

# Clinicopathological role of miR-30a-5p in hepatocellular carcinoma tissues and prediction of its function with bioinformatics analysis

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**Background:** It has been reported that deregulation or dysfunction of microRNAs (miRNAs) plays an essential part in the hepatocarcinogenesis. However, the contribution and mechanism of microRNA-30a-5p (miR-30a-5p) in hepatocellular carcinoma (HCC) remains largely unknown. Therefore, our aim was to investigate the clinicopathological role of miR-30a-5p in HCC tissues and explore its potential pathways in this study.

**Methods:** The expression of miR-30a-5p was measured in 95 HCC and adjacent noncancer tissues by real-time reverse transcription quantitative polymerase chain reaction. The relationship between miR-30a-5p expression levels and clinicopathological parameters was also analyzed. Furthermore, the potential target genes of miR-30a-5p were collected via online prediction and literature searching. Gene ontology and pathway enrichment analyses were used to identify the possible function of miR-30a-5p in HCC.

**Results:** Compared with adjacent noncancer tissues ( $2.23 \pm 0.77$ ), expression level of miR-30a-5p was significantly lower in HCC tissues ( $1.26 \pm 0.66$ ,  $P < 0.001$ ). MiR-30a-5p expression was evidently correlated with tumor nodes, metastasis, tumor-node-metastasis stage, portal vein tumor embolus, vascular invasion, and status of tumor capsule (all  $P < 0.05$ ). A total of 878 genes were finally used for the biological informatics analyses. These prospective target genes were highly enriched in various key pathways, for instance, Ubiquitin-mediated proteolysis, Axon guidance, Neurotrophin signaling pathway, Amyotrophic lateral sclerosis, and ErbB signaling pathway.

**Conclusion:** In conclusion, this study clarifies that the downregulation of miRNA-30a-5p might play a vital part in the incidence and progression of HCC via targeting various prospective genes and pathways. Future validation is required to further explore the prospective molecular mechanism of miR-30a-5p in HCC.

**Keywords:** miR-30a-5p, hepatocellular carcinoma, progression, target genes, gene ontology analysis, pathway analysis

## Introduction

Liver cancer is one of the most frequent cancers worldwide with a progressively increasing tendency of mortality and morbidity worldwide. According to statistical data, 28,410 new estimated cases of liver and intrahepatic bile duct cancer will occur in males in 2016, which ranks tenth and accounts for 3% of all malignancies. More importantly, liver and intrahepatic bile duct cancer is the leading cause of death in both males (estimated deaths 18,280, ranking fifth) and females (estimated deaths 8,890, ranking eighth, 3%) in the United States.<sup>1</sup> On a more serious note, in People's Republic of China liver cancers were the most commonly diagnosed cancer and the most prevalent cause of cancer-related death, after lung, stomach, and esophageal

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cancers (based on the statistics in 2015).<sup>2</sup> In liver cancer, hepatocellular carcinoma (HCC) is the most common class, which comprises about 90% of cases.<sup>3</sup> At the time of definitive diagnosis of HCC, the disease has frequently reached an advanced stage and curative treatment opportunities are often lost; thus, the recurrence rates of HCC still remain high.<sup>4</sup> Survival for HCC patients after diagnosis is not optimistic, with a median survival time about 6–20 months.<sup>5</sup> Furthermore, HCC is unevenly distributed in the world based on the different leading risk factors.<sup>3</sup> Genetic markers, including AFP, cannot be used due to the heterogeneity of HCC. Therefore, one of the more difficult tasks in clinical research of HCC is to attempt to discover novel markers to improve the diagnosis technique and to boost the survival prediction and treatment indication for HCC patients.<sup>5,6</sup>

MicroRNA (miRNA) is a class of noncoding RNAs that contain 19–25 nucleotides.<sup>7</sup> These miRNAs can target the 3′-untranslated region of mRNA, which subsequently leads to mRNA degradation or translational repression. miRNAs are widely involved in the biological functions such as cell proliferation, differentiation, and apoptosis.<sup>8</sup> On the basis of studies, it has been estimated that more than 60% human genes can be regulated by different miRNAs, and synchronously, one single miRNA can target various mRNAs to fulfill its biological functions.<sup>9</sup> Growing evidence has revealed that miRNAs play a vital role in HCC.<sup>10–13</sup> Previously, we found that overexpression of microRNA-30a-5p (miR-30a-5p; accession number: MIMAT0000087) in vitro could markedly inhibit cell growth and induce caspase-3/7 activity and apoptosis in four HCC cell lines HepG2, SMMC-7221, HepB3, and SNU449,<sup>14</sup> consistent with the in vitro finding by Liu et al<sup>15</sup> and Li et al.<sup>16</sup> They also detected the expression of miR-30a-5p in HCC samples; however, the number of patients involved was extremely small (n=63 in Liu et al<sup>15</sup> and n=16 in Li et al<sup>16</sup>). The clinicopathological significance of miR-30a-5p in HCC remains largely unclear. Thus, we were interested in analyzing the expression status of miR-30a-5p in HCC tissues by real-time reverse transcript-quantitative polymerase chain reaction (RT-qPCR), and also studying the correlations between miR-30a-5p expression and clinicopathological parameters of HCC. Further, we performed bioinformatics analysis to gather the possible target genes and potential pathways of miR-30a-5p in regulation gene network of HCC.

