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Review Article

Upregulated miR-18a-5p and its regulatory roles in hepatocellular carcinoma: a study based on bioinformatics analysis with miRNA-seq and miRNA-microarray data

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Abstract: Background: Expression levels of miR-18a-5p and its molecular mechanisms in hepatocellular carcinoma (HCC) remain poorly understood. This present study aimed to examine expression profiling of miR-18a-5p in HCC samples from available high throughput data and to assess possible related pathways of miR-18a-5p. Material and methods: Expression levels of precursor miR-18a and miR-18a-5p were extracted and analyzed from Cancer Genome Atlas (TCGA) data as well as different miRNA microarrays from Gene Expression Omnibus (GEO) and ArrayExpress databases. This study further conducted meta-analyses to determine clinical expression levels of miR-18a-5p in HCC, combining all data from miRNA-seq and miRNA microarrays with standard mean difference (SMD) and summarizing receiver operating characteristic (sROC) methods. Predicted genes from 12 online platforms were then collected and differentially expressed genes (DEGs) in both TCGA and GEO datasets were interacted. Furthermore, bioinformatics analyses of the intersecting genes were performed to identify possible pathways related to miR-18a-5p in HCC using Gene ontology (GO) classification, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, and protein-protein interaction (PPI) analyses. Results: Levels of precursor miR-18a were more upregulated in 371 HCC tissues (6.2620±1.46955) than in 50 adjacent liver tissues (5880±0.6416, P=0.0015). Results from meta-analyses with 902 HCC cases also indicated that levels of miR-18a-5p were obviously elevated in HCC tissues. AUC of sROC reached 0.88 (0.85-0.91), implying a certain distinguishing capacity of miR-18a-5p in HCC. In total, 78 genes were gathered to gain great possibilities of becoming prospective targets of miR-18a-5p in HCC. Additionally, GO analysis indicated the following centralized pathways for miR-18a-5p in HCC: striated muscle cell differentiation, cell fraction, and electron carrier activity. KEGG pathway analyses indicated that pathways of 'retinol metabolism' were the most centralized pathways for miR-18a-5p in HCC. CXCL12 and IGF1 could be core target genes of miR-18a-5p in HCC, as provided by the PPI network, whose mRNA levels were downregulated in HCC tissues from TCGA data. Conclusion: miR-18a-5p might contribute to tumorigenesis and progression of HCC via influencing various related pathways. CXCL12 and IGF1 could be novel targets of miR-18a-5p in HCC.

Keywords: miR-18a-5p, hepatocellular carcinoma, TCGA, GEO, target genes

Introduction

Hepatocellular carcinoma (HCC) is considered one of the leading malignancies, worldwide [1-6]. In 2017, it was determined that 28,920 deaths and 40,710 new cases would appear from HCC [7]. Regarding treatment for HCC, surgical resection, radiotherapy, chemotherapy, and liver transplantation have been considered effective methods. Applying chemotherapy for patients after liver transplantation (LT) for HCC has been helpful concerning prolonging relapse of HCC and survival of patients. However, radiotherapy lacks the ability of ameliorating HCC prognosis. Moreover, only approximately 15% of patients are ineligible for surgery. Furthermore, 80% of patients receiving surgery will relapse [8-14]. Additionally, HCC remains difficult to detect in the early stage without specific biomarkers, as patients often present with advanced tumor-node-metastasis (TNM) stages when they are definitively diag-

