Characterization of long non-coding RNA-associated ceRNA network in nasopharyngeal carcinoma

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Abstract: Nasopharyngeal carcinoma (NPC) is a common malignant tumor of the head and neck. The aim of this study was to construct long non-coding RNA (lncRNA)-associated competing endogenous RNA (ceRNA) regulation networks for NPC using bioinformatics methods. LncRNAs related to NPC was retrieved by searching Pubmed, MNDR v2.0, and LncRnaDisease database. The location of these lncRNAs was found in RNALocate and lncATLAS. For screened cytoplasmic lncRNAs, RAID v2 and miRTarBase were used to predict the possible ceRNA relationship between lncRNA-miRNA-mRNA. STRING and Cytoscape resources were used for systematical analysis of the molecular function, biological processes, and signal pathways of differentially expressed genes. So far 39 lncRNAs have been reported to be differentially expressed in NPC. Among them, 5 lncRNAs, namely AFAP1-AS1, DANCR, CCAT1, UCA1, and H19, were identified to be mainly cytoplasmic and had the potential to be ceRNAs. There have been strong evidences that 3 of the lncRNAs, namely CCAT1, UCA1, and H19, could bind to 11 miRNAs (miR-490-3p, miR-485-5p, miR-145-5p, miR-143-3p, miR-16-5p, miR-141-3p, miR-429, miR-200, miR-148a-3p, miR-22-3p, and miR-765-5p) and further regulate their corresponding downstream target mRNAs. A protein-protein interaction network of 356 nodes and 4676 edges was constructed, with proteins enriched in kinases and

positive regulator of cellular metabolism. After MCODE analysis, the proteins were further enriched to 6 nodes, namely DEAD-box helicase 53, high mobility group box-1 protein, CD40, CD28, interferon beta, and transferrin receptor. This study will provide novel insight for better understanding of lncRNA-associated ceRNA network and facilitate the exploration of new diagnostic and therapeutic markers for NPC.

Keywords: competing endogenous RNA, long non-coding RNA, nasopharyngeal carcinoma, bioinformatics analysis

Introduction

Nasopharyngeal carcinoma (NPC) is one of the common malignancies derived from nasopharynx epithelium. NPC is particularly prevalent in certain geographic areas such as Southern China (1). NPC usually has a concealed onset and is prone to high local metastasis and early distant metastasis. Even under the multimodality therapy including intensity-modulated radiotherapy (IMRT), the average 5-year survival rate of NPC has been remained at about 70% (2). Therefore, studying the occurrence and development of nasopharyngeal carcinoma from the molecular mechanism level is of great significance for improving the early diagnosis, selecting the best treatment plan, and predicting the prognosis of NPC

Long non-coding RNAs (lncRNAs) is a class of non-coding RNAs of larger than 200 nucleotides, and plays an important role in gene activation, silencing, nuclear transport and chromatin modification (3). At present, nuclear lncRNA and its function have been extensively studied, but the function of cytoplasmic lncRNA is relatively unclear. Recent advances have shown that lncRNA can bind to microRNAs (miRNAs) via the miRNA response element on mRNA, thus acting as competing endogenous RNA (ceRNA) to inhibit the regulation of miRNAs and indirectly regulate the expression of protein-coding genes (4).

With the rapid development of gene chip technology and high throughput sequencing technology, a large number of bioinformatics data of genome, proteome, transcriptome, and metabolome have been accumulated. The representative databases include GenBank of NCBI, Universal Protein Resource (UniProt), Gene Expression Omnibus (GEO), Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), etc. The results of individual studies rarely have overlaps. Bioinformatics takes these massive data as the research object, integrates, excavates and analyses them, and can extract the key genes and signal pathway information related to diseases, so as to help researchers select biomarkers more quickly and efficiently. Systematic analysis of lncRNA-associated ceRNA network is still lacking in NPC. Therefore, it is of great importance to explore those databases and construct such a

network by bioinformatics methods.

Materials and methods

Data collection

The profile data of differentially expressed lncRNA in NPC were obtained through the following ways: 1. Searching PubMed database (www.ncbi.nlm.nih.gov/pubmed) by using the keywords "nasopharyngeal carcinoma"/"nasopharynx carcinoma" + "lncRNA"/"long non-coding RNA"; 2. Searching MNDR v2.0 database (www.rna-society.org/mndr/index.html) for lncRNAs related with NPC (5); 3. Searching LncRNADisease database (www.cuilab.cn/lncrnadisease) for lncRNAs related with NPC (6).

The lncRNA-associated ceRNA network was constructed based on the following rules: 1. The subcellular localization of lncRNAs is mainly in cytoplasm; 2. There are strong evidences of interaction between lncRNAs and miRNAs; 3. There are strong evidences of interaction between miRNAs and targeted mRNAs. For differentially expressed lncRNAs reported in literatures, their subcellular localization was obtained from RNALocate database (www.rna-society.org/rnalcoate/index.html) (7) and lncATLAS database (lncatlas.crg.ed) (8). The lncRNAs that were shown by both databases to be mainly cytoplasmic were taken as potential ceRNA candidates. The interacted miRNA list of potential ceRNAs was obtained from RAID v2.0 (www.rna-society.org/raid/index.html) (9). The targeted mRNA data of those miRNAs were obtained from miRTarBase database version 7.0 (mirtarbase.mbc.nctu.edu.tw/php/index.php) (10).