## Materials and methods

### Tissue samples and RT-qPCR

A total of 95 patients from the First Affiliated Hospital of Guangxi Medical University, People's Republic of China (between March 2010 and December 2011) were included in

this study. The age of HCC patients ranged from 29 to 82 years, with a mean age of 52 years. The detailed clinicopathological information of patients is summarized in Table 1. Tissue samples were obtained from surgical resection of patients who had not received any treatment. The adjacent noncancer

**Table 1** Relationship between the expression of miR-30a-5p and clinicopathological parameters in HCC

Clinicopathological parameters	n	miR-30a-5p relevant expression ( $2^{-\Delta\Delta Cq}$ )		
		Mean $\pm$ SD	t	P-value
Tissue			-9.406	<0.001
Adjacent noncancer liver	95	2.2309 $\pm$ 0.7677		
HCC	95	1.2564 $\pm$ 0.6561		
Age			-0.466	0.642
<50	49	1.2869 $\pm$ 0.5617		
$\geq$ 50	46	1.2239 $\pm$ 0.7487		
Sex			-0.219	0.772
Male	75	1.2463 $\pm$ 0.6447		
Female	20	1.2945 $\pm$ 0.7132		
Differentiation			F=0.516	0.599
Well	6	1.5100 $\pm$ 0.7133		
Moderate	60	1.2535 $\pm$ 0.7175		
Poor	29	1.2100 $\pm$ 0.5033		
Size			1.967	0.052
<5 cm	18	0.9867 $\pm$ 0.3715		
$\geq$ 5 cm	77	1.3195 $\pm$ 0.6930		
Tumor nodes			3.137	0.002
Single	52	1.4400 $\pm$ 0.7169		
Multi	43	1.0344 $\pm$ 0.4970		
Metastasis			5.683	<0.001
-	46	1.6033 $\pm$ 0.7218		
+	49	0.9308 $\pm$ 0.3620		
Clinical TNM stage			2.373	0.020
I-II	22	1.5405 $\pm$ 0.8430		
III-V	73	1.1708 $\pm$ 0.5680		
Portal vein tumor thrombus			5.793	<0.001
-	63	1.4733 $\pm$ 0.6477		
+	32	0.8294 $\pm$ 0.4271		
Vascular invasion			4.606	<0.001
-	59	1.4520 $\pm$ 0.7140		
+	36	0.9358 $\pm$ 0.3756		
Tumor capsular infiltration			2.728	0.007
With complete capsule	45	1.4471 $\pm$ 0.7490		
Capsule infiltration or no capsule	50	1.0848 $\pm$ 0.5085		
HCV			0.022	0.983
-	63	1.2575 $\pm$ 0.6680		
+	32	1.2544 $\pm$ 0.6425		
HBV			0.248	0.805
-	17	1.2924 $\pm$ 0.8377		
+	78	1.2486 $\pm$ 0.6159		
AFP			-1.074	0.286
-	41	1.2783 $\pm$ 0.7029		
+	38	1.1297 $\pm$ 0.5008		

(Continued)

**Table 1** (Continued)

Clinicopathological parameters	n	miR-30a-5p relevant expression ( $2^{-\Delta Cq}$ )		
		Mean $\pm$ SD	t	P-value
Cirrhosis			-0.605	0.547
-	50	1.2952 $\pm$ 0.6449		
+	45	1.2133 $\pm$ 0.6729		
MTDH			1.213	0.228
-	50	1.3582 $\pm$ 0.6474		
+	39	1.1854 $\pm$ 0.6914		
nm23			-1.426	0.517
-	20	1.0715 $\pm$ 0.2330		
+	75	1.3057 $\pm$ 0.7219		
P53			-0.469	0.640
-	40	1.2193 $\pm$ 0.6632		
+	55	1.2835 $\pm$ 0.6557		
P21			-1.456	0.149
-	62	1.1853 $\pm$ 0.5942		
+	33	1.3900 $\pm$ 0.7504		
VEGF			-0.826	0.411
-	25	1.1632 $\pm$ 0.3472		
+	70	1.2897 $\pm$ 0.7350		
Ki-67 LI			-0.390	0.698
Low	47	1.2298 $\pm$ 0.6082		
High	48	1.2825 $\pm$ 0.7053		
MVD			-2.381	0.020
Low	47	1.0989 $\pm$ 0.5027		
High	48	1.4106 $\pm$ 0.7513		

**Note:** t, Student's t-test; F, ANOVA.

**Abbreviations:** SD, standard deviation; HCC, hepatocellular carcinoma; TNM, tumor-node-metastasis; HCV, hepatitis C virus; HBV, hepatitis B virus; AFP, alpha-fetoprotein; MTDH, metadherin; VEGF, vascular endothelial growth factor; LI, label index; MVD, microvessel density; ANOVA, analysis of variance.

hepatic tissues were taken at least 2 cm away from the border of the tumor observed by naked eyes and confirmed independently by two pathologists (Wen-ting Huang and Gang Chen) as being without cancer by microscopic analysis. This study was approved by the Ethical Committee of the First Affiliated Hospital of Guangxi Medical University, and written informed consent was obtained from each participant, according to the institutional guidelines of our hospital.

The expression of miR-30a-5p was detected by real-time RT-qPCR. First, total RNA (including miRNA) was extracted from all samples with the miRNeasy FFPE Kit (QIAGEN, the Netherlands), following the manufacturer's protocol as previously reported.<sup>17-23</sup> RNA concentrations were determined using the Nano Drop 2000. A combination of RUN6B and RUN48 was the housekeeping reference used in this study. The primers for miR-30a-5p, RNU6B, and RNU48 were included in TaqMan<sup>®</sup> MicroRNA Assays (4427975, Applied Biosystems, Life Technologies, Grand Island, NY, USA). Primer sequences were as follows: miR-30a-5p (Applied Biosystems, cat no 4427975-000417): UG

UAAACAUCCUCGACUGGAAG; RNU6B (Applied Biosystems, cat no 4427975-001093): CGCAAGGAUGACA CGCAAAUUCGUGAAGCGUCCAUUUUUU; RNU48 (Applied Biosystems, cat no 442975-001006): GAUGAC-CCCAGGUAACUCUGAGUGUGUCGCU GAUGCCAU-CACCGCAGCGCUCUGACC. The reverse primers were also included in the process of reverse transcription with TaqMan<sup>®</sup> MicroRNA Reverse Transcription Kit (4366596, Applied Biosystems) to a total volume of 10  $\mu$ L. Real-time qPCR to detect the miRNA level was performed using the Applied Biosystems PCR7900. The miR-30a-5p level in each sample was normalized to its internal references. The expression of miR-30a-5p level in the FFPE experiments was calculated with the formula  $2^{-\Delta Cq}$  as previously reported.<sup>17-23</sup>

