

Bioinformatics analysis of the expression and role of microRNA-221-3p in the head and neck squamous cell carcinoma

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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 Real-time RT-Polymerase Chain Reaction (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.

1. 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[1,2,3]. 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 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[12,13,14]. 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[18,19,20] 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[14,15,24,25,26]. 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.

2. Methods And Methodology

2.1. 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 cycler (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 $2^{-\Delta\Delta C_t}$ 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.

2.2. 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 Wan fang 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)

2.3. 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.

2.4. 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.

2.5. 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.

2.6. 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.

2.7. 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 ($X \pm s$) 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 log₂ to improve the normality of the measurements. $P < 0.05$ is considered a statistically significant value.

2.8. 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 UALCAN[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.

3. Results

3.1. 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 ($p < 0.05$) (*Figure 1*).

3.2. 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 (*Figure 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*.

3.3. 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 (*Figure 3*).

3.4. 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 TCGA 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 miR-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) (*Figure 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 *Figure 5 (A-R)*.

3.5. 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 (*Figure 6*).. The ROC curve is shown in *Figure 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) (*Figure 8*)..

3.6. 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*)..

3.7. 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 *Figure 9* and *Table 3*. The enriched GO and KEGG pathway category overlapping genes with $p < 0.05$ are shown in *Figure 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 *figure 11*.

3.8. 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 *Figure 12*. The Spearman's correlation analysis showed that the correlation of the certified target genes with miR-221-3p in HNSCC (*Figure 13*).

4. 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[38,39,40,41,42], a control the progression of the tumors. For example, miR-221-3p has been 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.

5. Conclusions

In our study, we found that miR-221-3p levels were apparently up-regulated in HNSCC compared to normal tissues and had certain diagnostic value in HNSCC, also provided insights into its potential molecular mechanisms in driving cancer progression. MAOB and UGT1A7 are **potentially** important targets of miR-221-3p, which plays an important role in HNSCC through the drug metabolism - cytochrome P450 signaling pathway. In conclusion, miR-221-3p may can be used as a non-invasive 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; RT-qPCR:Real-time RT-Polymerase Chain Reaction; 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

Availability of data and materials

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

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.

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Authors' contributions

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

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Tables

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

Table 2. The correlation between miR-221-3p expression levels and clinic characteristics based on TCGA database

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

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

Clinicopathological features	Terms	n	Mean±SD	p-value
Unpaired tissue	Normal	44	8.16±0.64	0.001
	HNSCC	480	8.55±0.78	
Gender	Male	383	8.45±0.79	0.001
	Female	141	8.71±0.68	
Age	<60	234	8.50±0.76	0.559
	≥60	290	8.54±0.78	
lymphovascular invasion	NO	232	8.54±0.81	0.429
	YES	292	8.49±0.75	
Clinical stage	I-II	206	8.63±0.76	0.011
	III-IV	318	8.45±0.78	
Neoplasm histologic grade	G1-G2	371	8.49±0.73	0.260
	G3-G4	156	8.58±0.82	
T stage	T1-T2	188	8.61±0.73	0.029
	T3-T4	336	8.46±0.79	
M stage	M0	498	8.51±0.78	0.967
	M1	29	8.51±0.57	
N stage	N0	246	8.53±0.76	0.572
	N1-N3	281	8.49±0.77	
Alcohol	NO	164	8.62±0.76	0.047
	YES	363	8.48±0.76	
HPV status	Negative	194	8.41±0.81	0.066
	Positive	330	8.55±0.81	
Perineural invasion	NO	198	8.53±0.81	0.693
	YES	329	8.50±0.80	
Smoking	<3	298	8.47±0.76	0.094
	≥3	229	8.58±0.77	

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
	CYP4X1 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▯CYP4X1	6.04E-06
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▯CYP4X1	8.86E-06

GO:0048037	cofactor binding	12	ALDH1A1 CRYM CYP3A5 CYP4B1 CYP4X1 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 CYP4X1 FMO2	1.46E-04
GO:0051393	alpha-actinin binding	3	LDB3 SYNPO2 XIRP2	2.37E-04
GO:0020037	heme binding	5	CYP3A5 CYP4B1 CYP4X1 MB STEAP4	3.88E-04
GO:0046906	tetrapyrrole binding	5	CYP3A5 CYP4B1 CYP4X1 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 CYP4X1 FMO2	1.24E-03
GO:0033130	acetylcholine receptor binding	2	LYNX1 PSCA	1.47E-03
GO:0016722	oxidoreductase activity, oxidizing metal ions	2	CP STEAP4	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	p-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

Figures

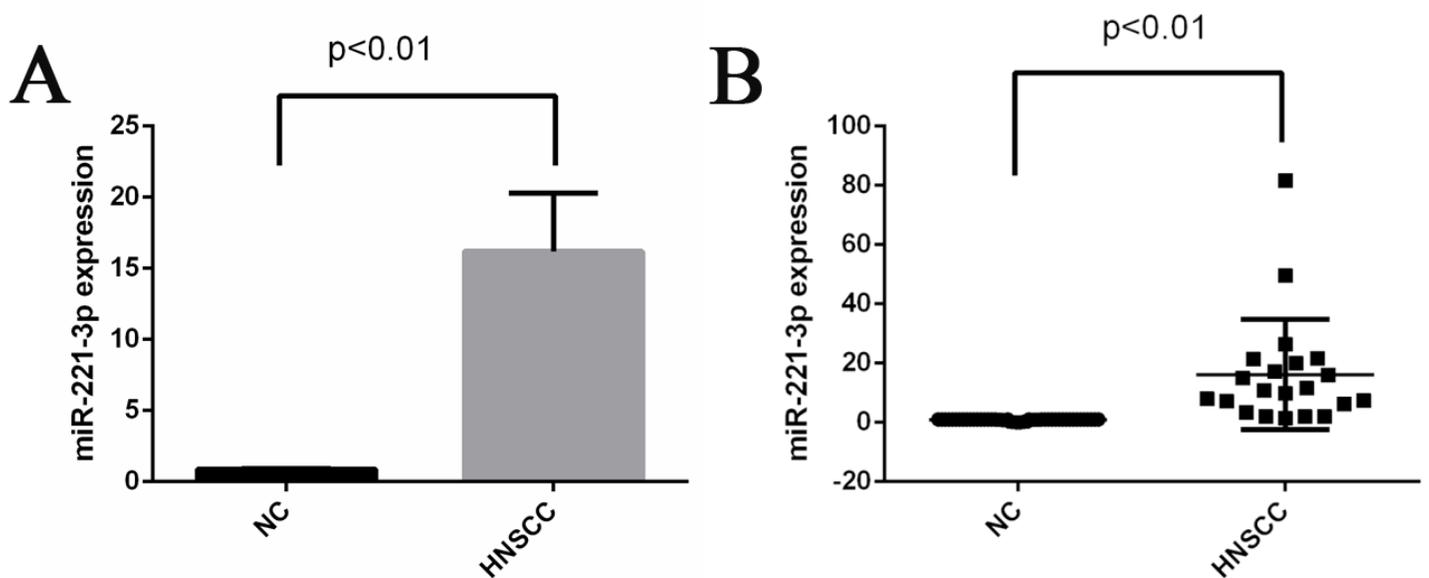


Figure 1

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).