Functional enrichment analysis

The mRNAs with strong evidence proof (reporter assay, Western blot, and qPCR) underwent functional enrichment analysis at the GO and KEGG levels using STRING version 11.0 resources (string-db.org) (11). The enrichment analysis was performed with whole human genome as background. Functional categories were visualized and clustered based on similar functions using Cytoscape version 3.7.1 (cytoscape.org),

and the functional modules were mined using the MCODE algorithm plugin in Cytoscape (12).

Results

The lncRNA-associated ceRNA network in NPC

By searching PubMed, MNDR v2.0, and LncRNADisease, totally 39 differentially expressed lncRNAs have been reported in NPC, as summarized in Table 1. Among them, 31 lncRNAs were upregulated and 8 lncRNAs were downregulated. The localization of these lncRNAs were shown in Table 2. Among these lncRNAs, the following 14 lncRNAs have not been reported for subcellular localization: N373932, ENST00000438550, ENST00000470135, CASC2, N375709, LNC1420, THOR, LET, LOC401317, NAG7, AF086415, AK056098, AK095147, AK294004. Some of the remaining lncRNAs can be expressed in both cytoplasm and nucleus. By combining the results from RNALocate and lncATLAS, we selected 5 lncRNAs expressed mainly in the cytoplasm for follow-up analysis. They are AFAP1-AS1, DANCR, CCAT1, UCA1, H19. By searching RAID v2.0, a list of potential interacted miRNAs was constructed (Table 3). So far, there remains 3 lncRNAs, namely CCAT1, UCA1, and H19, having strong evidences to interact with 13 miRNAs. The lncRNA/miRNA interaction pairs include: CCAT1/miR-490-3p, UCA1/miR-485-5p, UCA1/miR-145-5p, UCA1/miR-143-3p, UCA1/miR-16-5p, H19/miR-141-3p, H19/miR-148a-3p, H19/miR-22-3p, H19/miR-765-5p, H19/miR-200 family (miR-200a-3p, miR-200b-3p, miR-200c-3p), and H19/miR-429. The description of these interaction pairs were summarized in Table 4. The targeted mRNAs of these 13 miRNAs were further obtained from miRTarBase. Only mRNAs with strong targeting proof (reporter assay, Western blot, and qPCR) were selected. Therefore, a potential ceRNA network of lncRNA-miRNA-mRNA was listed in Table 5.