### Collection of prospective target genes of miR-30a-5p via bioinformatics approaches

To obtain a better picture of the potential mechanism of miR-30a-5p in the progression of HCC, the validated target genes of miR-30a-5p from literature screening were collected from PubMed, Wiley Online Library, Web of Science, Science Direct, Cochrane Central Register of Controlled Trials, Google Scholar, EMBASE, Ovid, and LILACS. Since the previous name of miR-30a-5p was miR-30a and there are several synonyms of miR-30a-5p available, the following searching key words were applied: “(miR-30a or miRNA-30a or microRNA-30a or miR30a or miRNA30a or microRNA30a or ‘miR 30a’ or ‘miRNA 30a’ or ‘microRNA30a’ or miR-30a-5p or miRNA-30a-5p OR microRNA-30a-5p) and (target\*).” Concurrently, target genes with “strong evidence” were also gathered from mirTarBase and TarBase databases. All the target genes from aforementioned sources verified by qPCR, Western blot, or luciferase reporter assay were integrated into “validated targets” of miR-30a-5p. To further acquire a more complete image of potential target genes of miR-30a-5p, we predicted the possible targets via 11 online methods, including MirTarBase, TarBase, Targetminer, polymiRTS, RNA22, microRNA.org, Pita, mirRNAMAP, Targetscan, miRDB, and Pictar-vert. Genes appearing more than five times were selected as the “predicted targets” of miR-30a-5p.

### Bioinformatics analyses with gene ontology (GO) and pathway enrichment

The genes merged from “validated targets” and “predicted targets” were evaluated for further GO and pathway analysis with the DAVID (<https://david.ncicrf.gov/>) and BINGO plug-in of Cytoscape. Genes were also mapped to the database of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway to identify the signaling. *P*-value <0.001

was of significance in GO analysis. Similarly,  $P < 0.001$  was considered significant in pathway analysis.

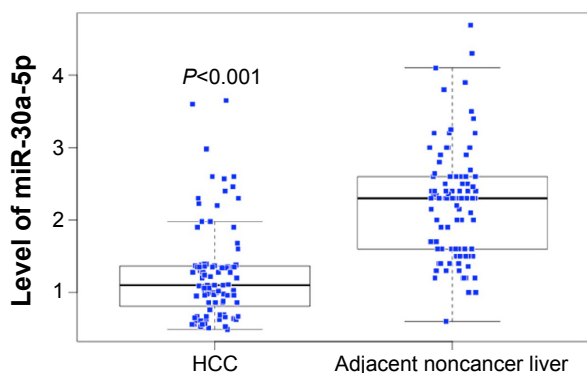
## Statistical analysis

All statistical analyses were conducted using SPSS version 20.0 (Armonk, NY, USA). The expression levels were presented as mean  $\pm$  standard deviation. Receiver operator characteristic curve (ROC) was applied to evaluate the diagnostic value.  $P$ -value  $< 0.05$  was considered of statistical significance.

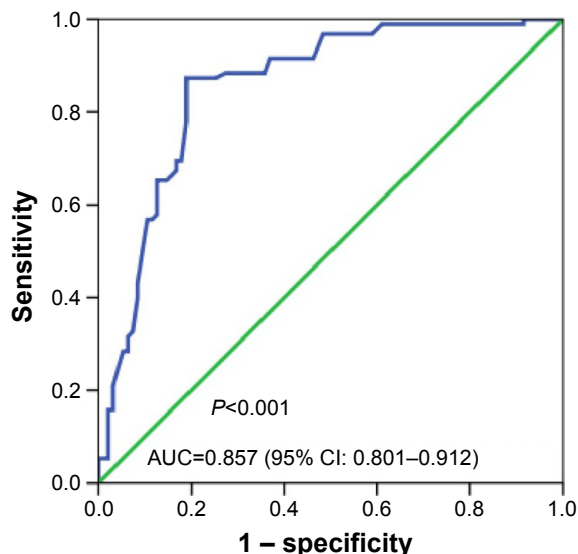
## Results

### Clinicopathological significance of miRNA-30a-5p in HCC tissues

In the studied population of HCC patients, the expression of miRNA-30a-5p was significantly downregulated in HCC tissues ( $1.2564 \pm 0.6561$ ) compared with that in adjacent non-cancer hepatic tissues ( $2.2309 \pm 0.7677$ ,  $P < 0.001$ , Figure 1). The area under the ROC curve of low miRNA-30a-5p was 0.857 (95% CI: 0.801–0.912;  $P < 0.001$ ; cut-off=1.395), with 87.4% sensitivity and 18.9% specificity in distinguishing the HCC from noncancerous liver tissues (Figure 2). The level of miRNA-30a-5p was also reduced in patients with tumors in multiple nodes, metastasis, tumor–node–metastasis (TNM) stage III or IV disease, portal vein tumor thrombus, vascular invasion, tumor capsule infiltration or no capsule, and low microvessel density, when compared with the counterparts (Table 1). The association of miRNA-30a-5p with clinicopathological parameters was further supported by Spearman analysis. From the results, miRNA-30a-5p was shown to be negatively associated with TNM stages ( $r = -0.213$ ;  $P = 0.038$ ), metastasis ( $r = -0.516$ ;  $P < 0.001$ ), tumor nodes ( $r = -0.317$ ;  $P = 0.002$ ), tumor capsule ( $r = -0.255$ ;  $P = 0.013$ ), portal vein tumor thrombus ( $r = -0.615$ ;  $P < 0.001$ ), and vascular invasion ( $r = -0.407$ ;  $P < 0.001$ ). We also investigated the relationship



**Figure 1** MiR-30a-5p expression in HCC and adjacent noncancer liver tissues. **Abbreviation:** HCC, hepatocellular carcinoma.

