Bioinformatics Analysis of the Expression and Role of microRNA-221-3p in the Head and Neck Squamous Cell Carcinoma

Ziyan Zhou

Guangxi Medical University First Affiliated Hospital, Guangxi Tumor Radiation Therapy Clinical Medical Research Center

Wenling Wu

Guangxi Medical University First Affiliated Hospital

Jixi Li

Guangxi Medical University First Affiliated Hospital; Guangxi Tumor Radiation Therapy Clinical Medical Research Center

Chang Liu

Guangxi Medical University

Zixi Xiao

Guangxi Medical University

Qinqiao Lai

Guangxi Medical University

Rongxing Qin

Guangxi Medical University

Mingjun Shen

Guangxi Medical University First Affiliated Hospital; Guangxi Tumor Radiation Therapy Clinical Medical Research Center

Shuo Shi

Guangxi Medical University First Affiliated Hospital

min kang (✉ kangmin@gxmu.edu.cn)

The First Affiliated Hospital of Guangxi Medical University

Research article

Keywords: HNSCC, miR-221-3p, RT-qPCR, TCGA, bioinformatics

Posted Date: December 31st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-136851/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Version of Record: A version of this preprint was published at BMC Cancer on April 12th, 2021. See the published version at <https://doi.org/10.1186/s12885-021-08039-5>.

Abstract

Background

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer subtype globally, associated with a high rate of morbidity and mortality. However, the target genes of miR-221-3p and the underlying mechanism involved in HNSCC were not known. Therefore, in the current study, we studied the role of miR-221-3p in the HNSCC.

Methods

Tissues collected from 48 control and 21 HNSCC patients were processed to check the differential expression of miR-221-3p by RT-qPCR. Overexpression of microRNA-221-3p (miR-221-3p) is significantly correlated to the onset and progression of HNSCC. We also conducted the meta-analysis of the cancer literature from the cancer genome atlas (TCGA) and the Gene Expression Omnibus (GEO) database to estimate the expression of miR-221-3p in HNSCC. The miR-221-3p target genes in the HNSCC were predicted with the miRWalk and TCGA databases, and functionally annotated via the Gene Ontology. Finally, Spearman's analysis was used to determine the role of the related target genes in important pathways involved in the development of HNSCC.

Results

We observed a significantly higher expression of miR-221-3p in HNSCC compared to the normal with a summary receiver operating characteristic (sROC) of 0.86(95% CI: 0.83,0.89). The KEGG and GO comprehensive analysis predicted that miR-221-3p might be involved in the development of HNSCC through the following metabolic pathways, viz Drug metabolism - cytochrome P450 UGT1A7 and MAOB may be important genes for the role of mir-221-3p.

Conclusions

Our results indicate that miR-221-3p may be used as a non-invasive and hypersensitive biomarker in the diagnosis. Thus, it can be concluded that miR-221-3p is an extremely important gene locus involved in the process of the deterioration and eventual tumorigenesis of HNSCC.

Background

Head and neck squamous cell carcinoma (HNSCC) encompassing the oral squamous cell carcinoma, oropharyngeal squamous cell carcinoma, hypopharyngeal squamous cell carcinoma and laryngeal squamous cell carcinoma is the sixth most common cancer worldwide, accounting for approximately 1–2% of all cancer deaths, with about 600,000 detection of new cases each year globally[1–3]. The treatment of patients at the early stage is relatively successful, but the overall survival rate of recurrent or metastatic HNSCC remains low and has barely seen any improvements in their survival for decades[4, 5]. Although the past three decades have seen several improvements in the diagnostic tools and treatment

regimens, the overall survival rate for the advanced (stage III-IV) head and neck squamous cell carcinoma (HNSCC) is only 65%[6, 7]. The current standard of care for such patients are surgery along with radiation and chemotherapy, but these have not significantly improved the 5-year survival of the HNSCC patients. There is an urgent need for the e main reason for the decline in patient survival is the lack of a powerful therapeutic target for the development of HNSCC[8, 9].

Recent years have seen an increasing concentration of research in, microRNA (miRNA)[10, 11], which are a type of highly conserved single-stranded non-coding RNA, containing 17 ~ 22 nucleotides and are involved in the process of tumorigenesis, cell survival, and chemosensitivity[12–14]. They bind the 3'-non-region (3'UTR) of different target mRNA (messenger RNA) genes to degrade or inhibit the mRNA translation of target genes associated with a tumor suppressor function[13, 15, 16]. In normal healthy individuals, miR-221-3p is observed to play a role in the process of vascular proliferation[17], while the tumor promoter, microRNA-221 is involved in the process of regulation of apoptosis of the tumor cell[18–20] and is associated with a variety of cancer types, including hepatocellular cancer[21], cutaneous melanoma[19], prostate cancer[20],non-small cell lung cancer[22].

Studies have shown that specific miRNA profiles can be identified between tumor tissue and adjacent healthy tissue in the HNSCC patients[2, 23]. For instance, studies have reported the link between miR-211 and vascular invasion during the incidence of HNSCC[14, 15, 24–26]. However, the definite target gene of the miR-211-3p and its biological mechanism of action are still not clear. Thus, in the current study, we investigated the expression of miR-221-3p in the HNSCC and attempted to explore the correlation between the two.