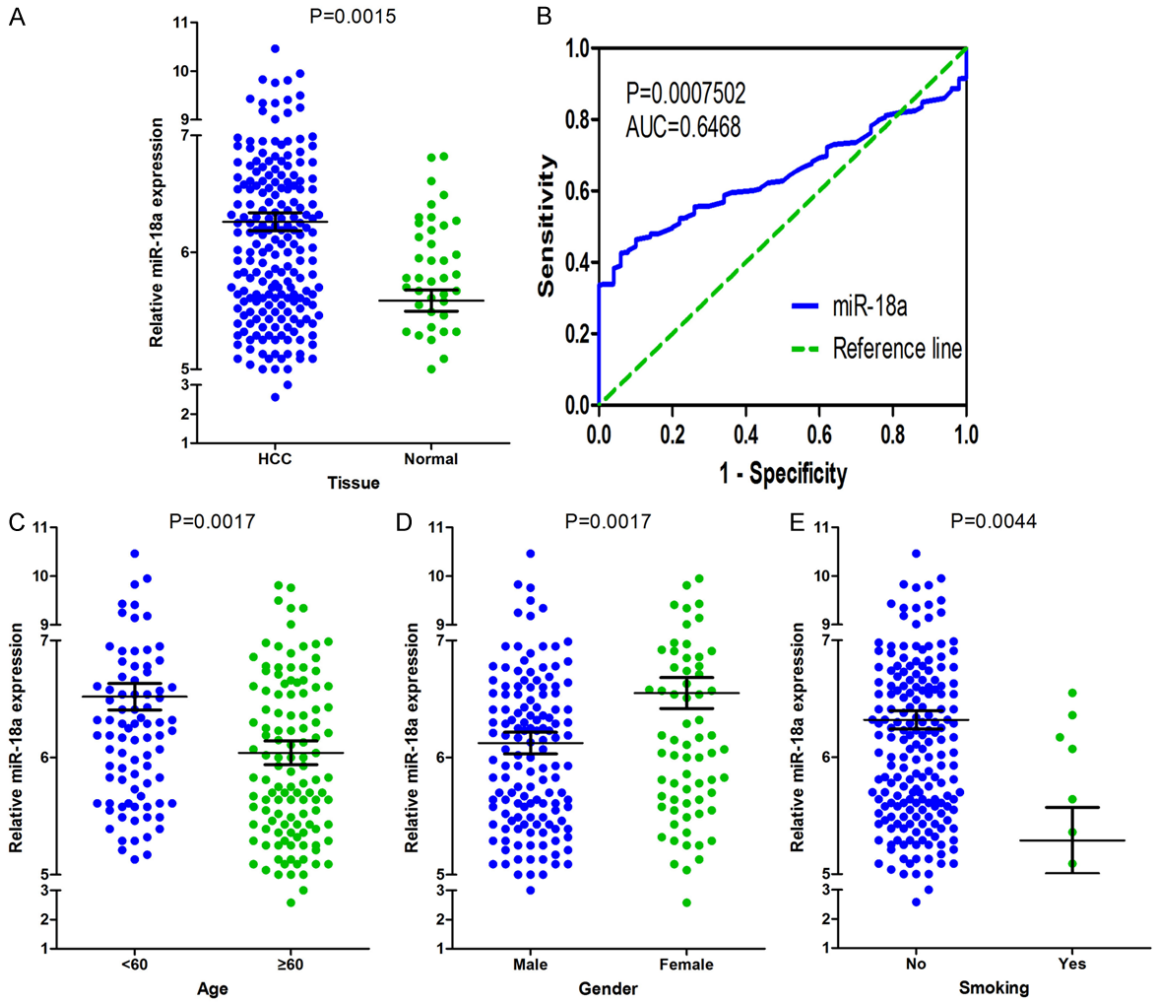

Figure 1. Clinical expression levels and prospective value of precursor miR-18a in HCC based on TCGA project. A. Expression of precursor miR-18a in HCC and non-cancerous liver tissues. B. Receiver operating characteristic (ROC) curve of precursor miR-18a in HCC. C. < 60 years old vs ≥ 60 years old. D. Male vs female. E. No smoking vs smoking.

Table 1. Expression of precursor miR-18a in various groups according to clinical pathological parameters of HCC in TCGA database

Clinical parameters		N	Precursor miR-18a expression		
			Mean ± SD	t	*P*-value
Tissue	Non-cancerous liver	50	5.5880±0.6416	3.200	0.0015*
	HCC	371	6.2620±1.4695		
Age (years)	< 60	170	6.5200±1.4767	3.149	0.0017*
	≥ 60	200	6.0412±1.4341		
Gender	Female	119	6.5515±1.4389	-2.647	0.0089*
	Male	252	6.1252±1.4667		
Tumor status	With tumor	113	6.2490±1.4520	-0.116	0.9085
	Tumor free	233	6.2296±1.4591		
Hepatitis B	No	246	6.2447±1.5098	-0.513	0.6088
	Yes	107	6.3277±1.3474		
Hepatitis C	No	297	6.2994±1.4861	0.785	0.4348
	Yes	56	6.1398±1.3250		