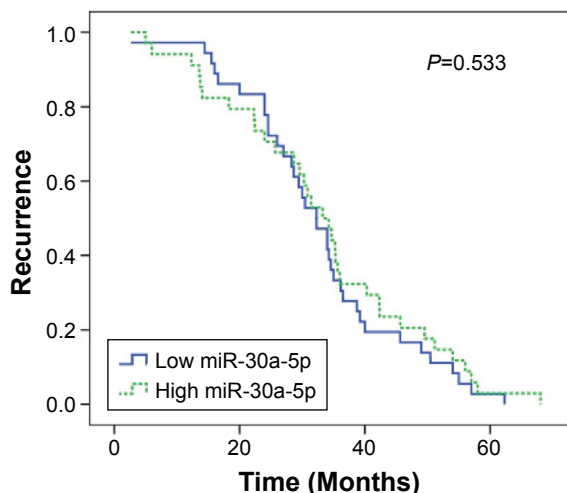


**Figure 2** ROC curve of miR-30a-5p expression in HCC. **Abbreviations:** AUC, area under the curve; CI, confidence interval; HCC, hepatocellular carcinoma; ROC, receive operator characteristic curve.

between miR-30a-5p and recurrence. Seventy patients in this study were followed up and all of them had recurrent disease. The follow-up time of HCC patients range from 2.68 to 68 months. The mean recurrent time was  $32.30 \pm 1.76$  months. We divided these 70 patients with follow-up information into two groups based on the cut-off of 0.98. There was no significant difference of the recurrent time between patients having high miR-30a-5p level ( $32.29 \pm 2.4$ ) and low level ( $33.25 \pm 2.78$ ,  $P = 0.533$ ; Figure 3).

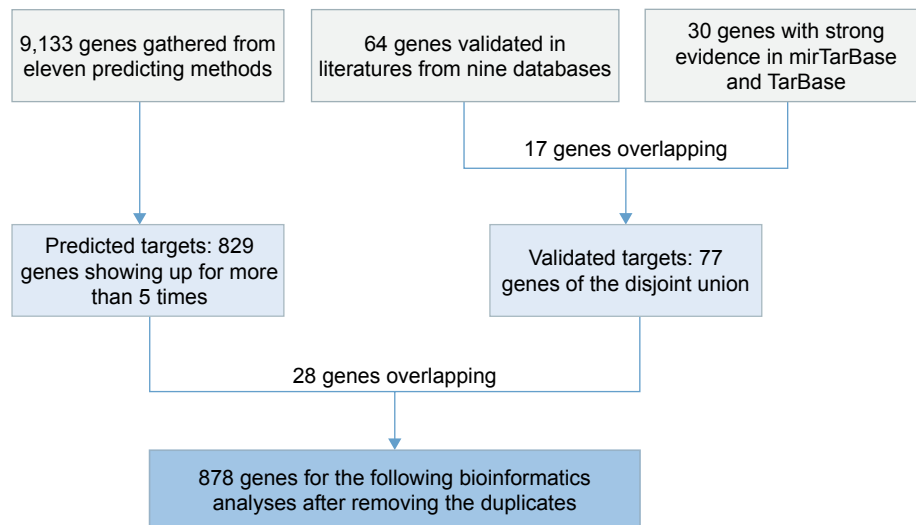
### Bioinformatics analysis of the potential target genes of miR-30a-5p

The confirmed target genes ( $n = 64$ ) of miR-30a-5p in the literature were recorded. In addition, another 30 genes with



**Figure 3** Relationship between miR-30a-5p expression and recurrence of HCC. **Abbreviation:** HCC, hepatocellular carcinoma.





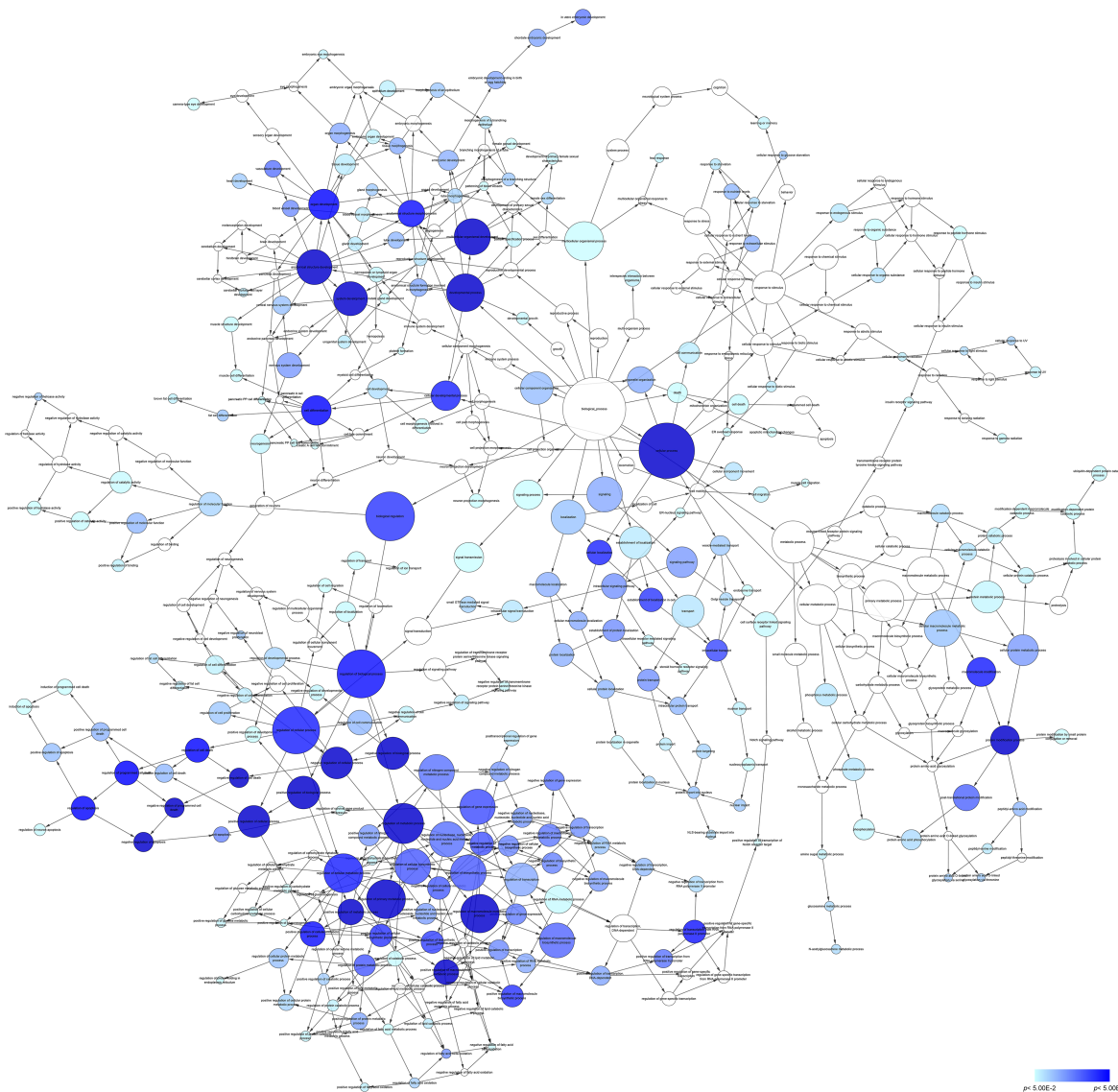
**Figure 4** Flowchart of the bioinformatics analysis of targets of miR-30a-5p.