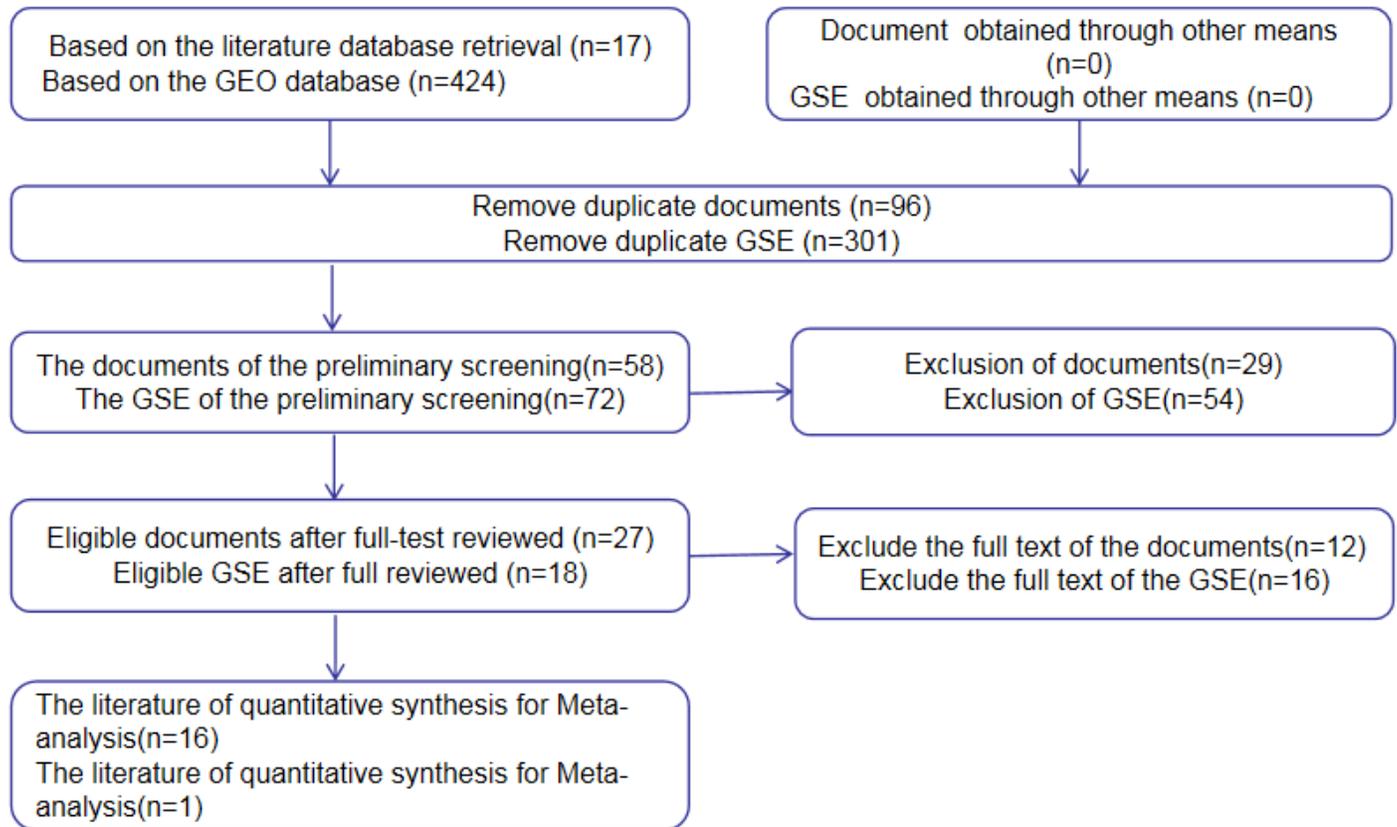


Figure 2

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.