Smoking	No	336	6.3195±1.4584	3.512	0.0044*
	Yes	17	5.2879±1.1658		
Cirrhosis	No	347	6.2641±1.4642	-0.609	0.5679
	Yes	6	6.6004±1.3379		
Vascular invasion	No	205	6.1341±1.4134	-1.262	0.2082
	Yes	111	6.3499±1.4706		
Race	Asian	161	6.4824±1.4199	F=2.224[a]	0.0850
	White	181	6.0821±1.4896		
	Black or African American	17	6.0734±1.4535		
	American Indian or Alaska native	2	6.4564±3.4739		
Pathologic Stage	Stage I-II	257	6.2949±1.4293	-0.030	0.9762
	Stage III-IV	87	6.3004±1.6897		
T	T1-2	275	6.2773±1.4264	0.228	0.8198
	T3-4	93	6.2344±1.6147		
Pathologic Stage	Stage I	172	6.2513±1.4182	F=0.213[a]	0.8873
	Stage II	85	6.3830±1.4559		
	Stage III	85	6.2893±1.6892		
	Stage IV	2	6.7715±2.3224		
T	T1	182	6.2486±1.4003	F=0.550[a]	0.6481
	T2	93	6.3336±1.4824		
	T3	80	6.3071±1.6405		
	T4	13	5.7867±1.4208		
N	No	254	6.3134±1.4986	-0.212	0.8455
	Yes	4	6.5602±2.3102		
M	No	269	6.3312±1.4844	-0.162	0.8816
	Yes	4	6.4447±1.3935		

a, One-way analysis of variance (ANOVA) was conducted. *P < 0.05 was considered statistically significant

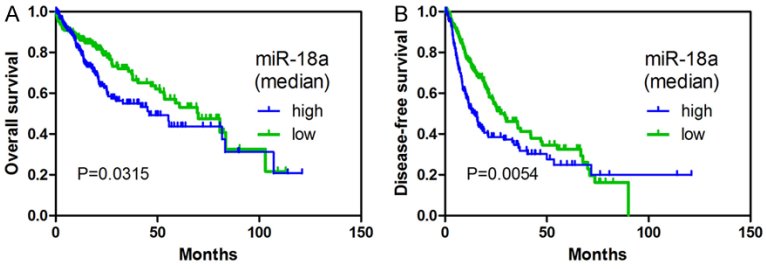

Figure 2. Kaplan-Meier curves for evaluating the prognosis value of precursor miR-18a in HCC based on TCGA project. A. Overall survival (OS) of precursor miR-18a in HCC. B. Diseased-free survival (DSF) of precursor miR-18a in HCC.

nosed [15-19]. Therefore, it is necessary to identify new molecular biomarkers for HCC.

MicroRNAs (miRNAs) are small non-coding single-stranded RNAs, around 22 nucleotides. These small RNAs could be potential new biomarkers for cancer detection or progression prediction [20-24]. Expression of miRNAs can influence tumor proliferation, apoptosis, metastasis, and relapse [25-30]. Thus, research of miRNAs can aid in identification of novel pathways regulating carcinogenesis and provide opportunities to discover biomarkers and therapeutic targets for cancers, including HCC [31-35]. Previous studies have discovered that miR-18a-5p is differentially expressed in non-small cell lung cancer, colorectal cancer, and prostate cancer [36-38]. However, there have been only several studies concerning the effects of miR-18a-5p in HCC. The clinical role of miR-18a-5p is not well known [39-42]. This present study aimed to assess expression data of the precursor miR-18a from The Cancer Genome Atlas (TCGA) Project to estimate clinical expression levels