strong evidence, which were validated by qPCR, Western blot, or luciferase assays, were provided by mirTarBase and TarBase databases. Seventeen genes were present at the same time in the literature and in the mirTarBase/TarBase database (*RUNX2*, *VIM*, *DTL*, *SEPT7*, *MTDH*, *Snai1*, *PRDM1*, *AVEN*, *FOXD1*, *BDNF*, *BECN1*, *TNRC6A*, *CDH1*, *BCL11A*, *HSPA5*, *EYA2*, and *SOX4*). Thus, the disjoint union of 77 genes were considered as “validated targets” of miR-30a-5p. Afterward, 11 online software predicted 9,133 target genes. To decrease the false positivity of target genes, 829 genes that came up more than five times were grouped into “predicted targets” of miR-30a-5p. After “validated targets” and “predicted targets” were combined, we finally had 878 genes for the following bioinformatics analyses (Figure 4).

According to the results of DAVID and BINGO in GO analysis, the target genes were found concentrated in the following biological pathways: regulation of transcription from RNA polymerase II promoter, positive regulation of cellular biosynthetic process, and positive regulation of biosynthetic process ( $P < 0.001$ , Figure 5). On the basis of cellular component, genes mostly assembled at the pathways of Golgi apparatus, insoluble fraction and membrane fractions, etc ( $P < 0.001$ , Figure 6). Genes prominently accumulated in four molecular functions, including transcription regulator activity, protein domain-specific binding, transcription activator activity, and acid-amino acid ligase activity ( $P < 0.001$ , Figure 7). Besides, KGEE pathway analysis showed that five pathways were significant, ie, Ubiquitin-mediated proteolysis, Axon guidance, Neurotrophin signaling pathway, Amyotrophic lateral sclerosis, and ErbB signaling pathway ( $P < 0.05$ , Figure 8).

## Discussion

Previously, we found that miR-30a-5p expression was lower in the HCC cell lines compared to the normal hepatic cells (data not shown), which was in agreement with the studies of Liu et al<sup>15</sup> and Li et al.<sup>16</sup> Thus far, only two groups have attempted to investigate the difference of miR-30a-5p level between HCC tissues and noncancerous liver tissues. Li et al<sup>16</sup> used RT-qPCR to detect miR-30a-5p expression in 16 pairs of HCC and their adjacent noncancerous tissues. Among 16 cases, 13 (81.25%) presented lower expression of miR-30a-5p in HCC tissues compared with matched noncancerous liver tissues. The results showed that miR-30a-5p expression in HCC tissues was significantly lower compared to adjacent noncancerous liver tissues. Besides, Liu et al<sup>15</sup> collected human HCC tissues and their paracancerous hepatic tissues from 63 patients undergoing resection and showed that miR-30a was downregulated in 87% (55/63) of the examined HCC tissues. In accordance with the aforementioned two reports, we also confirmed the striking downregulation of miR-30a-5p expression in HCC tissues, compared to the noncancerous liver controls, with a larger cohort of 95 pairs of clinical samples. The expression of miR-30-5p was only 56% that in the noncancerous liver, which indicates that during the process of HCC development, miR-30a-5p is lost. Hence, miR-30a-5p probably plays a tumor suppressive role in the carcinogenesis of HCC. Furthermore, ROC revealed that in this study, the diagnostic value of low miR-30a-5p level remained moderate with an area under the curve of 0.875. MiR-30a-5p might also act as a biomarker contributing in the screening of HCC. However, a larger study is warranted for confirmation of the diagnostic value of miR-30a-5p for HCC in the future.