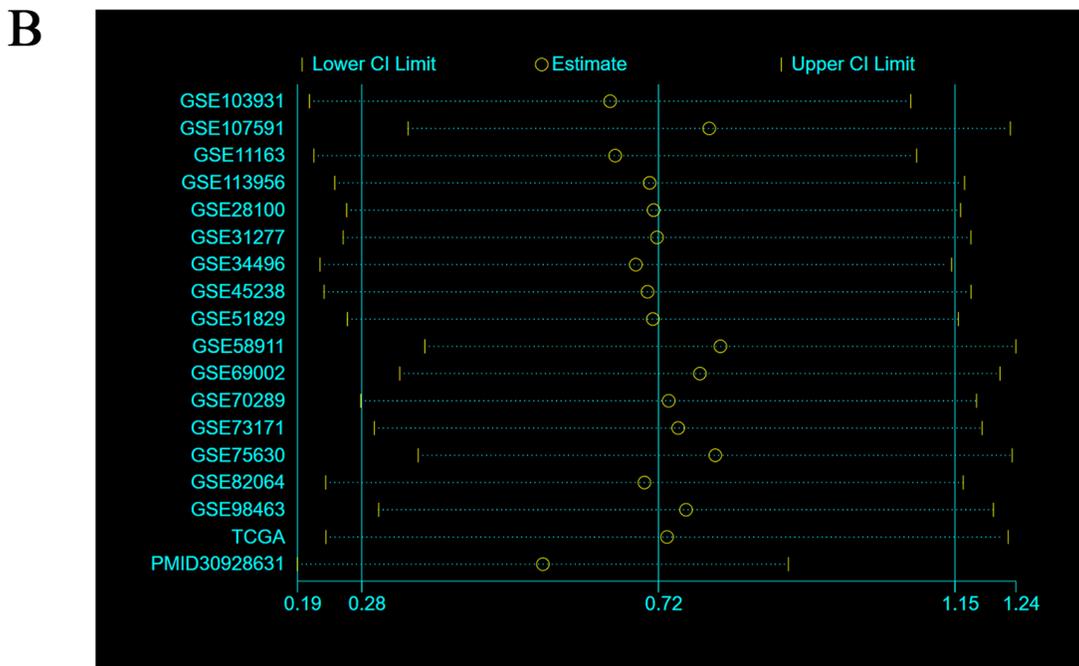
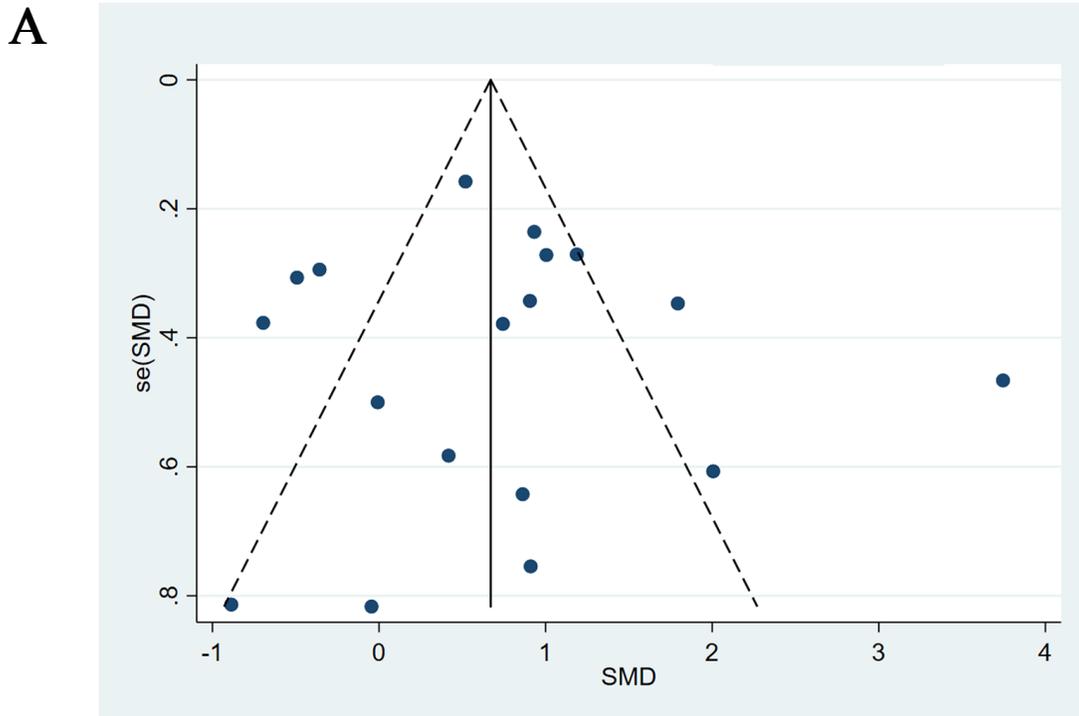


Figure 3

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.