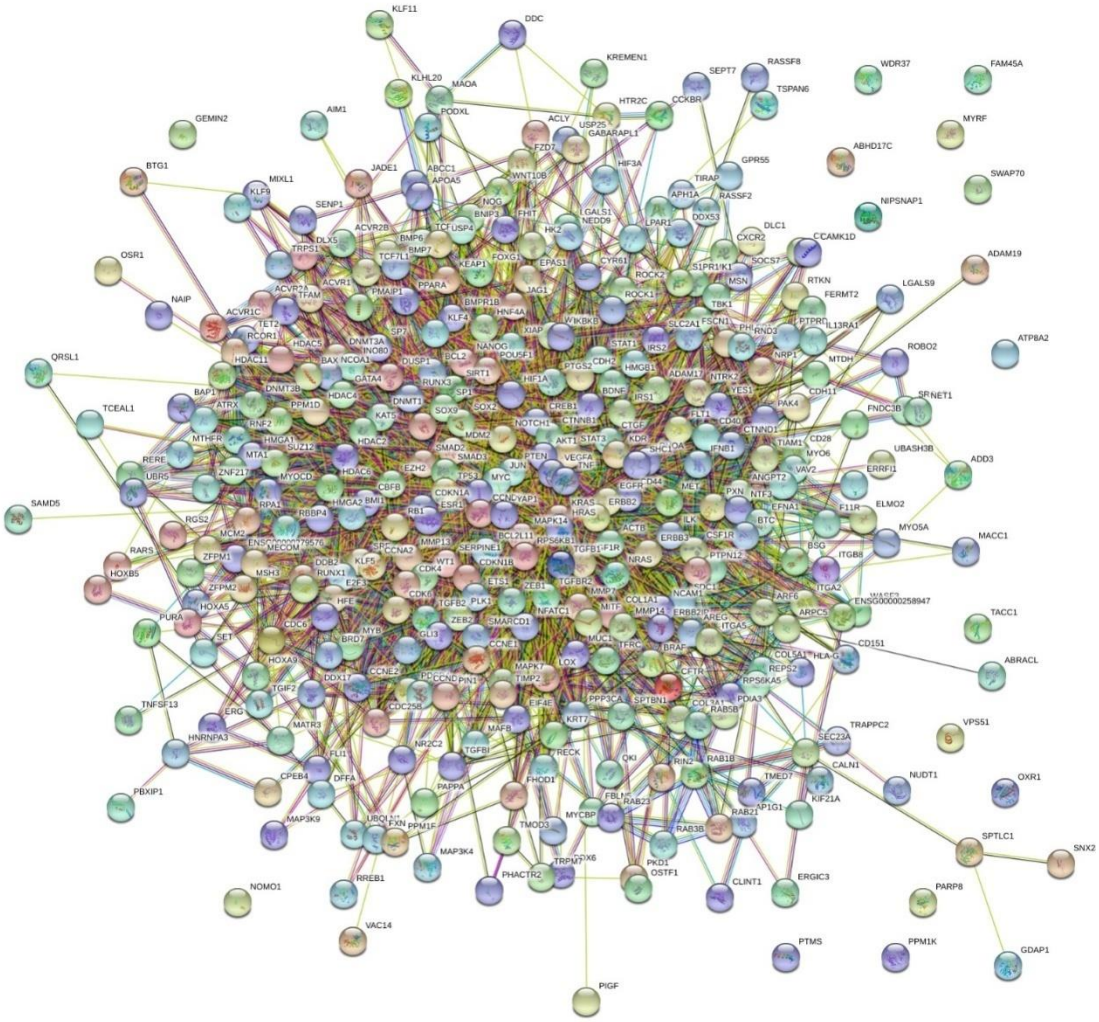

Figure 1. Protein-protein interaction network of targeted mRNAs

Functional characterization of ceRNA-associated mRNAs

Protein-protein interaction (PPI) network can provide valuable information for understanding cellular function and biological processes. Next, we performed functional analysis of those mRNAs using STRING online tool. A PPI network of 356 nodes and 4676 edges was constructed as shown in Figure 1. The average node degree is 26.3, and average local clustering coefficient is 0.497. The functional enrichment results were shown in Table 6. The functions of these proteins are mainly focused on protein binding (such as enzyme and subtract, ligand and receptor) and transcriptional regulator, and further positively regulate cellular metabolism. KEGG pathway is enriched in currently known pathways in cancer, which is not surprising. Enrichment analysis using other database (UniProt, PFAM, and INTERPRO) showed that these

proteins are mostly kinases involved in phosphorylation. By capturing the important modules using MCODE, we got a detailed module with 30 nodes and a simpler module with 6 nodes (Figure 2). The most enriched nodes are: DDX53 (DEAD-box helicase 53), HMGB1 (high mobility group box-1 protein), CD40, CD28, IFNB1 (interferon beta, IFN-β), TFRC (transferrin receptor). CD40, which is important for immune response, is the center of the module.

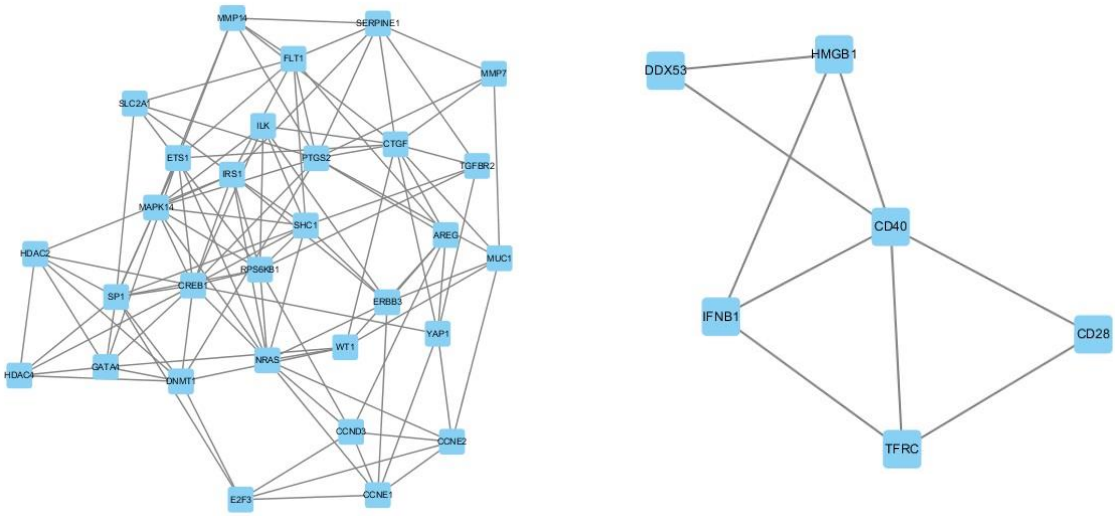

Figure 2. Functional enrichment of targeted mRNAs

Discussion

This study constructed an lncRNA-associated ceRNA network involving 3 lncRNAs and 13 miRNA in NPC. Target mRNAs enrichment showed that enriched in kinases and positive regulator of cellular metabolism. This constructed network will provide important clues for understanding the lncRNA-associated regulation in the development and progression of NPC.