Table 2. Gene Ontology (GO) analysis of overlapping genes of miR-18a-5p

Category	ID	Term	Count	P
GOTERM_BP_FAT	GO:0051146	Striated muscle cell differentiation	5	8.36E-04
GOTERM_BP_FAT	GO:0055114	Oxidation reduction	11	8.79E-04
GOTERM_BP_FAT	GO:0046395	Carboxylic acid catabolic process	5	0.001978343
GOTERM_BP_FAT	GO:0016054	Organic acid catabolic process	5	0.001978343
GOTERM_BP_FAT	GO:0042692	Muscle cell differentiation	5	0.002707722
GOTERM_BP_FAT	GO:0009611	Response to wounding	9	0.003727129
GOTERM_BP_FAT	GO:0005996	Monosaccharide metabolic process	6	0.004226822
GOTERM_BP_FAT	GO:0046700	Heterocycle catabolic process	4	0.006012789
GOTERM_BP_FAT	GO:0006006	Glucose metabolic process	5	0.00624081
GOTERM_BP_FAT	GO:0048146	Positive regulation of fibroblast	3	0.006858859
GOTERM_CC_FAT	GO:0000267	Cell fraction	16	8.57E-05
GOTERM_CC_FAT	GO:0005789	Endoplasmic reticulum membrane	7	0.001454113
GOTERM_CC_FAT	GO:0042175	Nuclear envelope-endoplasmic	7	0.001915071
GOTERM_CC_FAT	GO:0005615	Extracellular space	10	0.003917097
GOTERM_CC_FAT	GO:0044421	Extracellular region part	12	0.004022427
GOTERM_CC_FAT	GO:0005792	Microsome	6	0.004660224
GOTERM_CC_FAT	GO:0005626	Insoluble fraction	11	0.004685827
GOTERM_CC_FAT	GO:0044432	Endoplasmic reticulum part	7	0.005146712
GOTERM_CC_FAT	GO:0042598	Vesicular fraction	6	0.005266688
GOTERM_CC_FAT	GO:0031090	Organelle membrane	12	0.010678655
GOTERM_MF_FAT	GO:0009055	Electron carrier activity	8	1.03E-04
GOTERM_MF_FAT	GO:0020037	Heme binding	6	3.18E-04
GOTERM_MF_FAT	GO:0046906	Tetrapyrrole binding	6	4.27E-04
GOTERM_MF_FAT	GO:0019825	Oxygen binding	4	0.001224934
GOTERM_MF_FAT	GO:0005506	Iron ion binding	7	0.004012067
GOTERM_MF_FAT	GO:0070330	Aromatase activity	3	0.006670978
GOTERM_MF_FAT	GO:0048029	Monosaccharide binding	3	0.016542588
GOTERM_MF_FAT	GO:0015174	Basic amino acid transmembrane transporter activity	2	0.029220581
GOTERM_MF_FAT	GO:0030246	Carbohydrate binding	6	0.030112816
GOTERM_MF_FAT	GO:0008083	Growth factor activity	4	0.045016002

Only the top ten pathways were listed.

Table 3. KEGG pathways of overlapping genes of miR-18a-5p

Category	ID	Term	Genes	P
KEGG_PATHWAY	hsa00830	Retinol metabolism	CYP4A11, CYP2B6, ADH1B, CYP2A6, CYP1A2	7.82E-04
KEGG_PATHWAY	hsa00120	Primary bile acid biosynthesis	CYP39A1, CYP8B1, AKR1D1	0.006753459
KEGG_PATHWAY	hsa00982	Drug metabolism	CYP2B6, ADH1B, CYP2A6, CYP1A2	0.012377145
KEGG_PATHWAY	hsa00340	Histidine metabolism	AMDHD1, CNDP1, HAL	0.021425886
KEGG_PATHWAY	hsa00380	Tryptophan metabolism	KMO, CYP1A2, INMT	0.03899575
KEGG_PATHWAY	hsa00232	Caffeine metabolism	CYP2A6, CYP1A2	0.053812382
KEGG_PATHWAY	hsa04114	Oocyte meiosis	ADCY1, IGF1, IGF2, CAMK2B	0.054588385
KEGG_PATHWAY	hsa00980	Metabolism of xenobiotics by cytochrome P450	CYP2B6, ADH1B, CYP1A2	0.080266973
KEGG_PATHWAY	hsa05214	Glioma	PDGFRA, IGF1, CAMK2B	0.087287453

of precursor miR-18a in HCC. Moreover, this study analyzed expression levels of mature miR-18a-5p from microarrays in Gene Expression Omnibus (GEO) and ArrayExpress datasets, international databases with high-throughput data to accomplish new cognition

Table 4. Description of GEO microarrays in the present study

Dataset	First author	Country	Year	Platform	HCC			Non-cancerous liver			AUC
					N	Mean	SD	N	Mean	SD	
GSE57555	Taguchi Y	Japan	2015	GLP18044	5	-0.027433	0.025772	16	-0.048425	0.017063	0.0981
GSE69580	Hung C	Taiwan	2015	GLP10850	5	4.15107	3.766983	5	0.01	0	0.9000
GSE54751	Shen J	USA	2014	GLP18262	10	0.00672	0.005952	10	0.00206	0.000949	0.7750
GSE41874	Morita K	Japan	2013	GLP7722	6	1.674353	0.876924	4	0.423030	0.063714	1.000
GSE40744	Diaz G	USA	2013	GLP14613	26	3.891923	0.310944	19	4.037193	0.202711	0.7237
GSE21362	Sato F	Japan	2011	GLP10312	73	5.994827	1.965724	73	5.316681	1.279298	0.6077
GSE22058	Burchard J	USA	2010	GLP10457	96	0.699913	0.377676	96	0.282599	0.124170	0.8578
GSE10694	Li W	China	2008	GLP6542	78	11.591515	1.172031	88	10.972386	0.996767	0.7444
GSE21279	Hou J	China	2010	GLP9052	4	124.5	165.492	7	8.4286	3.99404	0.6071
GSE74618	Villanueva A	Spain	2016	GLP14613	218	2.691147	0.580318	10	2.381333	0.327875	0.6677
GSE12717	Su H	China	2008	GLP7274	10	10.850108	0.687869	6	9.163916	0.370029	1.000