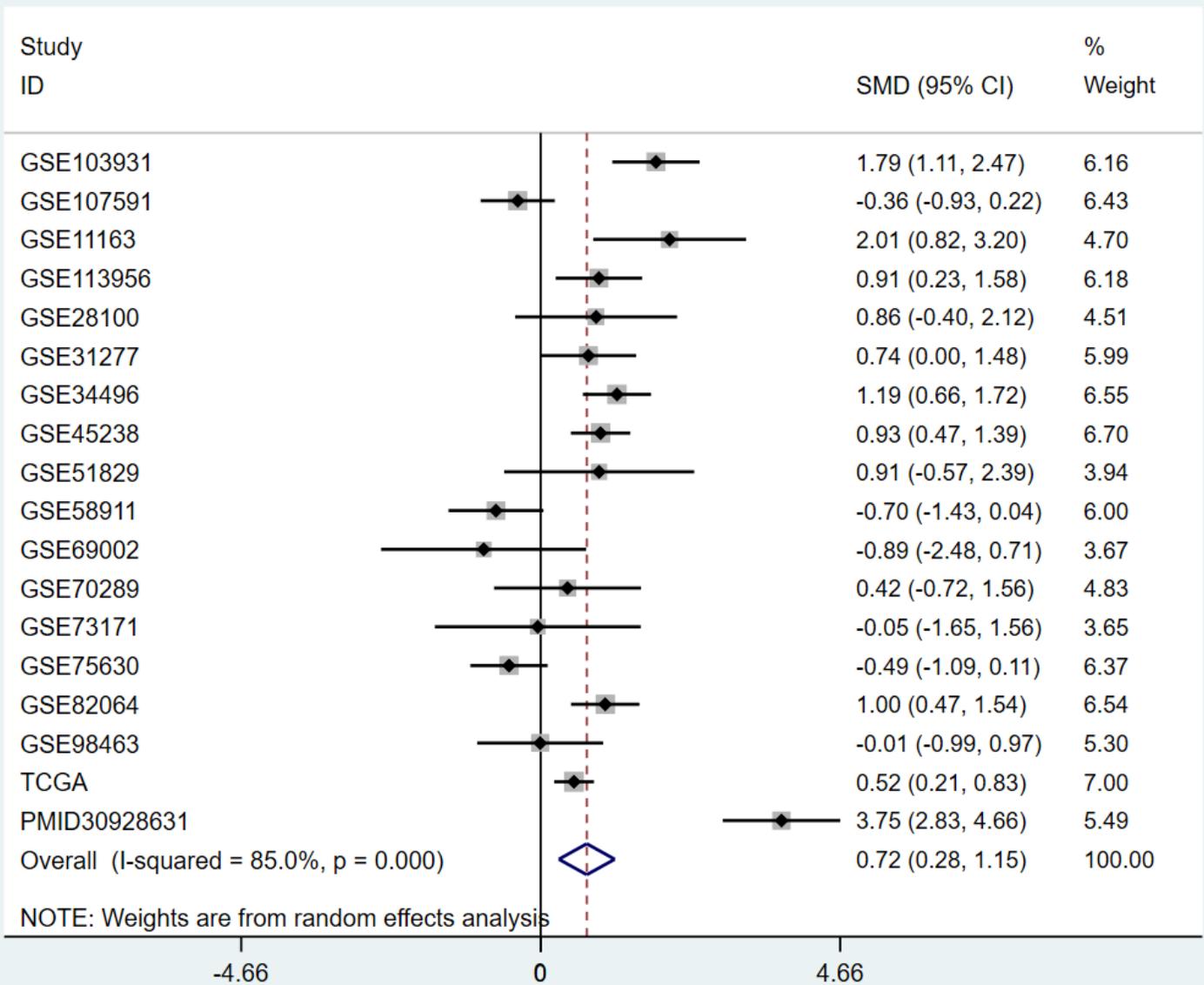


Figure 4

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

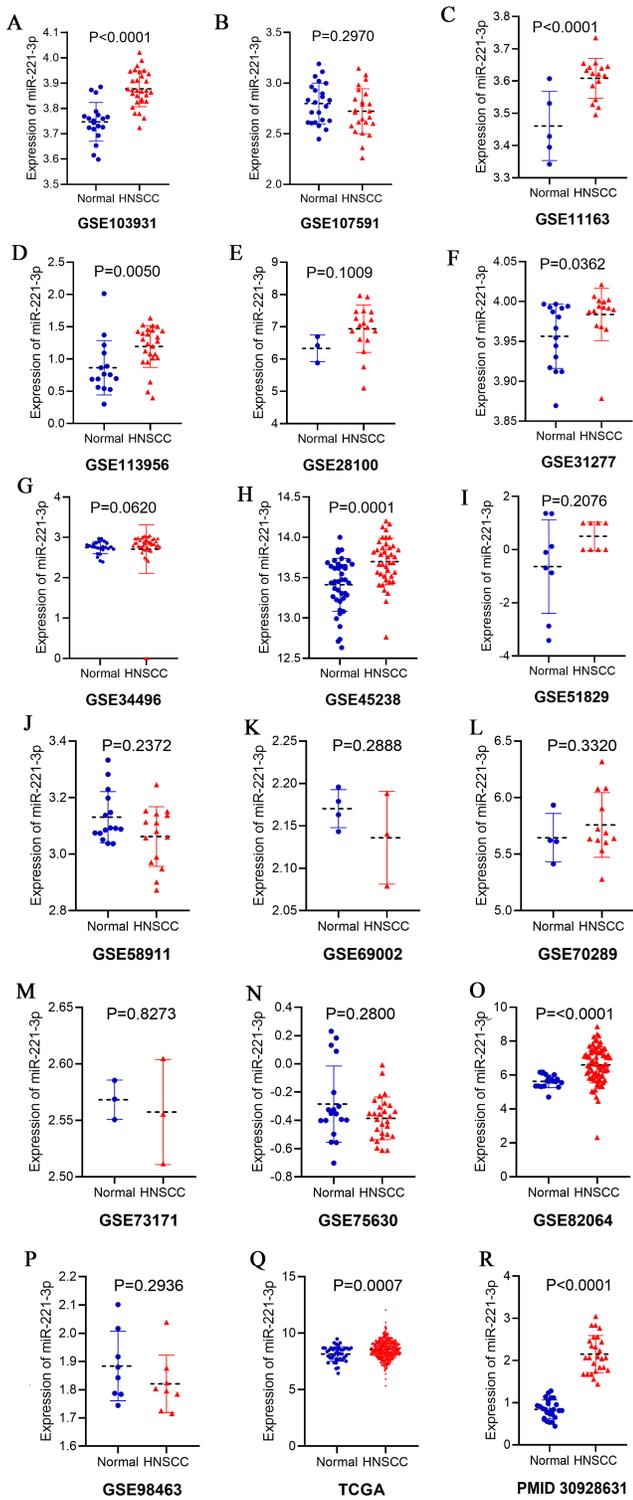


Figure 5

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

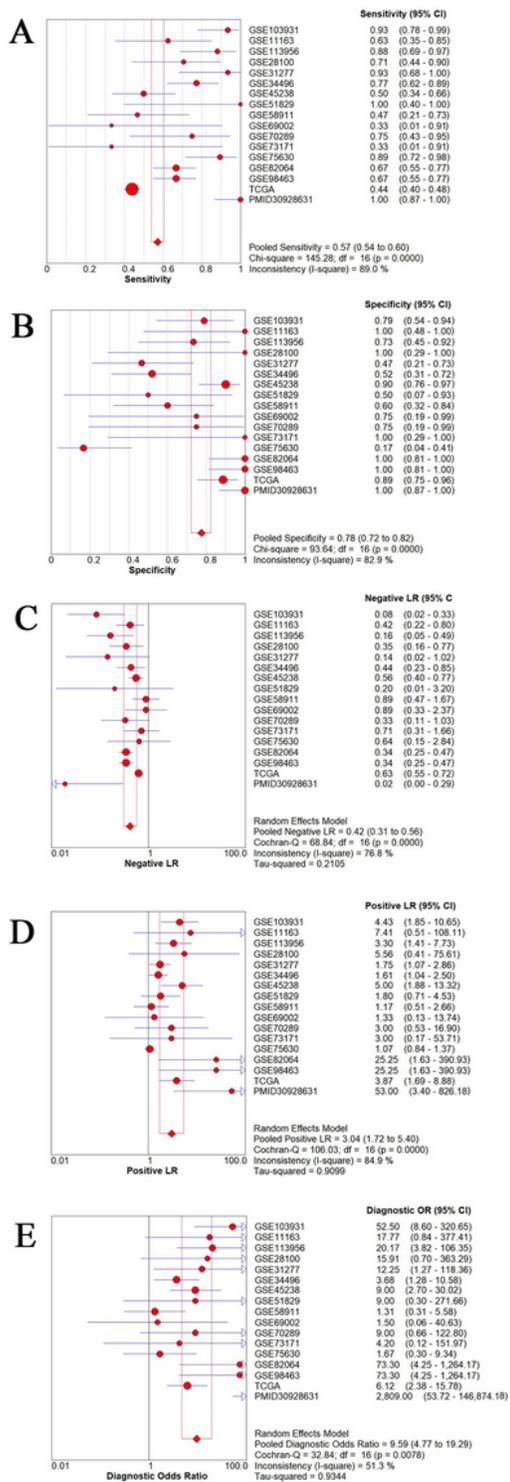


Figure 6