Because the PPI network involves hundreds of target mRNAs, we did not study the role of these genes in NPC one by one. However, we did search the literature of the 6 core ode enriched by MCODE. CD40 is a co-stimulatory protein found on antigen presenting cells. The binding of CD40 ligand (CD40L), also known as tumor necrosis factor-associated activation protein (TRAP), to CD40 activates antigen presenting process and induces a variety of downstream effects (13). Constitutive expression of CD40L was able to enhance efficacy of antitumor immunotherapy

(14,15). CD28 is a differentiation antigen expressed on T cells and provides co-stimulatory signals required for TH1/TH2 differentiation (16). CD28 also attracts a lot of attention in immunotherapy (17). HMGB1belongs to the high mobility group and is an important mediator of autophagy and apoptosis in cancer cells (18,19). DDX53 usually participates in ATP-dependent RNA unwinding, thus contributing to cancer-associated transcriptional regulation (20). INF-β is widely involved in innate immune response. Targeting INF-β has been applied as an important strategy in cancer therapy (21,22).TFRC is involved in the cellular uptake of iron and has been used as a target for treatment of cancer (23). Three of these proteins (CD40, CD28, INF-β) are key factors of immune response. This is not surprising because NPC is highly related with Epstein-Barr virus infection (24). HMGB1 and TRFC regulate the metabolism of cancer cells. DDX53 is a transcription regulator. This result may also imply that immune microenvironment plays an important role in tumorigenesis and indicate immunotherapy is worth exploring.

Because NPC is an endemic cancer, relatively fewer researchers have paid attention to it. Our study is the first to systematically investigate the function of lncRNA-associated ceRNA in NPC. Recently, Pei Li et al. reviewed the research advance of lncRNAs in NPC, including H19, HOTAIR, LOC401317, MALAT1, LET, AFAP-AS1, and ROR (25). This review is a good background supplement for this study. This study will provide novel insight for better understanding of lncRNA-associated ceRNA network and facilitate the exploration of new diagnostic and therapeutic markers for NPC.

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Table 1. Differentially expressed lncRNAs in nasopharyngeal carcinoma

LncRNA	Expression trend	Molecular function	Methods	Reference
C22orf32-1	Up	Proliferation, migration, invasion	Microarray, qRT-PCR	26,27
ROR	Up	Proliferation, metastasis, apoptosis, chemoresistance	qRT-PCR	28
AFAP1-AS1	Up	Proliferation, metastasis, migration, invasion, prognosis, targeting miR-423-5p	cDNA microarray, qRT-PCR	29,30
ANRIL/CKDN2B-AS1	Up	Proliferation, transformation, glucose uptake for gycolysis, induce side-population stem-like cancer cells	qRT-PCR	31
N373932	Up	Radioresistance	Next-generation sequencing, qRT-PCR	32
ZNF674-1	Down	Proliferation, migration, invasion,apoptosis	Microarray, qRT-PCR	27,33
AL355149.1-1	Up	Associated with male patients, reduced in recurrent NPC	Microarray, qRT-PCR	27
BCL2L11-3	Up	Increased in recurrent NPC	Microarray, qRT-PCR	27
ENST00000438550	Up	Associated with progression and metastasis	Microarray, qRT-PCR	34
ENST00000470135	Up	Proliferation, invasion, and migration, lymph node metastasis	Microarray, qRT-PCR	35
DANCR/ANCR	Up	Correlated with tumor size and TNM stage, proliferation, radioresistance, invasion and metastasis, associated with hypoxia phenotype	qRT-PCR	36,37
HOTAIR	Up	Proliferation, angiogenesis, associated with prognosis	qRT-PCR, in situ hybridization	38,39
CASC2	Down	Proliferation, apoptosis, targeting miR-18a-5p	qRT-PCR	40
CCAT1	Up	Proliferation, migration, invasion, apoptosis	qRT-PCR	41
UCA1	Up	Proliferation, migration, invasion, targeting miR-145	qRT-PCR	42
N375709	Up	Chemoresistance	Next-generation sequencing, qRT-PCR	43
LINC00319	Up	Proliferation, associated with poor prognosis, targeting miR-1207-5p	qRT-PCR	44
LINC1420	Up	Invasion and migration, associated	qRT-PCR	45

		with survival		
LINC00460	Up	Proliferation, tumorigenesis in xenograft assay, targeting miR-149-5p	qRT-PCR	46
THOR	Up	Proliferation, migration, invasion, stemness, chemoresistance	qRT-PCR	47
LET	Down	Proliferation, apoptosis, associated with survival	qRT-PCR	48,49
NEAT1	Down	Proliferation, invasion, radioresistance, metastasis, targeting miR-101-3p	Microarray, qRT-PCR	50
HNF1A-AS	Up	Proliferation, migration, EMT,	qRT-PCR	51
SNHG12	Up	Proliferation, migration, invasion, associated with prognosis	qRT-PCR	52
SNHG1	Up	Invasion, migration, metastasis, EMT, targeting miR-145a-5p	Microarray, qRT-PCR	53
FOXD2-AS1	Up	Proliferation, associated with prognosis, targeting miR-363-5p	qRT-PCR	54
PXN-AS1-L	Up	Proliferation, migration, invasion, associated with prognosis	qRT-PCR	55
MALAT1	Up	Proliferation, migration, radioresistance, stemness, targeting miR-1	qRT-PCR	56,57
XIST	Up	Proliferation, associated with prognosis, targeting miR-34a-5p	qRT-PCR	58
LOC401317	Up	Proliferation, apoptosis	qRT-PCR	59
H19	Up	Differentiation	Microarray,qRT-PCR, in situ hybridization	60
NAG7	Down	Growth, invasion, migration, adhension, associated with tumor size and lymph node metastasis	qRT-PCR	61-63
MUDENG	Up	Radioresistance	Microarray, qRT-PCR	64
AF086415	Down	Radioresistance	Microarray, qRT-PCR	64
AK056098	Up	Radioresistance	Microarray, qRT-PCR	64
AK095147	Down	Radioresistance	Microarray, qRT-PCR	64
AK294004	Up	Radioresistance	Microarray, qRT-PCR	64
RP1-179N16.3	Up	Radioresistance	Microarray, qRT-PCR	64
MEG3	Down	Proliferation, colony formation，	qRT-PCR	65