Methods

RT -qPCR

Tissue specimens were collected from 48 control and 21 HNSCC patients from the Department of Pathology, First Affiliated Hospital of Guangxi Medical University (Nanning, Guangxi Medical University), and processed for RT-qPCR with the SYBR-Green Master mix (Takara, Tokyo, Japan) in a ABI 7500 cyclor (Applied Biosystems) with the following conditions: initial denaturation at 95°C for 30 sec, followed by 40 cycles of denaturation at 95°C for 5 sec and annealing at 60°C for 34 sec. The differential expression of miR-21 in the HNSCC tissues relative to the negative control (NC) tissues was calculated using the 2- $\Delta\Delta$ Ct method, with U6 as the internal control. The primers of miR-221-3p and U6 were synthesized by TaKaRa (Dalian, Liaoning, China) and the sequences are as follows: miR-221-3p: Forward 5' - AGCUACAUUGUCUGCUGGGUUUC – 3' and Reverse 5' mRQ 3'; U6: Forward 5' - GGAACGATACAGAGAAGATTAGC-3' and Reverse 5' - TGGAACGCTTCACGAATTTGCG – 3'. All the experiments were repeated three times.

Literature search and selection strategy.

Literature search was conducted with the PubMed, Chinese Biomedical Literature Database (CBM), Science Direct, the China National Knowledge Infrastructure Database (CNKI), Web of Science, Wiley online library, EMBASE, the China Science and Technology Journal Database (VIP) and the Wanfang Database for this study. We searched the database from the earliest available data beginning to October 1, 2019. The following keywords were used: (HNSCC OR SCC OR "squamous cell cancer" OR "squamous cell carcinoma") AND (oropharynx OR oropharyngeal OR "head and neck" OR nose OR nasopharynx OR "nasal sinus" OR "nasal cavity" OR "oral cavity" OR hypopharynx OR oral OR laryngopharynx OR larynx OR laryngopharyngeal OR laryngeal OR pharyngeal OR tongue OR tonsil OR tonsillar OR cheek OR palatal OR "paranasal sinuses" OR buccal OR lip) AND (microRNA-221-3p OR miRNA-221-3p OR "miR 221-3p" OR "miRNA 221-3p" OR miRNA221-3p OR miR221-3p)

Selection criteria and data extraction.

The databases were searched independently by two researchers who selected the studies based on the following inclusion criteria: (1) comparison of HNSCC and non-cancerous tissues, (2) validation of miR-221-3p expression levels via reverse transcription quantitative PCR (RT-qPCR), (3) evaluation of the association between miR-221-3p expression and clinical outcomes, and (4) availability of sufficient data to calculate the mean, standard deviation (SD) and 95% confidence intervals (95% CI). The articles were eliminated if they met one of the following exclusion criteria: (1) irrelevant to the research focus, (2) inclusion of unqualified data, (3) publication language other than English or Chinese, (4) overlapping or duplicate publications, and (5) letters, reviews, comments, editorials, conference articles, laboratory studies or case reports. The reviewers independently appraised the quality of data in each eligible study and extracted the first author name, year of publication, country of origin, sample type, sample size and analysis method. For articles with incomplete information, the authors were contacted to obtain relevant information.

Microarray data collection from GEO.

Microarray data of HNSCC samples uploaded till October 1, 2019 was obtained from GEO database (<https://www.ncbi.nlm.nih.gov/gds/>) using the following keywords: (HNSCC OR SCC OR "squamous cell cancer" OR "squamous cell carcinoma") AND (oropharynx OR oropharyngeal OR "head and neck" OR nose OR nasopharynx OR "nasal sinus" OR "nasal cavity" OR "oral cavity" OR hypopharynx OR oral OR laryngopharynx OR larynx OR laryngopharyngeal OR laryngeal OR pharyngeal OR tonsil OR tonsillar OR tongue OR cheek OR palatal OR "paranasal sinuses" OR buccal OR lip) AND (microRNA OR miRNA OR "miR" OR "miRNA"). The inclusion criteria for the microarray datasets were: (1) comprising of data from HNSCC and non-cancerous tissues, (2) evaluation of the association between miR-221-3p expression and clinical outcomes, (3) availability of sufficiently data to calculate mean, SD and 95% CI. The exclusion criteria were: (1) unrelated to this study, (2) unqualified data, (3) publication language other than English.

RNA sequencing data selection from TCGA.

MiR-221-3p expression data of HNSCC and normal tissues, and the clinico-pathological parameters, were downloaded and extracted from the OncoLnc website (<http://www.oncolnc.org/>) (13). SPSS24.0 (Nanning, Guangxi Zhuang Autonomous Region, China) was used to determine the association between miR-221-3p expression levels and other factors.

Statistical analysis

Continuous variables and meta-analysis of the data of the available cancer literature, The Cancer Genome Atlas (TCGA) and GEO(Gene Expression Omnibus). The meta-analysis data was verified by PCR. Further, we used diagnostic meta-analysis to estimate the potential diagnostic value of miR-221-3p. In addition, the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses were used to comprehensively analyze the possible molecular mechanisms of miR-221-3p in HNSCC.

Comprehensive statistical analysis of the data

Data which were removed from our meta-analysis as well as TCGA data (Nanning, Guangxi Zhuang Autonomous Region, China) were analyzed using the STATA 15.0 software. Standard mean difference (SMD) and 95% confidence interval were used to estimate the expression value of miR-221-3p. In normal and HNSCC, scatter plots prepared with the GraphPad Prism 6 (Nanning, Guangxi Zhuang Autonomous Region, China) of the data of both the normal and HNSCC tissues were used to assess the expression level of miR-221-3p. Evidence of bias was assessed by the visual funnel plots and Egger's regression asymmetry test. In addition, the I^2 index was used to evaluate the potential heterogeneity of the selected data. When $I^2 > 50\%$ or $P < 0.05$, we used the fixed effect model; otherwise, the random effect model was used. SPSS 24.0 software (IBM, Somers, NY) was used for the statistical analysis. Mean $\pm$ SD ($\bar{X} \pm s$) and t test for comparisons between groups were applied all to the measurement data. The count rate was expressed as a percentage (%), and we used the χ^2 test for comparison. Based on true positive (TP), false positive (FP), false negative (FN) and true negative (TN) to determine the diagnostic value of miR-221-3p, we used the meta-disc software to calculate the specificity, sensitivity, likelihood ratio (negative and positive) receiver operating characteristic curve (sROC). We also did the sensitivity analysis to assess the differences between the sample sizes. Moreover, all the miR-221-3p expression data which included the TCGA sequence was normalized to $\log_2$ to improve the normality of the measurements. $P < 0.05$ is considered a statistically significant value.