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

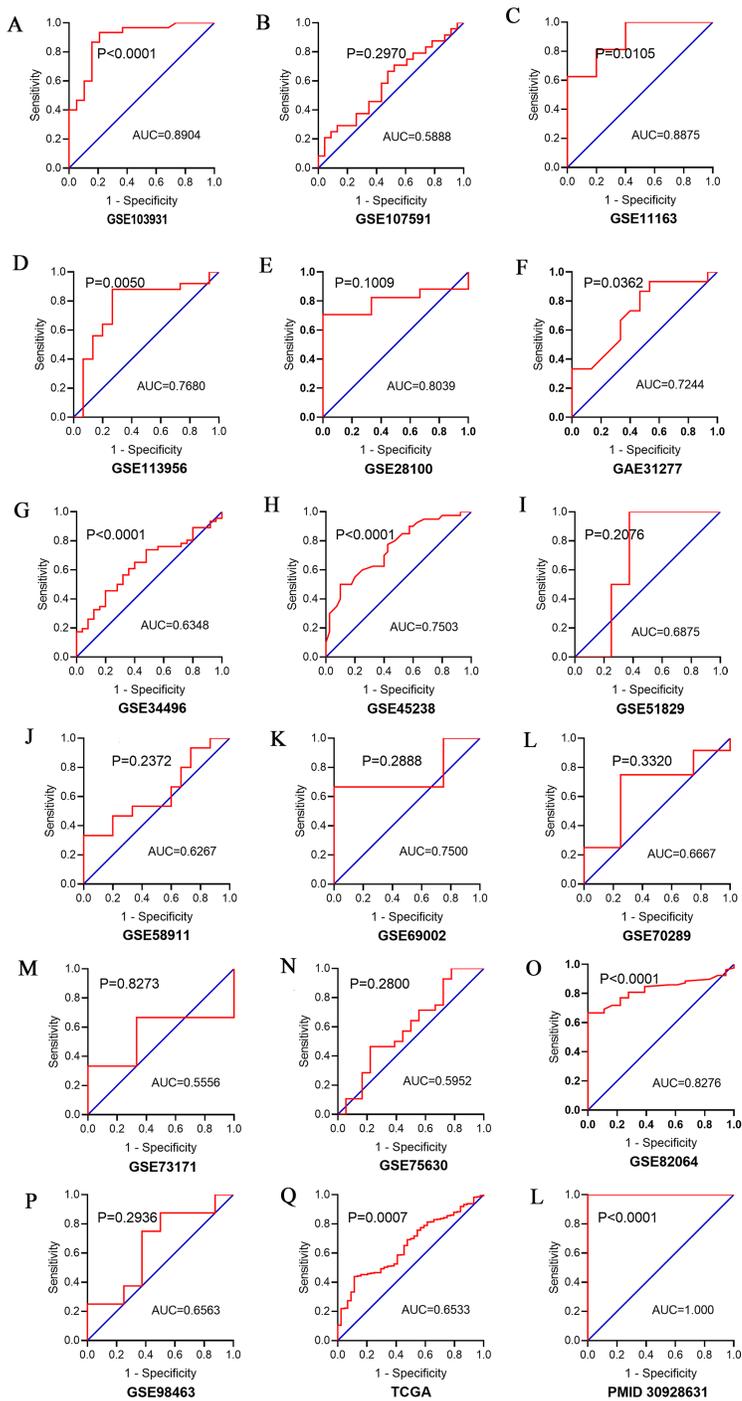


Figure 7

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

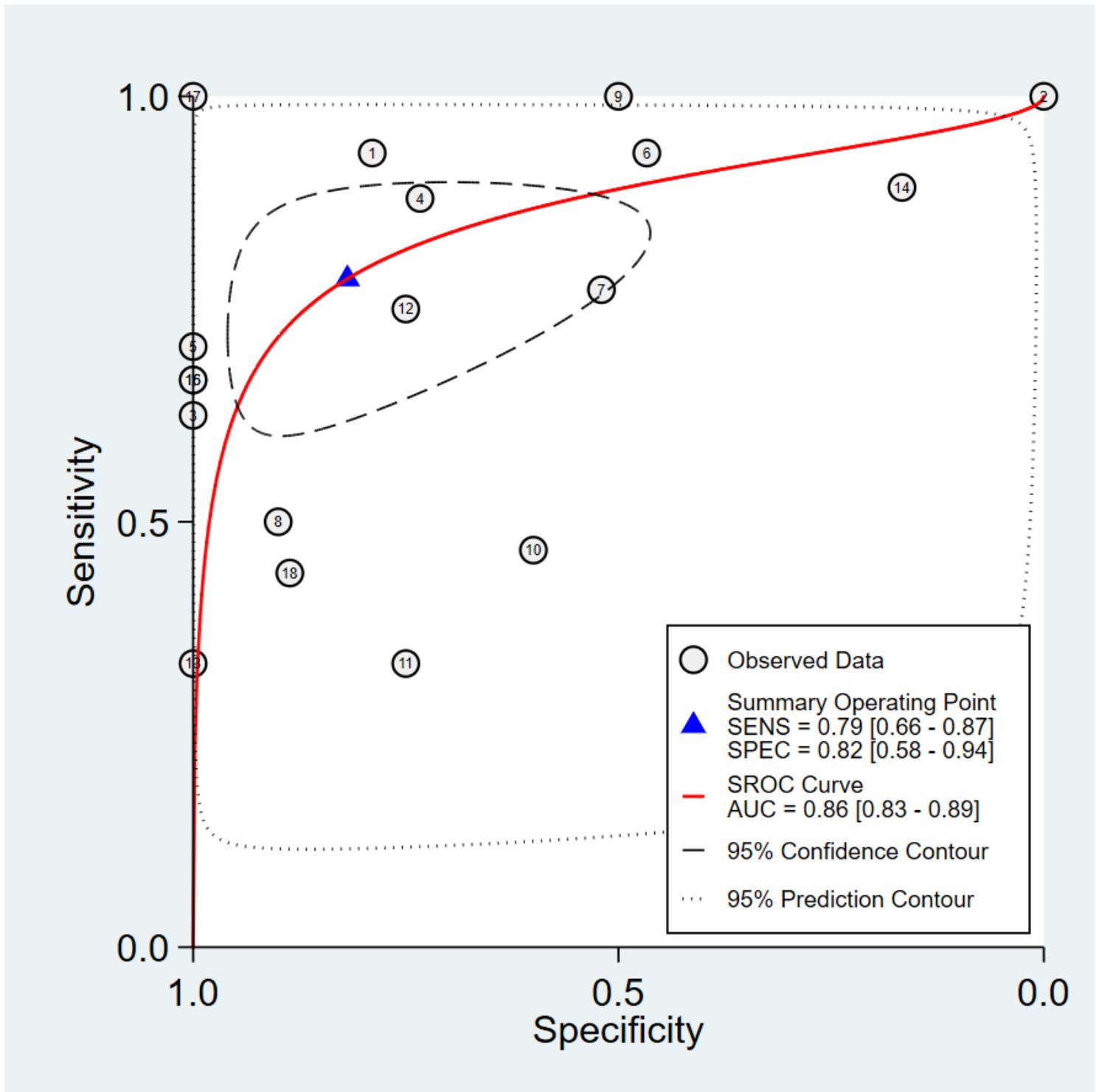


Figure 8

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.