	induction of cell cycle arrest, in vitro anchorage-independent growth, in vivo tumorigenicity	

Table 2. Localization of differentially expressed lncRNAs

LncRNA	Tissue	Subcellular Localization	
		RNALocate	lncATLAS
C22orf32/SMDT1	Colon cancer cell, myeloma cell	Cytosol	--
ROR	Breast, colon, embryonic stem cells	Cytoplasm	--
AFAP1-AS1	Myeloma cell	Cytosol	Cytoplasmic
ANRIL/CKDN2B-AS1	P493-6 cell, Hela cell, BGC-823, SGC-7901, NCM356	Nucleus	--
N373932	--	--	--
ZNF674-AS1	Colon cancer cell, K562 cells	Ribosome	Nucleus
AL355149.1-1	--	--	--
BCL2L11-3	Hela cells	Nucleus, cytoplasm	--
ENST00000438550	--	--	--
ENST00000470135	--	--	--
DANCR/ANCR	Colon cancer cell P493-6 cell Hela K562 cells	Cytosol Cytoplasm Cytoplasm, nucleus Cytoplasm, ribosome	Cytoplasmic
HOTAIR	Breast cancer cell, embryonic stem cell, gastric cancer cell, Hela cell, K562 cell Breast cancer cell, gastric cancer cell	Nucleus Cytoplasm	Nucleus
CASC2	--	--	Nucleus/Cytoplasmic
CCAT1	Colorectal cancer	Cytoplasm	Nucleus/Cytoplasmic
UCA1	Bladder cancer cell Colon cancer cell	Cytoplasm Ribosome	Cytoplasmic
N375709	--	--	--
LINC00319	--	--	Nucleus
LINC1420	--	--	--
LINC00460	--	--	Cytoplasmic
THOR	--	--	--
LET	--	--	--
NEAT1	Myeloma cell Fibroblast, lymphoblast, brain, Hela cell, colon cancer cell, prostate cancer cell, placenta	Cytosol Nucleus	Nucleus
HNF1A-AS	NSCC cell lines (both adenocarcinoma and squamous	Nucleus	--

	carcinoma subtypes)		
SNHG12	Colon cancer cell Myeloma cell	Nucleus Cytosol	Nucleus
SNHG1	Hela cell Colon cancer cell	Cytoplasm Nucleus	Nucleus
FOXD2-AS1	--	--	Nucleus
PXN-AS1-L	K562 cell	Ribosome, nucleus	--
MALAT1	Myeloma cell Fibroblast, lymphoblast, Hela, brain, colon cancer, Helacell	Cytosol Nucleus Exosome	Nucleus
XIST	Embryonic stem cell, somatic cell, lymphoblast, fibroblast, Hela cell, breast cancer cell, ovarian cancer cell	Nucleus	Nucleus
LOC401317	--	--	--
H19	Myeloma cell Hepatoma cell Plasma Breast cancer	Cytosol Cytoplasm Circulating Nucleus	Cytoplasmic
NAG7	--	--	--
MUDENG	Myeloma cell	Cytosol	--
AF086415	--	--	--
AK056098	--	--	--
AK095147	--	--	--
AK294004	--	--	--
RP1-179N16.3	Hela cell	Nucleus	--
MEG3	Hepatoma cell Brain tissue, lung fibroblast, Cerebellar Purkinje cell Myelomacell	Cytoplasm Nucleus Endoplasmic reticulum	--

--: No results.