Predicting target genes of mir-221 in HNSCC

The Gene Ontology (GO)[27]analysis was used to define biological processes (BP), molecular function (MF), and cellular components (CC), and the Kyoto Encyclopedia of Genes and Genomes (KEGG)[28] pathway enrichment analyses were conducted using R (version 3.6.1) to visualize the ClusterProfiler of R. The Search Tool for the Retrieval of Interacting Genes (STRING) (<https://string-db.org/>) was then used to establish a protein-protein interaction (PPI) network of the miR-221-3p target genes associated with the significantly enriched pathways. The expression levels of the miR-221-3p target genes in the HNSCC and non-tumor tissues were determined with the UALCOULD[29] (<http://ualcan.path.uab.edu/index.html>).

Finally, the Linkedomics[30] (<http://www.linkedomics.org/>) Spearman's analysis tool was applied to determine the correlation between the expression levels of the miR-221-3p and the potential target genes involved in notable signaling pathways.

Result

miR-221-3p is highly expressed in the tumor tissues

The RT-qPCR data clearly showed that miR-221-3p is highly expressed in the tissues of the nasopharyngeal carcinoma patients as compared to the control group($p < 0.05$) (Fig. 1).

Selection of relevant literature and microarray data extraction.

A total of 17 articles were retrieved from the initial search, and the following paper was selected after the full-text review based on the inclusion criteria (Fig. 2): Zhou Cheng. et al (PMID: 30928631)[31]. Sixteen out of 424 GEO microarray datasets – GSE11163, GSE103931, GSE107591, GSE113956, GSE28100, GSE31277, GSE34496, GSE45238, GSE51829, GSE58911, GSE69002, GSE70289, GSE73171, GSE75630, GSE82064, GSE98463 – met our inclusion criteria. The information is summarized in Table 1.

Table 1
The main features of included studies for this meta-analysis

Researcher	Year	Country	Cancer/normal	Methods	Sample
TCGA	2019	USA	523/44	qPCR	Tissue
GSE11163	2012	USA	16/5	qPCR	Tissue
GSE103931	2018	Taiwan	30/19	qPCR	Tissue
GSE107591	2018	USA	24/23	qPCR	Tissue
GSE113956	2019	China	25/15	qPCR	Tissue
GSE28100	2015	USA	17/3	qPCR	Tissue
GSE31277	2018	Brazil	15/15	qPCR	Tissue
GSE34496	2017	USA	44/25	qPCR	Tissue
GSE45238	2019	USA	40/40	qPCR	Tissue
GSE51829	2013	China	4/4	qPCR	Tissue
GSE58911	2018	USA	15/15	qPCR	Tissue
GSE69002	2017	USA	3/4	qPCR	Tissue
GSE70289	2018	USA	12/4	qPCR	Tissue
GSE73171	2017	Hong Kong	3/3	qPCR	Tissue
GSE75630	2016	Australia	28/18	qPCR	Tissue
GSE82064	2017	Switzerland	78/18	qPCR	Tissue
GSE98463	2018	Spain	8/8	qPCR	Tissue
Zhou Cheng. et al	2019	China	26/26	qPCR	Tissue

Bias risk assessment

The results of the visual funnel plots show a symmetrical shape, which indicate absence of any significant publication bias as a whole. Sensitivity analysis performed on the heterogeneity samples showed that there did not exist any significant difference between the studies (Fig. 3).

Expression and meta-analysis of miR-221-3p in the HNSCC From the total of 911 HNSCC samples and 289 normal samples of the Pubmed, GEO and TGCA datasets, we observed that miR-221-3p is overexpressed in the HNSCC compared to the normal tissues ($P < 0.05$). Which is consistent with the results obtained by RT-qPCR. Meta-analysis to explore the level of expression of mrR-221-3p in HNSCC indicate a high degree of heterogeneity between these studies. Therefore, the random-effect mode was selected and the combined standard deviation (SMD) was observed to be 0.717(95% CI :0.283, 1.152)

(Fig. 4). The meta-analysis data indicate that miR-221-3p expression is significantly upregulated in the HNSCC. In addition, the expression levels of mir-221-3p in HNSCC and normal tissues from each included dataset is shown in Fig. 5 (A-R).

Diagnostic value of miR-221-3p in HNSCC

The random-effects mode applied to analyze the diagnostic value of miR-221-3p for HNSCC showed a significant heterogeneity in the likelihood ratios (negative and positive) sensitivity and specificity analyses. Diagnostic meta-analysis results indicated that mixed specificity, sensitivity, likelihood ratio (negative and positive) and diagnostic odds ratio to be 0.78(95% CI:0.72–0.82),0.57(95% CI:0.54–0.60), 0.42(95% CI:0.31–0.56) and 3.04(95% CI:1.72–5.40) respectively (Fig. 6). The ROC curve is shown in Fig. 7. Based on the ROC of each study, the total ROC curve indicated that the area under the sROC curve (AUC) analysis is 0.86(95% CI: 0.83,0.89) (Fig. 8).