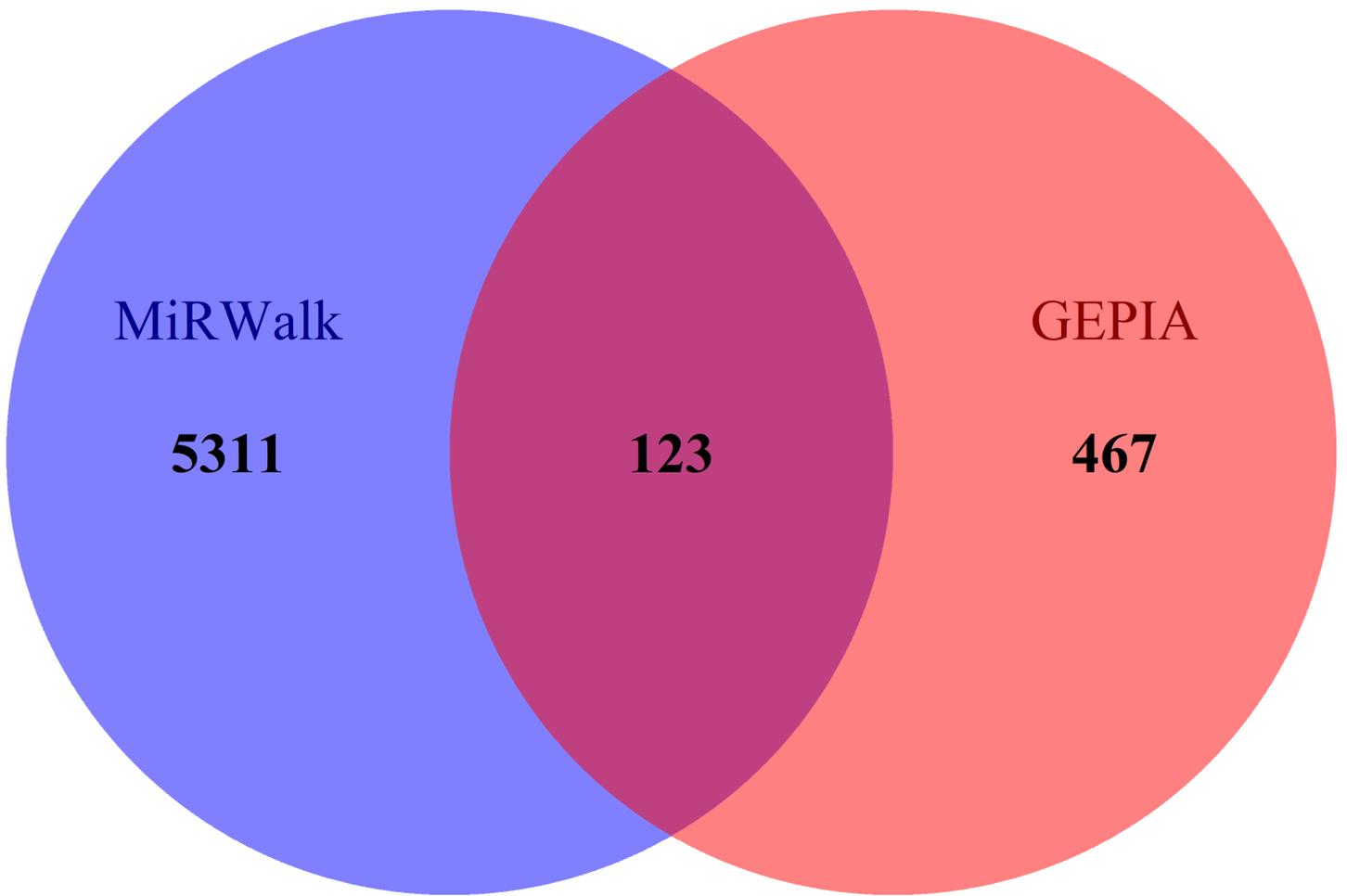
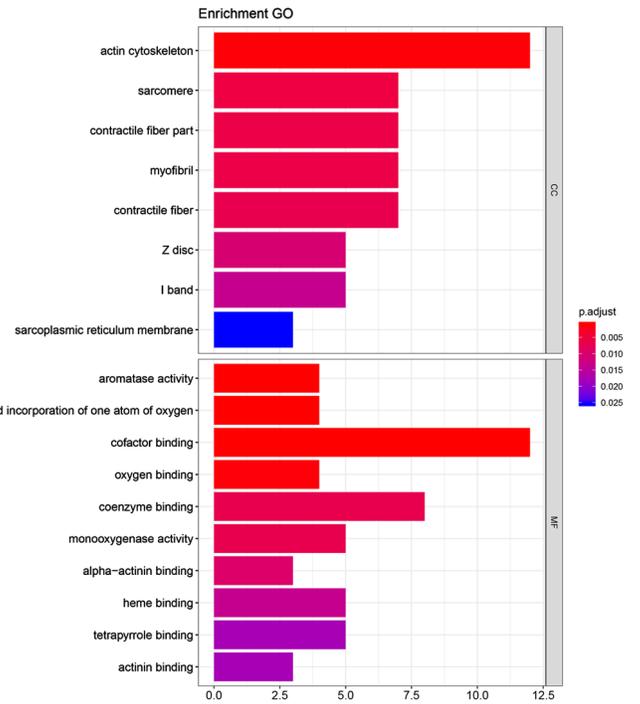


