise mechanisms through which circRNAs drive OSCC progression remain incompletely understood, and their application in targeted therapy is limited and underexplored. Thus, further elucidation of the molecular mechanisms underlying OSCC occurrence and progression could identify novel circRNAs as effective therapeutic targets, ultimately enhancing survival outcomes for OSCC patients.

Table 7: Top 10 miRNAs and their target mRNAs in the lncRNA-miRNA-mRNA ceRNA network.

miRNAs	Log2FC	Target mRNAs
hsa-miR-205-5p	5.7907	SPEG
hsa-miR-31-5p	3.6616	HSPB7, PPM1L
hsa-miR-95-3p	-3.0271	PUS1
hsa-miR-27a-5p	3.0008	PDE4DIP, PFKM, GAMT, ADCY1, CHID1, LDB3, CD36, PLCL1, PRKN, UBE2D4, FAM184B, KIAA1671
hsa-miR-21-3p	2.8524	PDLIM3, ASB8, UBE2D4, GPRC5B, MAP3K20, SOBP, CLSTN2, AKAP6, ADSSL1, SYNPO2
hsa-miR-210-3p	2.7971	PRKN, PPM1L, F8, FZD4
hsa-miR-628-3p	-2.7916	COL12A1, TIMELESS
hsa-miR-708-3p	2.612	ADHFE1
hsa-miR-148a-5p	2.6083	SEMA4F
hsa-miR-138-5p	2.5671	ACACB, PDE4DIP, SPATA20, ACADVL, ADHFE1, ADSSL1, MICAL3, SMARCD3, MYL3, IGDCC4, CAMK2B, FILIP1, SMARCD3, EPM2A, MFAP5, MAP2K6, SNED1, COQ9, SERPING1, UQCRC2, RGMA, BVES, TPM1, PPM1L, CRELD1, UBE2D4, MAP3K20, ANK2, PDK4, EZH1, NDUFS7, ACSL1, PDE7A, ABCA10, EPM2A, CFL2, GUCD1

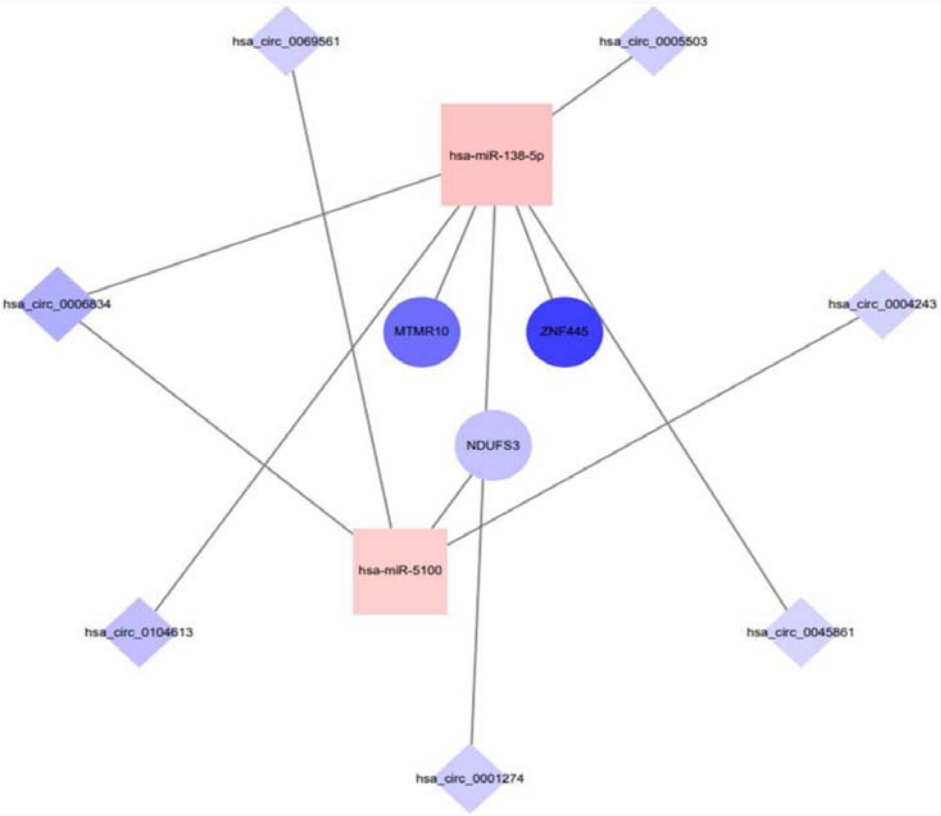

Figure 8: CircRNAs-miRNAs-mRNAs ceRNA network. Red nodes and blue node indicate upregulated and downregulated RNAs respectively. The depth of color is directly proportional to the absolute value of log2FC. Diamonds, squares, and circles represent circRNAs, miRNAs and mRNAs respectively.

LncRNAs and circRNAs have emerged as a significant focus in tumor biology and therapy, with increasing understanding of their biogenesis, characteristics, and functions. In addition, LncRNAs and circRNAs can be readily detected in saliva, especially in metastatic OSCC patients. Due to their distinct properties, they hold promise as biomarkers for diagnosing and prognosing OSCC, as well as potential targets or tools for treatment.

Conclusion

We described the expression profile of lncRNAs, circRNAs, miRNAs and mRNAs in OSCC. We also constructed the OSCC specific lncRNA-miRNA-mRNA and circRNA-miRNA-mRNA ceRNA regulatory network. Further elucidation of the molecular mechanisms underlying OSCC occurrence and progression could identify novel lncRNAs and circRNAs as effective therapeutic targets, ultimately enhancing survival outcomes for OSCC patients.

Funding

This study was supported by the Ningxia Natural Science Foundation of China (Grant Numbers: 2021AAC03377, 2021AAC03357 and 2022AAC03473).

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