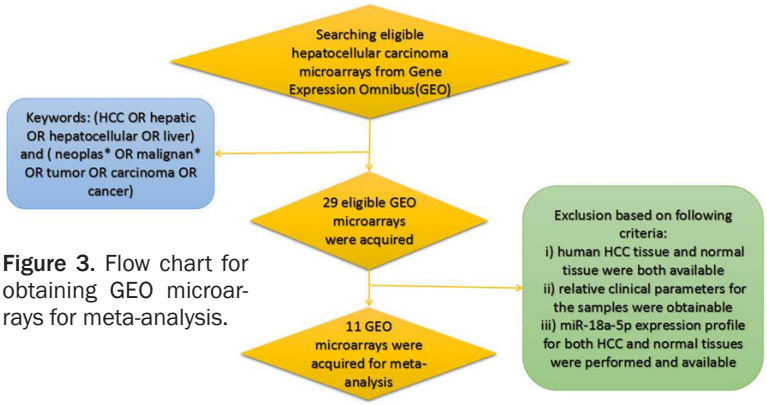

Figure 3. Flow chart for obtaining GEO microarrays for meta-analysis.

of molecular and genetic mechanisms of cancer using various genome technologies [43-47]. Afterward, comprehensive meta-analyses were performed to confirm expression levels of miR-18a-5p in HCC, combining all available high throughput data. The prospective molecular mechanism and regulatory network of miR-18a-5p in HCC was then investigated by performing GO and KEGG pathway analyses with predicted target genes. Finally, this study examined the protein-protein interaction (PPI) network to better understand related proteins of miR-18a-5p in HCC.

Methods and materials

Patients in TCGA database

Level 3 miRNA-Seq profiles and corresponding clinical information of HCC patients were downloaded from TCGA (http://cancergenome.nih.gov/). Data included miRNA profiling of 371 HCC samples and 50 non-cancerous liver samples analyzed on the Illumina HiSeq platform. This data was then log2-transformed for further evaluation. Expression levels of precursor miR-18a in HCC tissues and non-cancerous liver tissues were compared by independent-sample t-tests with SPSS 22.0 software. Receiver operating characteristic (ROC) curve was conducted to evaluate the distinguishing value of precursor miR-18a in HCC. Area under the curve (AUC) values > 0.7 implied a certain distinguishing value of the biomarker. Kaplan-Meier survival curves, including overall survival (OS) and diseased-free survival (DFS) with the log rank test, were used to estimate the prognostic value of precursor miR-18a in HCC patients. Additionally, hazard ratios (HRs) of survival curves were calculated by SPSS 22,0. Variation of expression of precursor miR-18a in different groups, according to clinical parameters of HCC patients, was analyzed using independent-sample t-tests.

Retrieval of HCC-related microarrays in GEO and ArrayExpress datasets

Eligible HCC microarrays were acquired from GEO and ArrayExpress up through December 30, 2017, according to the following searching keywords: (HCC OR hepatic OR hepatocellular OR liver) and (neoplas * OR malignan * OR tumor OR carcinoma OR cancer). Additionally, the microarrays met the following crite-