Table 3. Potential interacted miRNAs of 5 ceRNA candidates

LncRNA	Evidence type	Interactor	Score
AFAP1-AS1	Strong	--	--
	Week	--	--
	Computational prediction	miR-520c-3p, miR-302a-3p, miR-302e, miR-384, miR-302c-3p, miR-372-3p, miR-302b-3p, miR-520d-3p, miR-520a-3p, miR-302d-3p, miR-520e, miR-373-3p, miR-520b	0.1828
DANCR	Strong	--	--
	Week	miR-758-3p, miR-496, miR-216a-5p, miR-33b-5p, miR-142-3p, miR-216b-5p, miR-378a-3p, miR-422a, miR-135b-5p, miR-135a-5p, miR- 33a-5p	0.6308
		miR-106a-5p	0.5483
	Computational prediction	miR-148a-3p, miR-185-5p, miR-125b-5p, miR-338-3p, miR-874-3p, miR-504-5p, miR-34c-5p, miR-449b-5p, miR-145-5p, miR-125a-5p, miR-449a	0.1828
CCAT1	Strong	miR-490-3p	0.8808
	Week	--	--
	Computational prediction	--	--
UCA1	Strong	miR-485-5p miR-145-5p miR-143-3p miR-16-5p	0.9612 0.9526 0.9026 0.8808
	Week	--	--
	Computational prediction	miR-122-5p, miR-18b-5p, miR-185-5p, miR-28-5p, miR-204-5p, miR-708-5p, miR-203a-3p, miR-193a-3p, miR-454-3p, miR-18a-5p, miR-590-3p, miR-495-3p, miR-193b-3p, miR-96-5p, miR-135a-5p, miR-135b-5p, miR-296-3p, miR-1271-5p, miR-9-5p, miR-137, miR-134-5p	0.1828
H19	Strong	miR-141-3p miR-148a-3p miR-22-3p miR-675-5p miR-200a-3p, miR-200b-3p, miR-200c-3p, miR-429	0.982 0.956 0.9526 0.9526 0.8808
	Week	miR-152-3p, miR-194-5p, miR-301a-3p, miR-19b-3p, miR-148b-3p, miR-150-5p, miR-342-3p, miR-19a-3p, miR-130b-3p, miR-422a, miR-301b-3p, miR-130a-3p, miR-454-3p, miR-370-3p, miR-216b-5p	0.6308
		miR-196a-5p, miR-106a-5p, miR-196b-5p	0.5483
	Computational prediction	miR-491-5p, miR-326, miR-330-5p	0.1828

--: No results.

Table 4. Descriptions of interaction between lncRNAs and miRNAs

LncRNA/ miRNA	Evidence support	Description	Reference
CCAT1/ miR-490-3p	RT-PCR, Reporter assay	CCAT1 was upregulatedand miR-490 was downregulated in gastric cancer. miR-490 plays an important role in CCAT1-mediated pro-motion of GC cell migration	66
UCA1/ miR-485-5p	RNA immunoprecipitation, RT-PCR, reporter assay	UCA1 functions as an endogenous sponge by directly binding to miR-485-5p, thus regulating matrix metallopeptidase 14 expression, a target gene of miR-485-5p	67
UCA1/ miR-145-5p	Western blot, RT-PCR, reporter assay	UCA1 is a target of miR-145 and UCA1 can also inhibit miR-145 expression to upregulate ZEB1/2. UCA1 and miR-145 form a reciprocal repression regulation loop to regulate bladder cancer progression.	68
UCA1/ miR-143-3p	RNA immunoprecipitation, RT-PCR,	UCA1 is present in Ago2-containing RNA-induced silencing complex (RISC) through association with miR-143, thus modulating growth and apoptosis of breast cancer cells	69
UCA1/ miR-16-5p	RT-PCR, reporter assay	UCA1 contains the miR-16-5p binding site. UCA1 regulates GLS2 expression through interfering with miR-16, ad represses ROS formation in bladder cancer cells.	70
H19/ miR-141-3p	Immunoprecipitation, reporter assay, RT-PCR, western blot	1. H19 functions as a sponge for miR-141 and miR-22, and leads to regulation of their shared target gene β-catenin and osteogenesis. 2. H19 regulates miR-141 and its target gene ZEB1. H19 and miR-141 compete with each other and affect their target genes in gastric cancer.	1. 71 2. 72
H19/ miR-148a-3p	MTT assay, RT-PCR, CLIP-seq	H19 is an inhibitory regulator of miR-148a-3p and promotes laryngeal squamous cell carcinoma progression via miR-148a-3p/DNMT1 axis.	73
H19/ miR-22-3p	Immunoprecipitation, reporter assay, RT-PCR	H19 functions as a sponge for miR-141 and miR-22, and leads to regulation of their shared target gene β-catenin and osteogenesis.	71
H19/ miR-765-5p	Western blot, RT-PCR, reporter assay	1. H19 promotes glioma cell invasion by deriving miR-675, thus regulating its target Cadherin 13. 2. There is a feedback loop between H19 and its encoded miR-675-5p during osteoblast differentiation.	1. 74 2. 71
H19/ miR-200a-3p, miR-200b-3p, miR-200c-3p, miR-429	Western blot, RT-PCR	H19 can activate miR-200 family by increase histone acetylation, thus contributing to mesenchymal-to-epithelial transition and to the suppression of hepatocellular carcinoma metastasis.	75