Figure 9

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

A

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



B

KEGG pathway

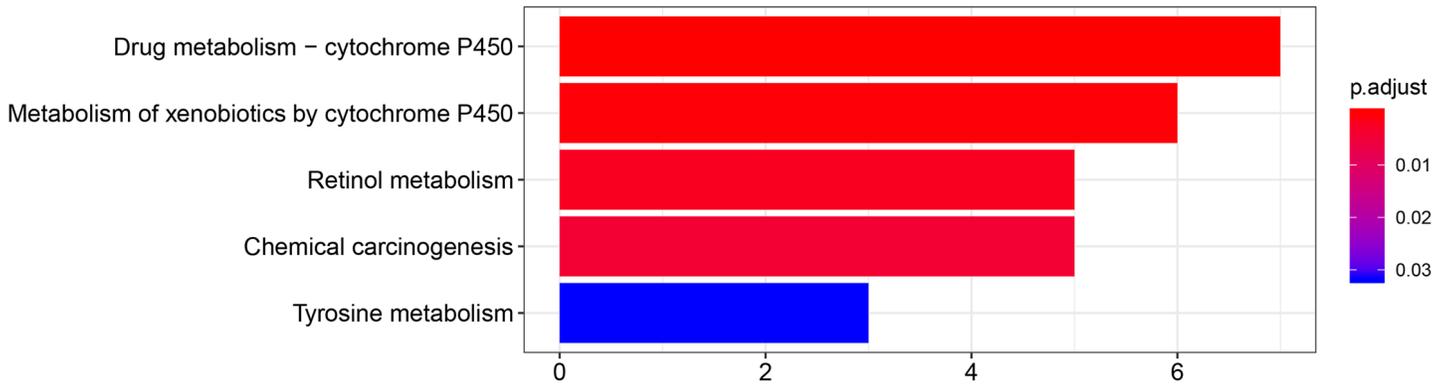


Figure 10

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.

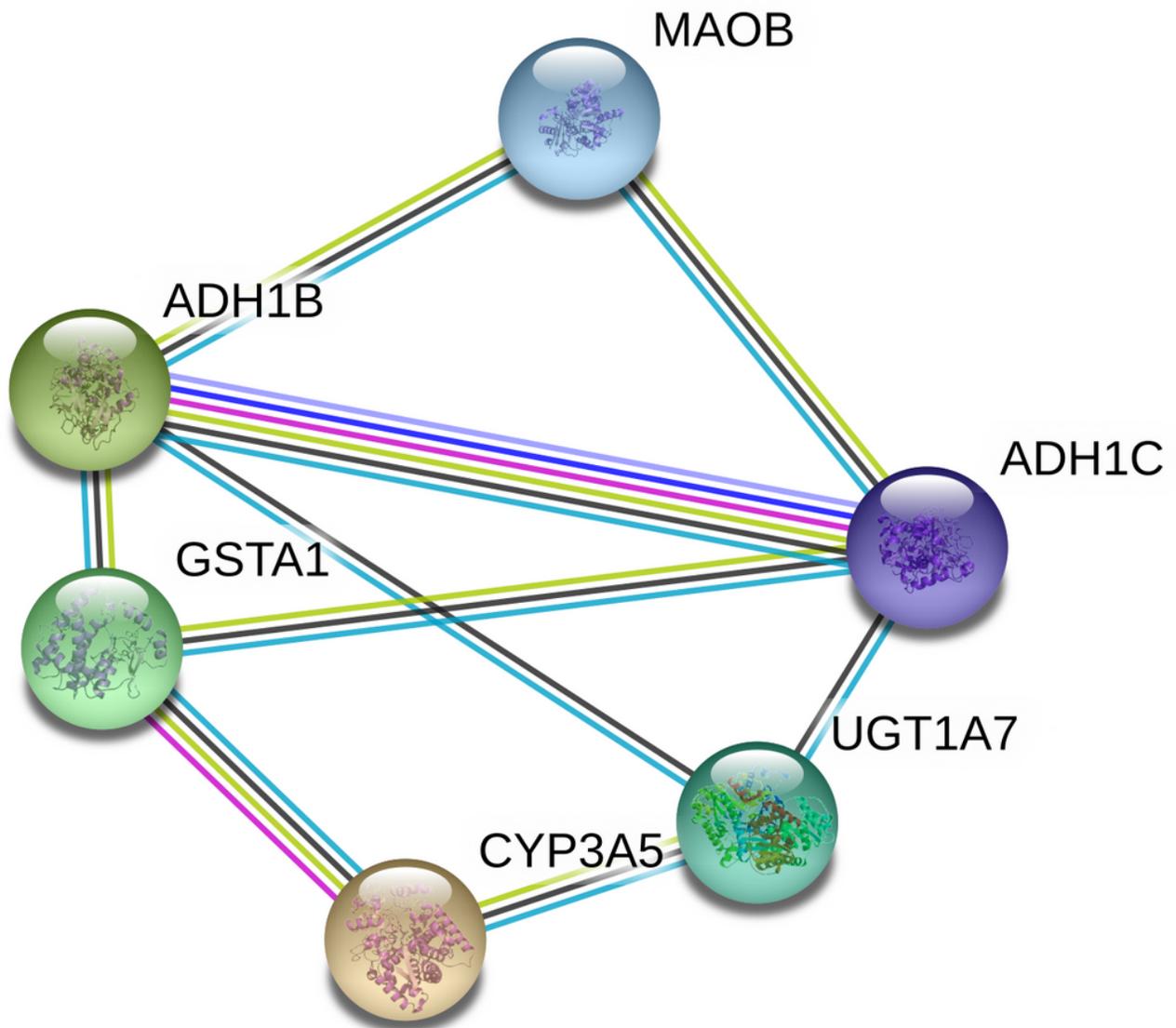


Figure 11

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.