**Figure 5** Network analysis with the prospective target genes of miR-30a-5p of BP.

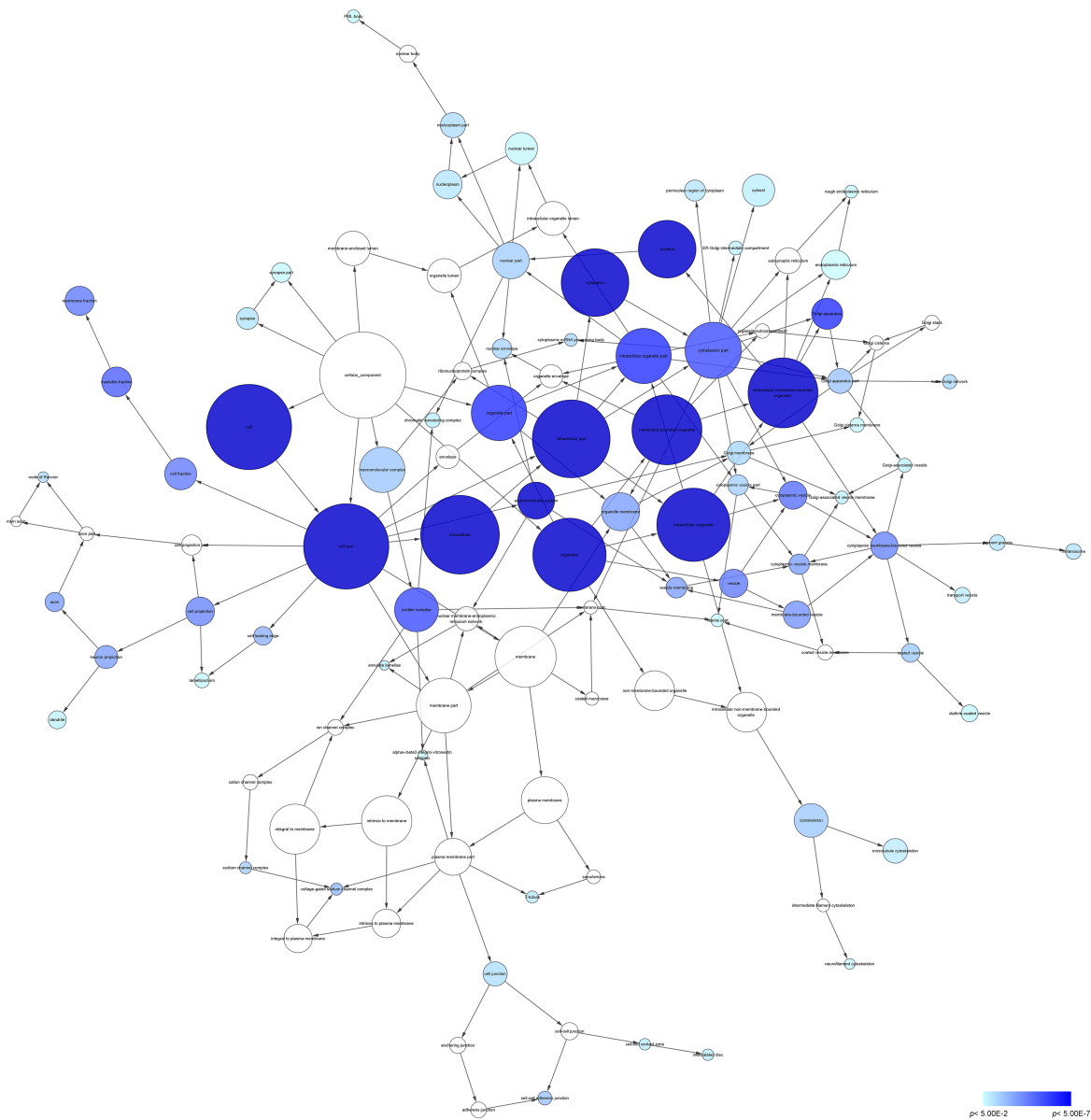
**Notes:** The intensity of the color indicates P-value size, node refers to pathways, and the node size is representative of the number of genes.

**Abbreviations:** BP, biological process; UV, ultraviolet.

miRNAs are particularly stable in body fluids, including serum or plasma, which makes them preferable biomarkers for the early diagnosis in HCC.<sup>24,25</sup> To date, only one study has identified and evaluated miR-30a-5p as a HCC-associated plasma miRNA in HCC. The result showed that miR-30a-5p was one of the significantly overexpressed miRNAs in the hepatitis B virus-positive HCC patients compared with the hepatitis B virus-positive cancer-free controls,<sup>26</sup> which is a contrary phenomenon compared to its reduced expression level in HCC tissues. The cause of this contradiction needs further investigation.

With regard to the correlation between miR-30a-5p and clinical parameters of HCC, Liu et al's<sup>15</sup> study was the only

one that explored whether miR-30a-5p downregulation was associated with clinical features or prognosis of HCC patients. A relationship between reduced miR-30a expression and intrahepatic metastasis, advanced TNM stage, and high Edmonson pathological classification was noted. Additionally, lower miR-30a level was associated with shorter disease-free survival, and multivariate analysis confirmed that low miR-30a-5p level was an independent predictor for shorter disease-free survival of HCC patients (HR =3.2;  $P=0.002$ ). In this study, we found a similar relationship between low miR-30a-5p and the deterioration of HCC, which is associated with the status of metastasis, number of tumor nodes, condition of tumor capsule, portal vein tumor thrombus, and