Table 5. CeRNA network of lncRNA-miRNA-mRNA in nasopharyngeal carcinoma

lncRNA	miRNA	mRNA
CCAT1	miR-490-3p	ERGIC3, SMARCD1, CCND1, PAPPA
UCA1	miR-485-5p	TP53, HIF3A, APOA5
	miR-145-5p	BNIP3, KLF5, SOX2, KLF4, MUC1, MYO6, CDKN1A, STAT1, YES1, CBFB, PPP3CA, CLINT1, IRS1, PARP8, TMOD3, HOXA9, EGFR, FSCN1, MYC, FLI1, DFFA, IFNB1, TIRAP, POU5F1, IGF1R, KRT7, PPM1D, MYRF, CPEB4, FZD7, ROBO2, SRGAP1, EIF4E, CDK4, VEGFA, SERPINE1, IRS2, ITGB8, SWAP70, ESR1, NUDT1, JAG1, NEDD9, PAK4, DDX17, ERG, NRAS, ILK, CTGF, SOCS7, MDM2, ADAM17, CDH2, HDAC2, RTKN, F11R, MYO5A, NANOG, ABHD17C, APH1A,PODXL, TSPAN6, MIXL1, ABRACL, MMP14, KREMEN1, NIPSNAP1, JADE1, AP1G1, PIGF, FAM45A, EPAS1, ETS1, RREB1, COL5A1, CD44, BRAF, ACTB, SOX9, SMAD3, TGFBR2, CTNND1, SP1, TNFSF13, CDK6, DDX6, ARF6, ADD3, PADL1, PHACTR2, SNX24, KIF21A, SAMD5, CRYBG1, HMGA2, DDC, ANGPT2, ROCK1, E2F3, RPS6KB1, CD28, NFATC1, VPS51, ADAM19, ABCC1, SPTBN1, SENP1, NAIP, SET, RPA1, MCM2, SPTLC1, MYOCD, MTDH, TUG1, CAMK1D, HIF1A, FXN, CRNDE, PXN, MSH3, CD40, TGFB2, SMAD2, SP7, HDAC11, CFTR
	miR-143-3p	KRAS, MYO6, DNMT3A, FNDC3B, MAPK7, COL1A1, HRAS, FSCN1, HK2, SERPINE1, FHIT, MACC1, PTGS2, JAG1, AKT1, MDM2, BCL2, MMP13, SDC1, RREB1, CD44, KLF5, BRAF, IGF1R, GABARAPL1, TNF, NR2C2, IL13RA1, COL3A1, DDX6, LIMK1, HNF4A
	miR-16-5p	BML1, HMGA1, ACVR2A, PDCD4, RAB21, WT1, CDK6, CCND3, CCND1, CCNE1, RARS, PURA, PTGS2, ITGA2, FGF2
H19	miR-141-3p	ZEB2, ZEB1, DLX5, BAP1, KLF5, STK3, TGFB2, SFPQ, CLOCK, BRD3, UBAP1, PTEN, ZFPM2, TRAPPC2B, EIF4E, CTBP2, CDYL, ACVR2B, MAPK14, PPARA, NROB2, YWHAG, ELAVL4, MAPK9, TFDP2, E2F3, SHC1, VAC14, TCF7L1, ELMO2, RASSF2, KLHL20, RIN2, SEPT7, HOXB5, ERBIN, KLF11, PTPRD, WDR37, STAT4, YAP1, CDC25C, HDGF, KEAP1, TAZ, TIAM1, TM4SF1, CDC25A, PHLPP1, PHLPP2, TET3, TET1, MAP4K4, HNRNPD, ZMPSTE24
	miR-429	ZEB2, ZEB1, RERE, WASF3, ZFPM2, BCL2, XIAP, OSTF1, SOX2, KLHL20, PTPRD, ELMO2, ERBIN, BAP1, WDR37, VAC14, TCF7L1, HOXB5, RASSF2, RIN2, KLF11, SEPT7, SHC1, MYC, MYB, DNMT1, EZH2, RBBP4, RASSF8
	miR-200	TUBB3, BMI1, PTPN12, DLX5, ZEB2, BAP1, GEMIN2, CTNNB1, ZEB1, RERE, ETS1, FIN1, PDCD4, GATA4, WASF3, ZFPM2, TRAPPC2B, MATR3, UBE2L, JAG1, PTPN12, GDAP1, RNF2, ROCR3, BRD7, E2F3, ACVR2B, MAPK14, MSN, NTRK2, VEGFA, FLT1, KDR, RND3, ERRFI1, FHOD1, PPM1F, CCNE2, BCL2, XIAP, KEAP1, TIMP2, FBLN5, NCAM1, SRF, IKBKB, KLF9, TBK1, PMAIP1, NTF3, SMAD2, SMAD3, YAP1, CREB1, LPAR1, ENDRA, RHOA, KLHL20, PTPRD, ELMO2, ERBIN, WDR37, VAC14, SHC1, TCF7L1, RASSF2, RIN2, HOXB5, SEPT7, KLF11, SHC1, MYB, ETS1, TFAM,