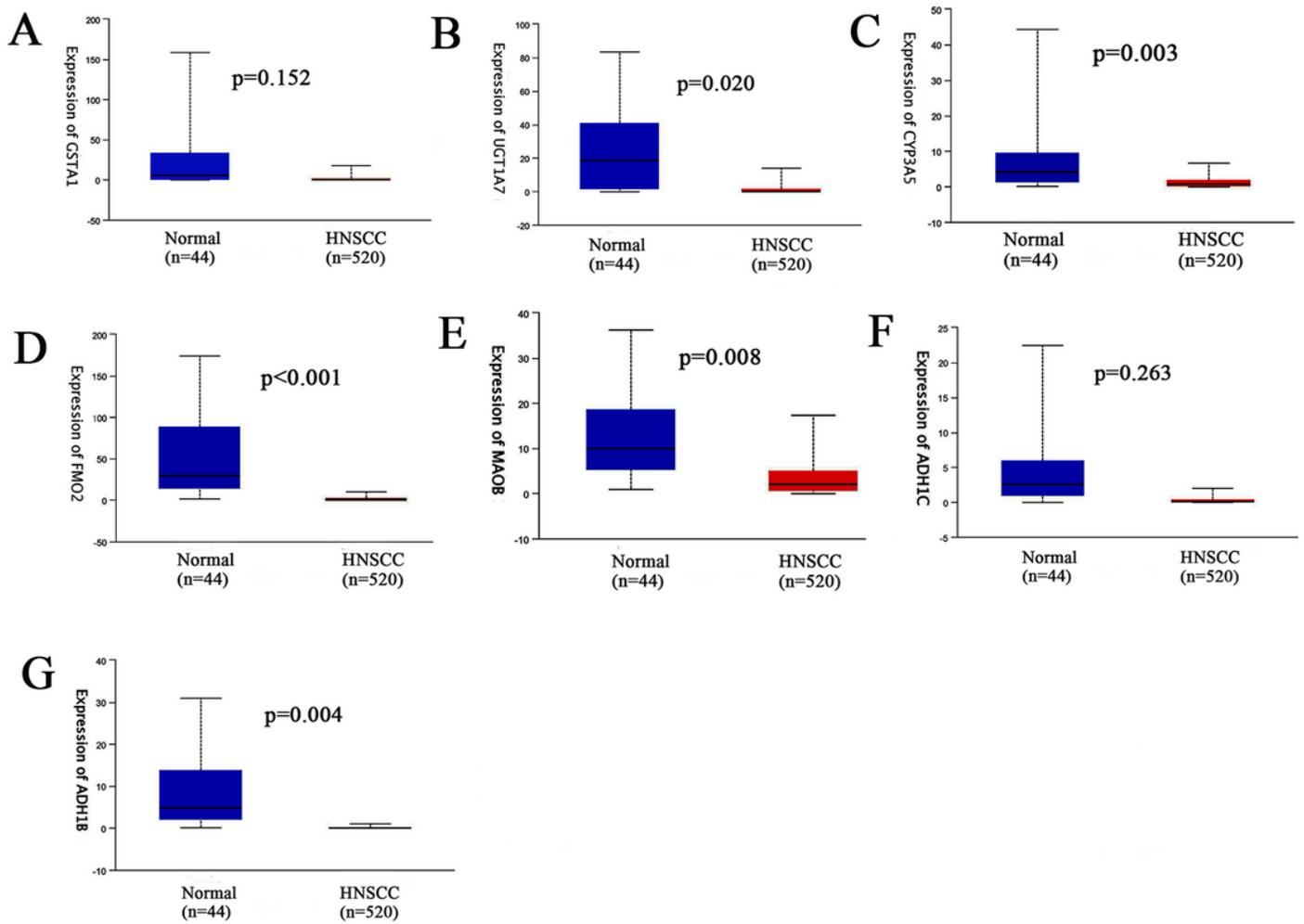


Figure 12

The expression levels of miR-221-3p target genes in HNSCC. A-G pictures represents the expression of the following genes: GSTA1, UGT1A7, CYP3A5, FMO2, MAOB, ADH1C, ADH1B.

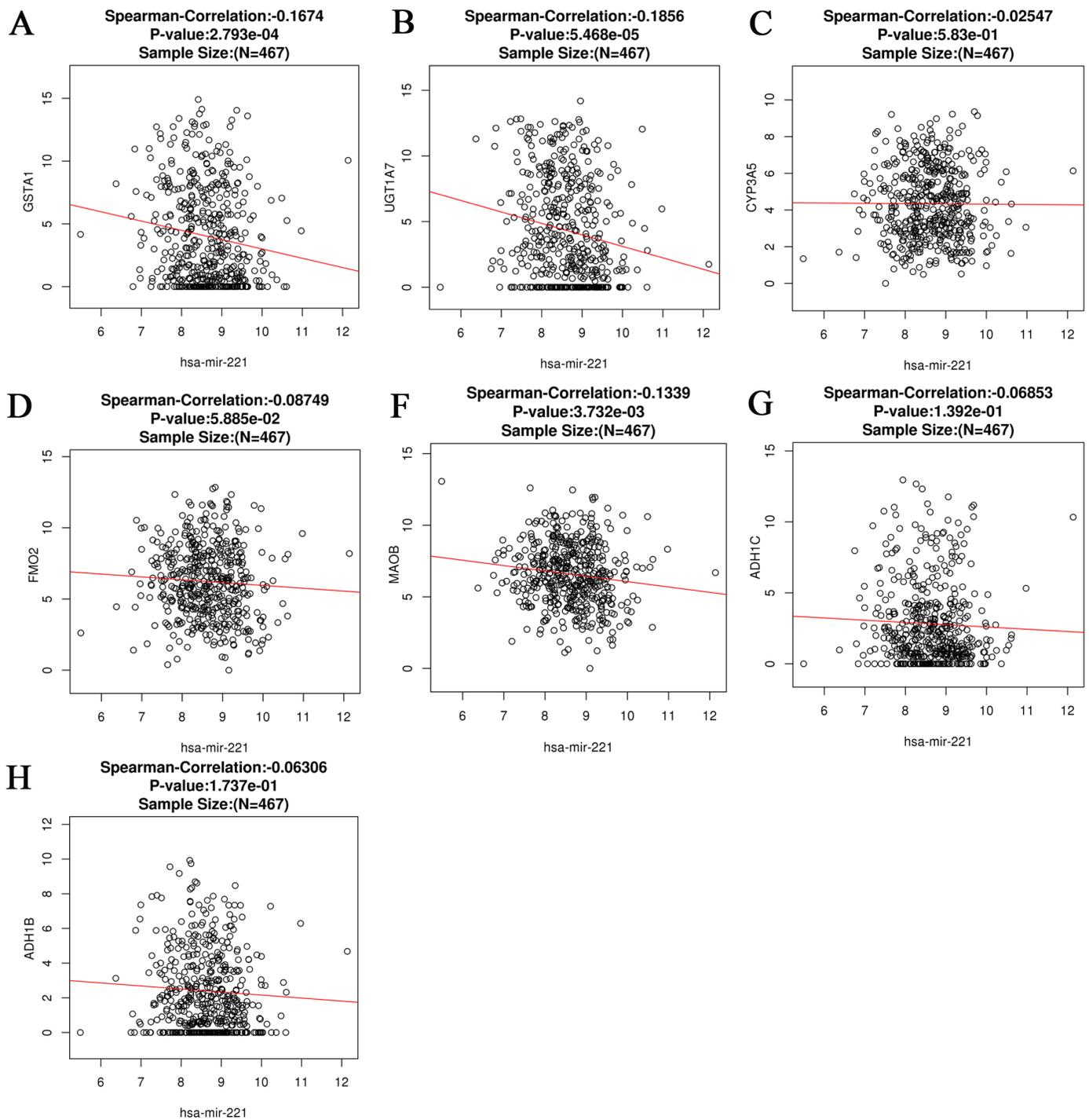


Figure 13

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.