Clinical -pathological features of HNSCC based on the TCGA

Based on the sample data of the HNSCC extracted from the TCGA database, we analyzed 527 samples, which indicate the absence of any statistically significant difference between miR-221-3p expression and age, lymphovascular invasion,Neoplasm histologic grade or any other clinical-pathological features. However, it indicated a statistically significant difference in the expression of miR-221-3p that mainly refer to the different tissue, gender and clinical stage, even alcohol. (Table 2).

Table 2

The correlation between miR-221-3p expression levels and clinic characteristics based on TCGA database

Clinicopathological features	Terms	n	Mean $\pm$ SD	p-value
Unpaired tissue	Normal	44	8.16 $\pm$ 0.64	0.001
	HNSCC	480	8.55 $\pm$ 0.78	
Gender	Male	383	8.45 $\pm$ 0.79	0.001
	Female	141	8.71 $\pm$ 0.68	
Age	< 60	234	8.50 $\pm$ 0.76	0.559
	$\geq$ 60	290	8.54 $\pm$ 0.78	
lymphovascular invasion	NO	232	8.54 $\pm$ 0.81	0.429
	YES	292	8.49 $\pm$ 0.75	
Clinical stage	I-II	206	8.63 $\pm$ 0.76	0.011
	III-IV	318	8.45 $\pm$ 0.78	
Neoplasm histologic grade	G1-G2	371	8.49 $\pm$ 0.73	0.260
	G3-G4	156	8.58 $\pm$ 0.82	
T stage	T1-T2	188	8.61 $\pm$ 0.73	0.029
	T3-T4	336	8.46 $\pm$ 0.79	
M stage	M0	498	8.51 $\pm$ 0.78	0.967
	M1	29	8.51 $\pm$ 0.57	
N stage	N0	246	8.53 $\pm$ 0.76	0.572
	N1-N3	281	8.49 $\pm$ 0.77	
Alcohol	NO	164	8.62 $\pm$ 0.76	0.047
HPV status	YES	363	8.48 $\pm$ 0.76	0.066
	Negative	194	8.41 $\pm$ 0.81	
	Positive	330	8.55 $\pm$ 0.81	
Perineural invasion	NO	198	8.53 $\pm$ 0.81	0.693
	YES	329	8.50 $\pm$ 0.80	

Notes: HNSCC, Head and neck squamous cell carcinoma; T stage, size or direct extent of the primary tumor; N stage, degree of spread to regional lymph nodes; M stage, presence of distant metastasis; SD, standard deviation

Clinicopathological features	Terms	n	Mean ± SD	p-value
Smoking	< 3	298	8.47 ± 0.76	0.094
	≥ 3	229	8.58 ± 0.77	
Notes: HNSCC, Head and neck squamous cell carcinoma; T stage, size or direct extent of the primary tumor; N stage, degree of spread to regional lymph nodes; M stage, presence of distant metastasis; SD, standard deviation				

The prospective target genes of miR-221 in HNSCC

By using MiRWalk2.0[32] (<http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/>), and GEPIA(<http://gepia.cancer-pku.cn/index.html>) analysis, we retrieved the number of the differentially expressed genes to be 5311 and 467 respectively. Bioinformatics analysis showed a total of 117 overlap genes which were involved, as shown in Fig. 9 and Table 3. The enriched GO and KEGG pathway category overlapping genes with $p < 0.05$ are shown in Fig. 10, Table 4 and Table 5. For the cellular component, the identified target genes are mostly enriched in the actin cytoskeleton, sarcomere, contractile fiber part and the molecular function is mainly concerned with the aromatase activity, oxidoreductase activity, and cofactor binding. KEGG enrichment analysis showed that miR-221-3p plays a significant role in HNSCC through a variety of pathways, including the drug metabolism pathways of the cytochrome P450 signaling pathway. The protein-protein interaction (PPI) network of drug metabolism - cytochrome P450-related genes is shown in the Fig. 11.

Table 3
The promising target genes of miR-221-3p in HNSCC with TCGA and MiRWalk

Sources of genes	Genes
TCGA and MiRWalk	AADAC ACPP ADH1B ADH1C ALDH1A1 AMOT ANKRD35 ANXA1 AQP3 ASB5 ATP2A1
	BCAS1 C2orf54 C7 CA3 CAB39L CAPN14 CAPN5 CAPN6 CD24 CEACAM6 CEACAM7 CGNL1 CHRDL1 CLU COBL CP CRISP3 CRYM CXCL17 CXCR2 CYP2F1 CYP3A5 CYP4B1
	CYP4 × 1 DEPTOR EHF EIF1AY EMP1 ENDOU FAM189A2 FAM3D FMO2 FOXA1 FUT3 GABRP GATM GBP6 GCNT3 GGT6 GPD1 GPT2 GPX3 GSTA1 HLF HMGCS2 HPGD
	HSPB8 IL1RN KALRN KLHL41 KRT23 KRT78 LDB3 LYNX1 MANSC1 MAOB MAPT MB METTL7A MGLL MUC21 MUC4 MYL2 MYZAP NCCRP1 NDRG2 NFIX PADI1
	PADI2 PAX9 PCP4L1 PDK4 PEBP4 PI16 PLEKHA6 PPP1R1A PPP1R3C PSCA PTN RAET1E RNF222 RRAGD SCIN SELENBP1 SERPINB13 SFRP1 SH3BGRL2 SLC16A7 SLC5A1
	SMPX SORBS1 SORBS2 STEAP4 SYNPO2 THSD4 TMEM45B TNNI1 TPRG1 TRDN TTC9 UGT1A7 UPK1A VSIG10L XIRP2 ZBTB7C ZSCAN18

Notes: HNSCC: Head and neck squamous cell carcinoma; TCGA: The Cancer Genome Atlas.