		QRSL1, UBASH3B, DUSP1, USP25, EFNA1, SUZ12, RND3, WNT1, DNMT3A, SP1, TP53, MSN, CFL2, CDH11, SEC23A, DNMT1, EZH2, PTEN, KDR, HFE, DLC1, HNRNPA3, ATRX, ZNF217, FERMT2, RAB21, RAB3B, RAB1B, RAB23, OXR1, STAT3, TRPS1, FOXG1, NOG, HOXA5, NOTCH1, AREG, BTC, ZFPM1, PIN1, CDKN1B, KRAS, LOX, PHLPP1, SEC23A, SUZ12, ROCK2, DDX53, UBQLN1, CDK6, RECK, GLI3, OSR1, PKD1, JUN, MAPK7
	miR-148a-3p	DNMT1, HLA-G, TGIF2, DNMT3B, NR1L2, RPS6KA5, CCKBR, IRS1, ACVR1, BCL2, TMED7, PBXIP1, CDC25B, MAP3K4, MMP7, WNT10B, MYC, CDKN1B, SERPINE1, ITGB8, VAV2, ITGA5, ROCK1, RUNX3, SMAD2, MET, BCL2L11, PDIA3, MAFB, ERRFL1, S1PR1, USP4, MAP3K9, INO80, TGFB2, IKBKB, BAX, QKI, NRP1
	miR-22-3p	BMP7, PPARA, SER1, HDAC4, TFRC, MYCBP, ACVR1C, BDNF, HTR2C, MAOA, RGS2, NCOA1, ARPC5, ERBB3, TCEAL1, PTMS, PTEN, HMGB1, IRF5, RCOR1, SP1, RAB5B, TET2, CDKN1A, WNT1, CD151, CYR61, SIRT1, LGALS1, HIF1A, BSG, CSF1R, BMPR1B, BMP6, NET1, TIAM1, BTG1, SLC2A1, TCF7, LGALS9, TRPM7, MMP14, SNAL1, MALAT1, CXCR2, CCNA2, MTHFR, UBR5, KAT5, TACC1, NTRK2, MTA1, MECOM， AKT1, MTDH, ERBB2, ACLY, PLK1, PPM1K
	miR-675-5p	RB1, RUNX1, CALN1, NOMO1, TGFBI, MITF, CDC6, REPS2, ATP8A2, TGFB1, GPR55, DDB2, HDAC4, HDAC5, HDAC6

Table 6. Functional enrichment of targeted mRNAs

Key term	Description	Count in gene set	FDR
Biological Process (GO)			
GO:0048522	Positive regulation of cellular process	248 of 4898	1.58e-64
GO: 0031325	Positive regulation of cellular metabolic process	198 of 3060	6.92e-62
GO: 0009893	Positive regulation of metabolic process	204 of 3280	6.92e-62
GO: 0048518	Positive of biological process	255 of 5459	5.65e-61
GO: 0010604	Positive regulation of macromolecular metabolic process	196 of 3081	3.34e-60
Molecular function (GO)			
GO: 0005515	Protein binding	258 of 6605	2.48e-46
GO: 0005488	Binding	322 of 11878	1.60e-33
GO: 0019899	Enzyme binding	123 of 2197	5.97e-29
GO: 0044212	Transcription regulatory region DNA binding	70 of 829	1.27e-23
GO: 0008134	Transcription factor binding	59 of 610	1.93e-22
Cellular component (GO)			
GO: 0070013	Intracellular organelle lumen	185 of 5162	2.02e-21
GO: 0031981	Nuclear lumen	159 of 4030	2.02e-21
GO: 0043227	Membrane-bounded organelle	292 of 11244	1.40e-20
GO: 0005654	Nucleoplasm	142 of 3446	1.50e-20
GO: 0044428	Nuclear part	163 of 4359	2.49e-20
KEGG pathways			
Hsa05206	microRNAs in cancer	51 of 149	5.82e-42
Hsa05200	Pathways in cancer	71 of 515	1.64e-36
Hsa05205	Proteoglycans in cancer	44 of 195	7.61e-30
Hsa05165	Human papillomavirus infection	47 of 317	5.60e-25
Hsa05225	Hepatocellular carcinoma	36 of 163	3.73e-24
UniProt keywords			
KW-0597	Phosphoprotein	251 of 8066	5.52e-26
KW-0832	Ubiquitin conjugation	117 of 2380	8.65e-22
KW-9995	Disease	151 of 3799	9.15e-21
KW-0656	Proto-oncogene	34 of 228	9.34e-18
Kw-0539	Nucleus	171 of 5200	5.99e-16
PFAM protein domains			
PF07714	Protein tyrosine kinase	35 of 481	6.70e-09
PF00069	Protein kinase domain	35 of 482	6.70e-09
PF00850	Histone deacetylase domain	5 of 11	0.00099
PF02984	Cyclin, C-terminal domain	5 of 17	0.0034
PF01030	Receptor L domain	4 of 7	0.0034
INTERPRO protein domains and features			
IPR000719	Protein kinase domain	35 of 485	1.56e-08
IPR011009	Protein kinase-like domain superfamily	36 of 529	1.87e-08
IPR017441	Protein kinase, ATP binding site	30 of 380	2.06e-08
IPR001245	Ser-Thr/Tyr-protein kinase, catalytic domain	17 of 131	2.85e-07
IPR000333	Ser/Thr protein kinase, TGFB receptor	6 of 12	8.23e-05