**Figure 6** Network analysis with the prospective target genes of miR-30a-5p of CC.

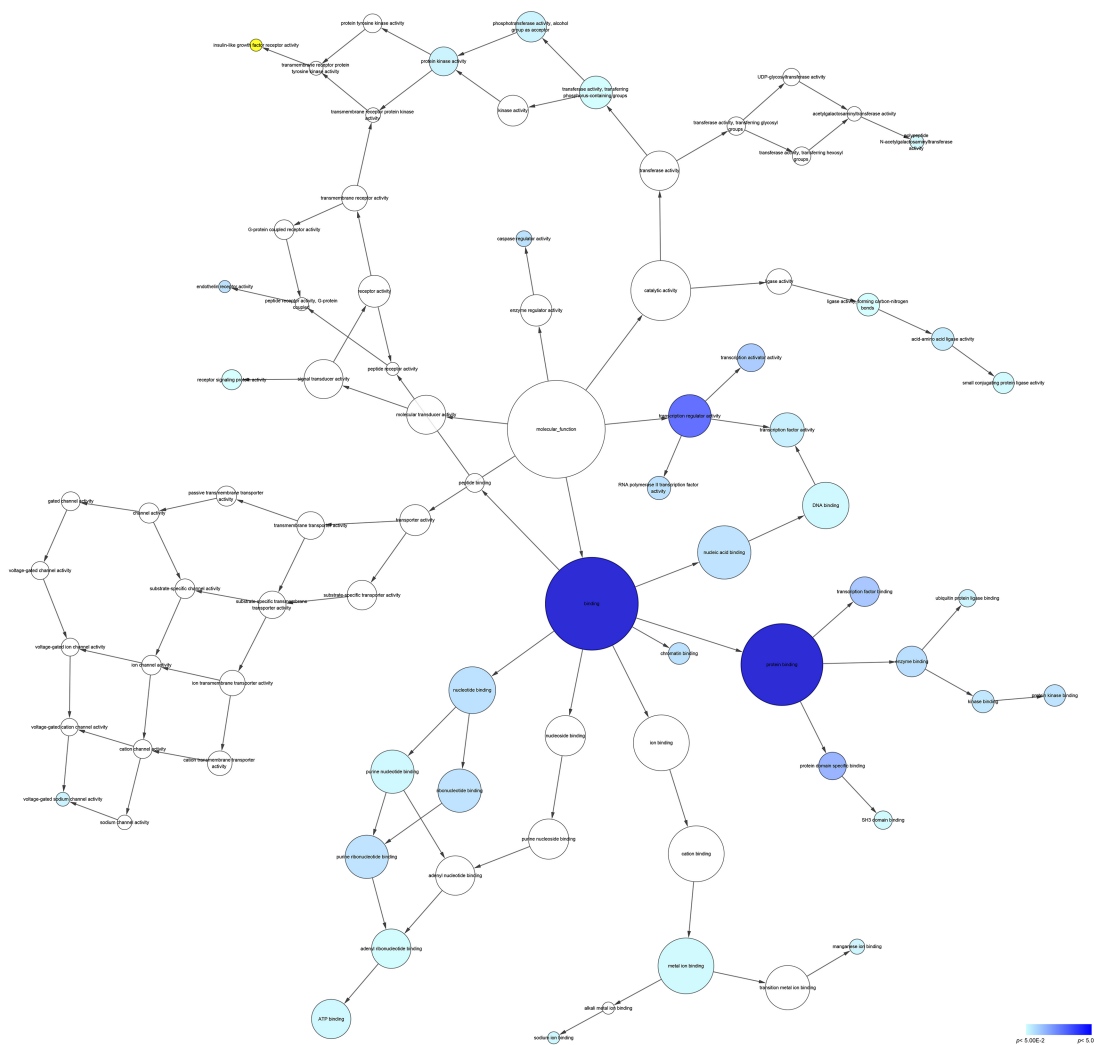
**Notes:** The intensity of the color indicates *P*-value size, node refers to pathways, and the node size is representative of the number of genes.

**Abbreviations:** CC, cellular component; PML, promyelocytic leukemia.

vascular invasion, although no significant correlation of miR-30a-5p was noted with recurrence. Collectively, the data of this study, together with the finding of Liu et al,<sup>15</sup> suggest that downregulation of miR-30a-5p may play an important part in the development and progression of HCC.

The clinicopathological significance of miR-30a-5p in HCC has also been verified with *in vitro* experiments. Previously, our group performed *in vitro* work to investigate the role of miR-30a-5p on the biological function of HCC cell lines HepG2, SMMC-7221, HepB3, and SNU449. We transfected miR-30a-5p inhibitor and miR-30a-5p mimic into

HCC cell lines and found that miR-30a-5p mimic could obviously inhibit cell growth and also induce caspase-3/7 activity and apoptosis.<sup>14</sup> Li et al<sup>16</sup> also reported similar results that miR-30a-5p overexpression in HCC cells significantly inhibited cell proliferation, suppressed colony-forming efficiency, induced apoptosis *in vitro*, and also reduced HepG2 tumor growth *in vivo*. Furthermore, gain- and loss-of-function studies by Liu et al<sup>15</sup> demonstrated that downregulation of miR-30a-5p facilitated tumor cell migration, invasion, and epithelial–mesenchymal transition. In summary, miR-30a-5p could influence several malignant phenotypes including



**Figure 7** Network analysis with the prospective target genes of miR-30a-5p of MF. **Notes:** The intensity of the color indicates P-value size, node refers to pathways, and the node size is representative of the number of genes. **Abbreviations:** MF, molecular function; UDP, uridine diphosphate.

cell growth, migration, invasion, apoptosis, and epithelial–mesenchymal transition of HCC cells.

Next, the molecular mechanism of miR-30a-5p is of great interest. Almost at the same time, Li et al<sup>16</sup> and our group both reported that metadherin (MTDH)/AEG-1 was one of the direct target genes of miR-30a-5p, which was confirmed by luciferase assay. Besides, Liu et al<sup>15</sup> identified SNAIL also as a direct target of miR-30a-5p. These two genes, MTDH/AEG-1 and SNAIL, are by far the only validated targets of miR-30a-5p in HCC. Since one single miRNA can target dozens of genes to fulfill its biological and clinical function, the relevant gene network and molecular mechanism of miR-30a-5p remain undefined. Thus, the bioinformatics analysis was performed to comprehensively understand the prospective targets and pathways of miR-30a-5p. Various

pathways have been shown with GO and KEGG analyses, which suggests that it could be a complicated process for miRNA-30a-5p to regulate different signaling pathways. This is also accordance with the accepted idea that HCC is a cancer, implying the multistep process of activating oncogenes and inactivating tumor suppressor genes. As the strong connection of miRNA-30a-5p expression and tumor progression was noted in this study, we paid more attention to the pathways related to cell grow, cell invasion, and metastasis. Among all the significant pathways shown by GO and KEGG analyses, some have been widely studied in HCC. For example, PIK3CD, TP53, FOXO3, IRS1, AKT1, MAPK1 in both Neurotrophin signaling pathway and ErbB signaling pathway have been confirmed to play vital roles in HCC.<sup>27–34</sup> Since these potential targets genes of miR-30a-5p