Table 4
Predictive target genes of miR-221-3p with GO analysis

GO ID	GO term	Count (%)	Gene symbol	P-value
Cellular component				
GO:0015629	actin cytoskeleton	12	AMOT▯ANXA1▯ CGNL1▯COBL▯ KALRN▯MYL2▯ MYZAP▯SCIN▯ SORBS1▯ SORBS2▯ SYNPO2▯XIRP2	6.22E-06
GO:0030017	sarcomere	7	KLHL41▯LDB3▯ MYL2▯MYZAP▯ SORBS2▯ SYNPO2▯XIRP2	6.06E-05
GO:0044449	contractile fiber part	7	KLHL41▯LDB3▯ MYL2▯MYZAP▯ SORBS2▯ SYNPO2▯XIRP2	1.12E-04
GO:0030016	myofibril	7	KLHL41▯LDB3▯ MYL2▯MYZAP▯ SORBS2▯ SYNPO2▯XIRP2	1.32E-04
GO:0043292	contractile fiber	7	KLHL41▯LDB3▯ MYL2▯MYZAP▯ SORBS2▯ SYNPO2▯XIRP2	1.90E-04
GO:0030018	Z disc	5	LDB3▯MYZAP▯ SORBS2▯ SYNPO2▯XIRP2	3.44E-04
GO:0031674	I band	5	LDB3▯MYZAP▯ SORBS2▯ SYNPO2▯XIRP2	5.21E-04
GO:0033017	sarcoplasmic reticulum membrane	3	ATP2A1▯ KLHL41▯TRDN	1.11E-03
Molecular function				
GO:0070330	aromatase activity	4	CYP2F1▯ CYP3A5▯ CYP4B1▯CYP4 × 1	6.04E-06

Notes: GO: Gene Ontology

GO ID	GO term	Count (%)	Gene symbol	P-value
GO:0016712	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen, reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen	4	CYP2F1 CYP3A5 CYP4B1 CYP4 × 1	8.86E-06
GO:0048037	cofactor binding	12	ALDH1A1 CRYM CYP3A5 CYP4B1 CYP4 × 1 FMO2 GPD1 GPT2 HPGD MAOB MB STEAP4	9.13E-06
GO:0019825	oxygen binding	4	CYP2F1 CYP3A5 CYP4B1 MB	1.73E-05
GO:0050662	coenzyme binding	8	ALDH1A1 CRYM FMO2 GPD1 GPT2 HPGD MAOB STEAP4	1.30E-04
GO:0004497	monooxygenase activity	5	CYP2F1 CYP3A5 CYP4B1 CYP4 × 1 FMO2	1.46E-04
GO:0051393	alpha-actinin binding	3	LDB3 SYNPO2 XIRP2	2.37E-04
GO:0020037	heme binding	5	CYP3A5 CYP4B1 CYP4 × 1 MB STEAP4	3.88E-04
GO:0046906	tetrapyrrole binding	5	CYP3A5 CYP4B1 CYP4 × 1 MB STEAP4	5.87E-04
GO:0042805	actinin binding	3	LDB3 SYNPO2 XIRP2	6.30E-04
GO:0016705	oxidoreductase activity, acting on paired donors, with incorporation or reduction of molecular oxygen	5	CYP2F1 CYP3A5 CYP4B1 CYP4 × 1 FMO2	1.24E-03
GO:0033130	acetylcholine receptor binding	2	LYNX1 PSCA	1.47E-03

Notes: GO: Gene Ontology

GO ID	GO term	Count (%)	Gene symbol	<i>p</i> -value
GO:0016722	oxidoreductase activity, oxidizing metal ions	2	CPSTEAP4	2.14E-03
GO:0030674	protein binding, bridging	5	MAPT SORBS1 SORBS2 SYNPO2 TRDN	2.20E-03
Notes: GO: Gene Ontology				

Table 5
KEGG pathway of validated target genes of miR-221-3p

KEGG ID	KEGG term	Count (%)	Gene symbol	<i>p</i> -value
hsa00982	Drug metabolism - cytochrome P450	7	GSTA1, UGT1A7, CYP3A5, FM02, MAOB, ADH1C, ADH1B	3.18E-07
hsa00980	Metabolism of xenobiotics by cytochrome P450	6	GSTA1, UGT1A7, CYP3A5, CYP2F1, ADH1C, ADH1B	8.10E-06
hsa00830	Retinol metabolism	5	UGT1A7, ALDH1A1, CYP3A5, ADH1C, ADH1B	6.43E-05
hsa05204	Chemical carcinogenesis	5	GSTA1, UGT1A7, CYP3A5, ADH1C, ADH1B	1.69E-04
hsa00350	Tyrosine metabolism	3	MAOB, ADH1C, ADH1B	1.54E-03
Notes: KEGG: Kyoto Encyclopedia of Genes and Genomes				

Validation of the miR-221-3p target genes in the drug metabolism - cytochrome P450 signaling pathway

Seven target genes associated with miR-221-3p are also involved in the drug metabolism - cytochrome P450 pathway and are down-regulated. The expression value of each of the target genes are shown in Fig. 12. The Spearman's correlation analysis showed that the correlation of the certified target genes with miR-221-3p in HNSCC (Fig. 13).

Discussion

The Drug metabolism - cytochrome P450 signaling pathway is an essential signal transduction pathway in cells. It is an important biological function of cell survival, proliferation and apoptosis. For example, Liping Wang and his colleagues suggest[33] that inhibition of CYP3A5 in the cytochrome P450 Drug metabolism can drive the migration, proliferation, and invasion of HNSCC cells. Dongfang Wang.et al

show that the cytochrome P450 is correlated with the overall survival and vascular invasion of Hepatocellular carcinoma (HCC) patients. Additionally, inhibiting the P450 pathway on drug metabolism - cytochrome can increase the efficacy of the HNSCC[34]. In the target genes of mir-221-3p, MAOB and UGT1A7 is associated with the mir-221-3p and drug-cytochrome P450 signaling pathways, are down-regulated in the HNSCC($P < 0.05$). MAOB is regarded as novel biomarkers for accurate Prostate cancer diagnosis and treatment[35]. In addition, a large number of valuable epidemiological studies have shown that UGT1A7 affects individuals' susceptibility to various cancers, such as pancreatic cancer[36],gastrointestinal carcinomas[37].Now our study suggests that miR-221-3p may play an important role

as a gene that promotes the development of HNSCC, by reducing the expression of MAOB and UGT1A7 pathways in HNSCC.Of course, our study has some limitations. The target gene of mir-221-3p have not been experimentally confirmed and we believe that our conclusions will need to be confirmed by clinical or molecular biological methods in the nearly future.

Understanding the pathogenesis of HNSCC and identifying new gene therapy programs are the focus of recent studies. Many recent studies have reported that the miRNAs with different expression patterns in different tumors[38–42], a control the progression of the tumors. For example, miR-221-3p has be regarded as a tumor biomarker that can be used to assess the clinical prognosis of breast cancer[15]. In healthy humans, it has been shown that miR-221-3p plays a role in the process of vascular proliferation, while the tumor promoter, miR-221-3p regulates the apoptosis of tumor cells. Now, the current study focused on the expression pattern of miR-221-3p in HNSCC, identifying the exact target gene of this miRNA and its biological mechanism of action.

In our study, we found that miR-221-3p levels were apparently up-regulated in HNSCC compared to normal tissues, also provided insights into its potential molecular mechanisms in driving cancer progression. MAOB and UGT1A7 are potentially important targets of miR-221-3p. MiR-221-3p may can be used as a non-invasive and hypersensitive biomarker in the diagnosis and is an extremely important gene locus involved in the process of the deterioration and eventual tumorigenesis of HNSCC.

Abbreviations

HNSCC: Head and neck squamous cell carcinoma; miR: microRNA; PPI: protein-protein interaction; GO: Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GEO: Gene Expression Omnibus; TCGA: The cancer genome atlas; DAVID: Database for Annotation, Visualization and Integrated Discovery; BiNGO: Biological Networks Gene Oncology tool; BP: biological process; CC: cellular component; MF: molecular function.

Declarations

Ethics approval and consent to participate

All procedures were approved by the Ethics Committee of Guangxi Medical University (Nanning, China). Written informed consent was obtained from all patients or their families prior to enrolment in the present study.

Consent for publication

Not applicable.

Competing interests

The authors declared that they have no competing interests.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 81460460,81760542), The Research Foundation of the Science and Technology Department of Guangxi Province, China (grant No. 2016GXNSFAA380252, 2018AB61001 and 2014GXNSFBA118114), the Research Foundation of the Health Department of Guangxi Province, China (No. S2018087). The funding body had no role in the design of the study and collection, analysis, and interpretation of data and in writing this manuscript.

Authors' contributions

MK designed the study and accessed the relevant information, and ZZ and LWW collected and analyzed the data. XJL, XZX and CL were involved in statistical analysis. QQL, JMS, XRQ and SS critically revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

References

1. Avissar M, Christensen BC, Kelsey KT, Marsit CJ. MicroRNA expression ratio is predictive of head and neck squamous cell carcinoma. *Clin Cancer Res*. 2009;15(8):2850–5.
2. Allen B, Schneider A, Victoria B, Nunez Lopez YO, Muller M, Szewczyk M, Pazdrowski J, Majchrzak E, Barczak W, Golusinski W, et al. Blood Serum From Head and Neck Squamous Cell Carcinoma Patients Induces Altered MicroRNA and Target Gene Expression Profile in Treated Cells. *Frontiers in oncology*. 2018;8(undefined):217.