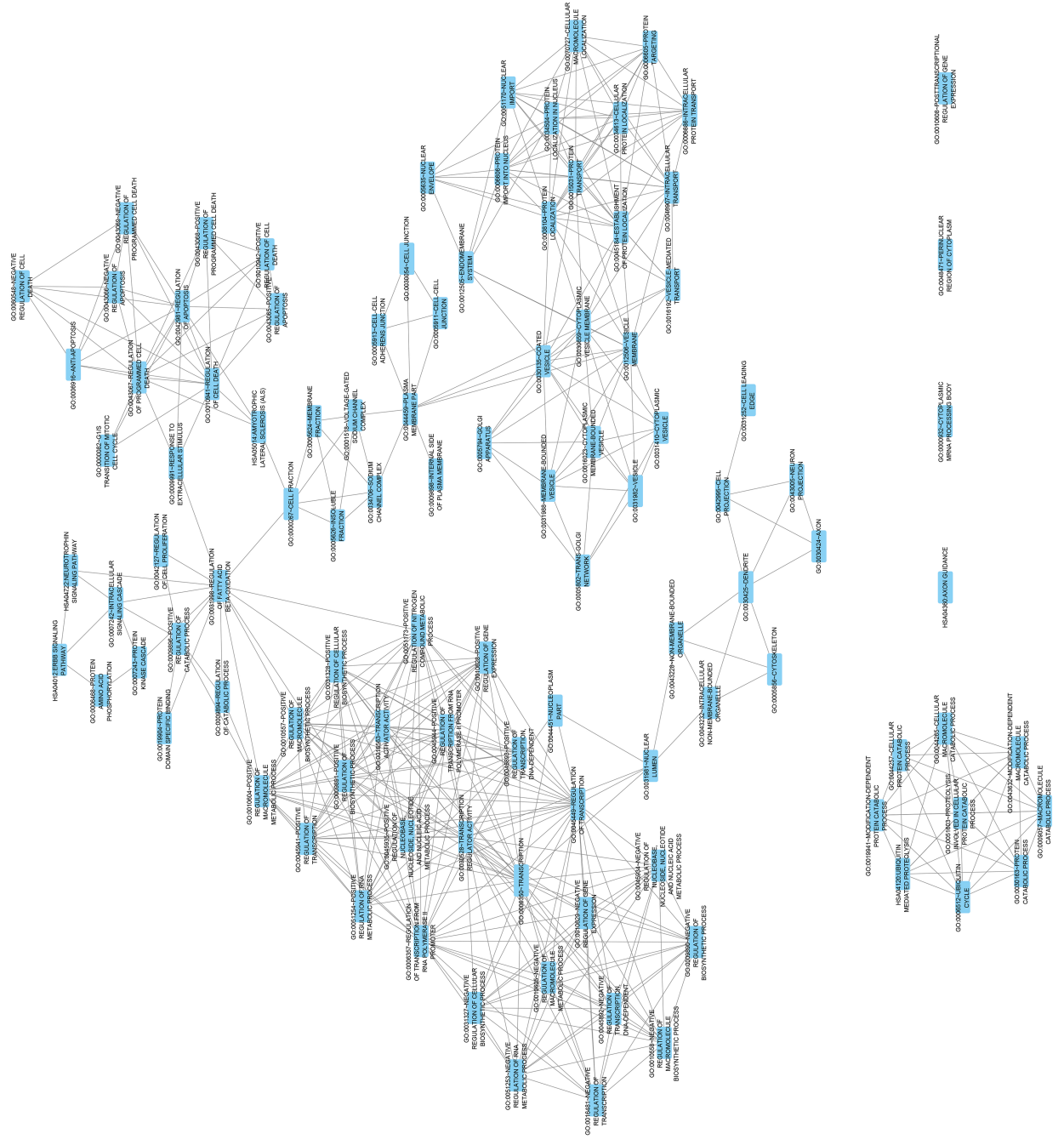


Figure 8 Pathways of the prospective target genes of miR-30a-5p by KEGG analysis. Abbreviation: KEGG, Kyoto Encyclopedia of Genes and Genomes.

have never been confirmed to date, future verification studies are expected to verify more target genes and related pathways of miR-30a-5p in HCC.

Autophagy has been considered as a prospective target to enhance the efficiency of conventional chemotherapeutics for HCC.<sup>35,36</sup> MiR-30a-5p, a powerful inhibitor of autophagy by restraining Beclin-1, was found to be interfering with the effectiveness of sorafenib-mediated apoptosis by an autophagy-dependent pathway in renal cell carcinoma cells.<sup>37</sup> Sorafenib is by far the only and standard systematic therapy drug for the treatment of advanced HCC, and its clinical benefits remain modest. Little is known of the biomarker to predict the efficiency of sorafenib on HCC, or the molecular mechanism of the drug resistance.<sup>38,39</sup> MiR-30a-5p might be a hopeful link to connect sorafenib-induced autophagy activation and HCC resistance.

It should be emphasized that limitation exists in this study. First, the survival data are missing due to the fact that most patients were lost after they were discharged from our hospital. The prognostic value of miR-30a-5p in HCC needs further verification. Second, even though thousands of prospective genes have been predicted in this study, only two genes (MTDH/AEG-1 and SNAI1) have been experimentally validated. More target genes are expected to be verified in the future work. Third, the retrospective nature of this study is itself a limitation. Prospective studies with a larger sample size are needed for further confirmation of the role of miR-30a-5p in HCC. Moreover, our research only aimed at HCC patients of Chinese yellow race. Since racial variations might have impact on the molecular signature of HCC, studies with a larger sample size and including other races are required in the future.

## Conclusion

This study clarifies that the downregulation of miRNA-30a-5p might play a vital role in the incidence and progression of HCC via targeting various prospective genes and pathways. Future validation is required to further explore the molecular mechanism of miR-30a-5p in HCC.

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## Disclosure

The authors report no conflicts of interests in this work.

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