3. Peitzsch C, Nathansen J, Schniewind SI, Schwarz F, Dubrovskaya A. Cancer Stem Cells in Head and Neck Squamous Cell Carcinoma: Identification, Characterization and Clinical Implications. *Cancers*. 2019;11(5):616.
4. Ausoni S, Boscolo-Rizzo P, Singh B, Da Mosto MC, Spinato G, Tirelli G, Spinato R, Azzarello G. Targeting cellular and molecular drivers of head and neck squamous cell carcinoma: current options and emerging perspectives. *Cancer Metastasis Rev*. 2016;35(3):413–26.
5. Britt EL, Raman S, Leek K, Sheehy CH, Kim SW, Harada H. Combination of fenretinide and ABT-263 induces apoptosis through NOXA for head and neck squamous cell carcinoma treatment. *PLoS One*. 2019;14(7):e0219398.
6. Koole K, Clausen MJ, van Es RJ, van Kempen PM, Melchers LJ, Koole R, Langendijk JA, van Diest PJ, Roodenburg JL, Schuurin E, et al. FGFR Family Members Protein Expression as Prognostic Markers in Oral Cavity and Oropharyngeal Squamous Cell Carcinoma. *Molecular diagnosis therapy*. 2016;20(4):363–74.
7. Tang XX, He J, Li B, Zheng Y, Li KJ, Zou S, Chen L. Efficacy and Safety of Gefitinib in Patients with Advanced Head and Neck Squamous Cell Carcinoma: A Meta-Analysis of Randomized Controlled Trials. *Journal of oncology* 2019, 2019(undefin):6273438.
8. Cao W, Liu J, Xia R, Lin L, Wang X, Xiao M, Zhang C, Li J, Ji T, Chen W. X-linked FHL1 as a novel therapeutic target for head and neck squamous cell carcinoma. *Oncotarget*. 2016;7(12):14537–50.
9. Ahmad P, Sana J, Slavik M, Slampa P, Smilek P, Slaby O. MicroRNAs Involvement in Radioresistance of Head and Neck Cancer. *Dis Markers*. 2017;2017:8245345.
10. Orosz E, Gombos K, Riedling T, Afiakurue P, Kiss I, Pytel J, Gerlinger I, Szanyi I. Comparative miRNA Expression Profile Analysis of Squamous Cell Carcinoma and Peritumoral Mucosa from the Meso- and Hypopharynx. *Cancer Genomics Proteomics*. 2017;14(4):285–92.
11. Qureshi R, Sacan A. A novel method for the normalization of microRNA RT-PCR data. *BMC Med Genom*. 2013;6(Suppl 1):14.
12. Irani S. miRNAs Signature in Head and Neck Squamous Cell Carcinoma Metastasis: A Literature Review. *Journal of dentistry*. 2016;17(2):13.
13. Wang H, Tang Y, Yang D, Zheng L. MicroRNA-591 Functions as a Tumor Suppressor in Hepatocellular Carcinoma by Lowering Drug Resistance through Inhibition of Far-Upstream Element-Binding Protein 2-Mediated Phosphoinositide 3-Kinase/Akt/Mammalian Target of Rapamycin Axis. *Pharmacology* 2019:1–14.
14. Hussein S, Mosaad H, Rashed HE, El-Anwar MW. Up-regulated miR-221 expression as a molecular diagnostic marker in laryngeal squamous cell carcinoma and its correlation with Apaf-1 expression. *Cancer Biomark*. 2017;19(3):279–87.
15. Zhou L, Jiang F, Chen X, Liu Z, Ouyang Y, Zhao W, Yu D. Downregulation of miR-221/222 by a microRNA sponge promotes apoptosis in oral squamous cell carcinoma cells through upregulation of PTEN. *Oncol Lett*. 2016;12(6):4419–26.

16. Deng X, Ma L, Wu M, Zhang G, Jin C, Guo Y, Liu R. miR-124 radiosensitizes human glioma cells by targeting CDK4. *J Neurooncol.* 2013;114(3):263–74.
17. Rippe C, Blimline M, Magerko KA, Lawson BR, LaRocca TJ, Donato AJ, Seals DR. MicroRNA changes in human arterial endothelial cells with senescence: relation to apoptosis, eNOS and inflammation. *Exp Gerontol.* 2012;47(1):45–51.
18. Gillies JK, Lorimer IA. Regulation of p27Kip1 by miRNA 221/222 in glioblastoma. *Cell Cycle.* 2007;6(16):2005–9.
19. Felicetti F, Errico MC, Bottero L, Segnalini P, Stoppacciaro A, Biffoni M, Felli N, Mattia G, Petrini M, Colombo MP, et al. The promyelocytic leukemia zinc finger-microRNA-221/-222 pathway controls melanoma progression through multiple oncogenic mechanisms. *Cancer Res.* 2008;68(8):2745–54.
20. Ambs S, Prueitt RL, Yi M, Hudson RS, Howe TM, Petrocca F, Wallace TA, Liu CG, Volinia S, Calin GA, et al. Genomic profiling of microRNA and messenger RNA reveals deregulated microRNA expression in prostate cancer. *Cancer Res.* 2008;68(15):6162–70.
21. Li J, Wang Y, Yu W, Chen J, Luo J. Expression of serum miR-221 in human hepatocellular carcinoma and its prognostic significance. *Biochem Biophys Res Commun.* 2011;406(1):70–3.
22. Ergun S, Arman K, Temiz E, Bozgeyik I, Yumrutas O, Safdar M, Dagli H, Arslan A, Oztuzcu S. Expression patterns of miR-221 and its target Caspase-3 in different cancer cell lines. *Mol Biol Rep.* 2014;41(9):5877–81.
23. Wald AI, Hoskins EE, Wells SI, Ferris RL, Khan SA. Alteration of microRNA profiles in squamous cell carcinoma of the head and neck cell lines by human papillomavirus. *Head Neck.* 2011;33(4):504–12.
24. Yang CJ, Shen WG, Liu CJ, Chen YW, Lu HH, Tsai MM, Lin SC. miR-221 and miR-222 expression increased the growth and tumorigenesis of oral carcinoma cells. *J Oral Pathol Med.* 2011;40(7):560–6.
25. Liu CJ, Kao SY, Tu HF, Tsai MM, Chang KW, Lin SC. Increase of microRNA miR-31 level in plasma could be a potential marker of oral cancer. *Oral Dis.* 2010;16(4):360–4.
26. Wong TS, Liu XB, Wong BY, Ng RW, Yuen AP, Wei WI. Mature miR-184 as Potential Oncogenic microRNA of Squamous Cell Carcinoma of Tongue. *Clin Cancer Res.* 2008;14(9):2588–92.
27. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, et al. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium Nature genetics. 2000;25(1):25–9.
28. Kanehisa M, Goto S, Sato Y, Furumichi M, Tanabe M. KEGG for integration and interpretation of large-scale molecular data sets. *Nucleic acids research.* 2012;40(Database issue):D109–14.
29. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, Varambally S. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. *Neoplasia (New York NY).* 2017;19(8):649–58.
30. Vasaikar SV, Straub P, Wang J, Zhang B. LinkedOmics: analyzing multi-omics data within and across 32 cancer types. *Nucleic acids research.* 2018;46(D1):D956–d963.

31. Cheng Z, Xing D. Ginsenoside Rg3 inhibits growth and epithelial-mesenchymal transition of human oral squamous carcinoma cells by down-regulating miR-221. *Eur J Pharmacol.* 2019;853:353–63.
32. Dweep H, Gretz N. miRWalk2.0: a comprehensive atlas of microRNA-target interactions. *Nat Methods.* 2015;12(8):697.
33. Wang LP, Chen WH, Zha J, Yan YY, Wei YX, Chen XL, Zhu XX, Ge LH. miR543 acts as a novel oncogene in oral squamous cell carcinoma by targeting CYP3A5. *Oncol Rep.* 2019;42(3):973–90.
34. Ruwali M, Dhawan A, Pant MC, Rahman Q, Khurana SM, Parmar D. Clinical Management of Head and Neck Cancer Cases: Role of Pharmacogenetics of CYP2 and GSTs. *Oncol Res Treat.* 2016;39(4):221–6.
35. Xu N, Wu Y-P, Ke Z-B, Liang Y-C, Cai H, Su W-T, Tao X, Chen S-H, Zheng Q-S, Wei Y, et al: Identification of key DNA methylation-driven genes in prostate adenocarcinoma: an integrative analysis of TCGA methylation data. *Journal of Translational Medicine* 2019, 17(1).
36. Yilmaz L, Borazan E, Aytekin T, Baskonus I, Aytekin A, Oztuzcu S, Bozdog Z, Balik A. Increased UGT1A3 and UGT1A7 Expression is Associated with Pancreatic Cancer. *Asian Pac J Cancer Prev.* 2015;16(4):1651–5.
37. Cengiz B, Yumrutas O, Bozgeyik E, Borazan E, Igci YZ, Bozgeyik I, Oztuzcu S. Differential expression of the UGT1A family of genes in stomach cancer tissues. *Tumor Biology.* 2015;36(8):5831–7.
38. Chen W, Fu W, Deng Q, Li Y, Wang K, Bai Y, Wu X, Li G, Wang G, Huang J, et al. Multiple metals exposure and chromosome damage: Exploring the mediation effects of microRNAs and their potentials in lung carcinogenesis. *Environ Int.* 2019;122:291–300.
39. Dong XT, Liu YD. Expression and significance of miR-24 and miR-101 in patients with advanced gastric cancer. *Oncol Lett.* 2018;16(5):5769–74.
40. Jin FF, Yang R, Wei Y, Wang D, Zhu YN, Wang XH, Lu YS, Wang YB, Zen K, Li LM. HIF-1 α -induced miR-23a approximately 27a approximately 24 cluster promotes colorectal cancer progression via reprogramming metabolism. *Cancer Lett.* 2019;440–441:211–22.
41. Jing PY, Zhao N, Xie NL, Ye MX, Zhang Y, Zhang ZP, Li MY, Lai XF, Zhang J, Gu ZP: miR-24-3p/FGFR3 Signaling as a Novel Axis Is Involved in Epithelial-Mesenchymal Transition and Regulates Lung Adenocarcinoma Progression. *J Immunol Res* 2018, 2018:2834109.
42. Lang B, Shang C, Meng LR. Targeted Silencing of S100A8 Gene by miR-24 to Increase Chemotherapy Sensitivity of Endometrial Carcinoma Cells to Paclitaxel. *Med Sci Monit.* 2016;22:1953–8.

Figures

Figure 1

MiR-221-3p levels in HNSCC and NC tissue specimens. RT-qPCR was used to detect miR-221-3p was significantly up-regulated in the tumor tissues of an HNSCC cohort ($n = 48$) relative to control tissues ($n = 21$) ($P < 0.01$).

Figure 2

The flow chart of literature search and selection of relevant studies. sixteen out of 424 GEO microarray datasets met our inclusion criteria. At the same time, a total of 17 articles were retrieved from the initial search, and one papers (Zhou Cheng. et al) were selected after the full-text review based on the inclusion criteria.

Figure 3

Bias analysis. (A) Funnel plots of published data bias for meta-analysis of miR-21. (B) Sensitivity analysis for meta-analysis of miR-221-3p.

Figure 4

Forest plots. The combined SMD of miR-221-3p expression levels in HNSCC samples was 0.717(95% CI :0.283, 1.152).

Figure 5

The expression data of miR-221-3p in HNSCC from TCGA, each included GEO and the literature.

Figure 6

The diagnostic value of miR-221-3p in HNSCC. (A) sensitivity; (B) specificity; (C) PLR; (D) NL; and (E) diagnostic odds ratio.

Figure 7

Receiver operating characteristic (ROC) curves showing the diagnostic value of miR-221-3p in HNSCC.

Figure 8

Summary ROC (SROC) curve showing the diagnostic performance of miR-221-3p in HNSCC. The sROC curve AUC was 0.86 (95% CI: 0.83-0.89), indicating high predictive power.

Figure 9

Venn diagram showing overlapping miR-221-3p target genes.

Figure 10

Enrichment analysis of GO and KEGG pathways of miR-221-3p target genes. A. Gene ontology (GO) of candidate genes; B. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

Figure 11

PPI network. The nodes represent target genes and the lines are associations between genes, which showing the connections among the 7 miR-221-3p targets associated with the Drug metabolism - cytochrome P450 pathway.

Figure 12

The expression levels of miR-221-3p target genes in HNSCC. A-G pictures represent the expression of the following genes: GSTA1, UGT1A7, CYP3A5, FM02, MAOB, ADH1C, ADH1B.

Figure 13

Spearman analysis. A-G show the association between miR-221-3p expression levels and the 7 identified target genes involved in the Drug metabolism - cytochrome P450 pathway.