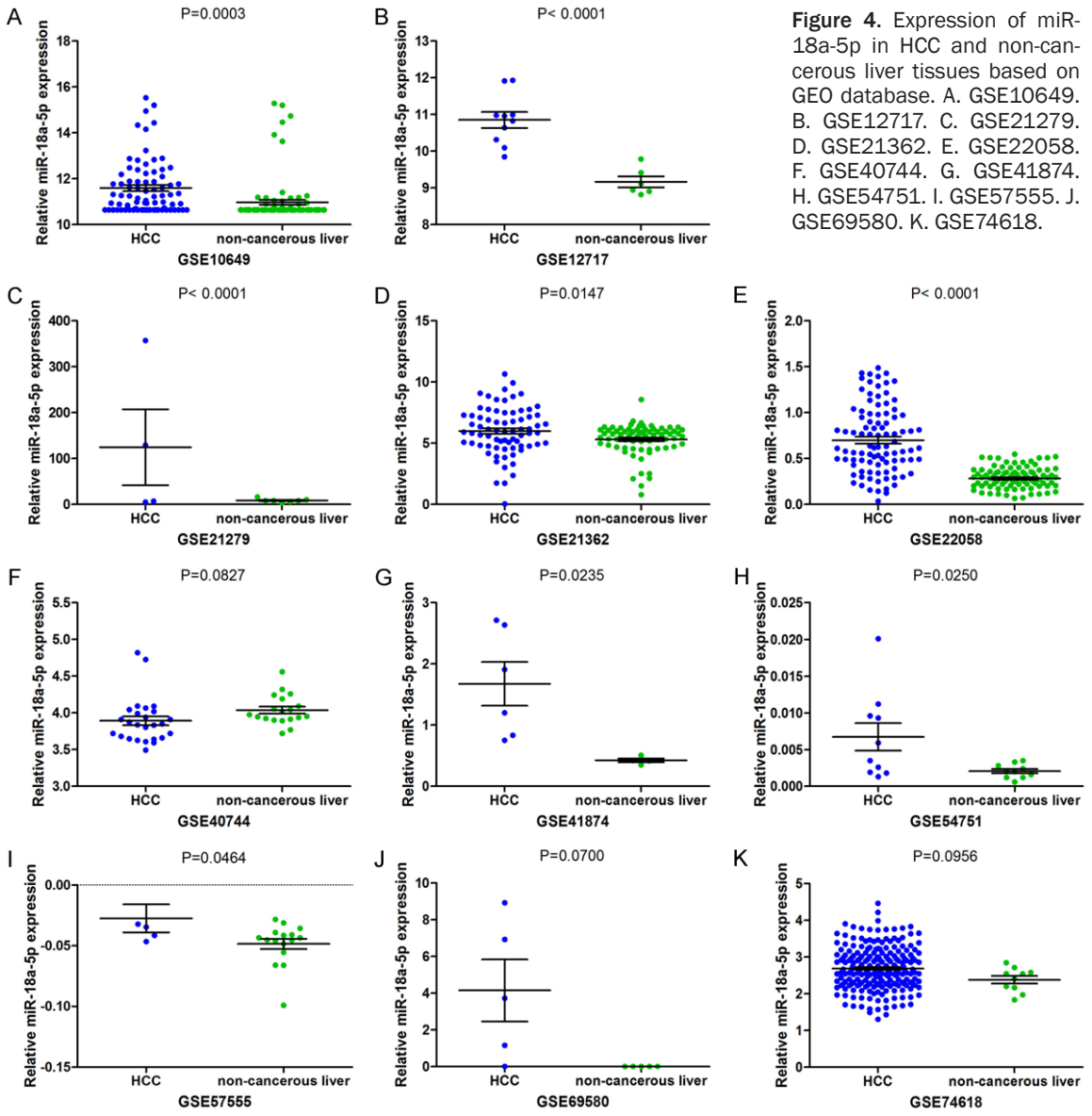

Figure 4. Expression of miR-18a-5p in HCC and non-cancerous liver tissues based on GEO database. A. GSE10649. B. GSE12717. C. GSE21279. D. GSE21362. E. GSE22058. F. GSE40744. G. GSE41874. H. GSE54751. I. GSE57555. J. GSE69580. K. GSE74618.

ria: 1) Human HCC and non-cancerous tissues were both available, 2) Relative clinical parameters for the samples were obtainable, and 3) miR-18a-5p expression profiles for both HCC and non-cancerous tissues were available for further meta-analysis.

Retrieval of differentially expressed genes (DEGs) of TCGA and GEO

DEGs of TCGA from Gene Expression Profiling Interactive Analysis (GEPIA) were acquired, according to the following condition: *p*-value < 0.05 and log2 fold change < 1. GEO datasets which contained mRNA profiling data for HCC were collected. To ensure uniformity of these microarray data, only microarrays carried out with the platform of GPL570 (Affymetrix Human Genome U133 Plus 2.0 Array) were selected. A total of 10 HCC microarrays sets were gained with human tissue samples for analysis by Gene-Cloud of Biotechnology Information (GCBI), according to the following criteria: *p*-value < 0.05 and fold-change < 1.5, as previously reported [48].

Retrieval of prospective target genes of miR-18a-5p

miRWalk databases were used to search prospective miR-18a-5p target genes with experimental validation. This database integrates the

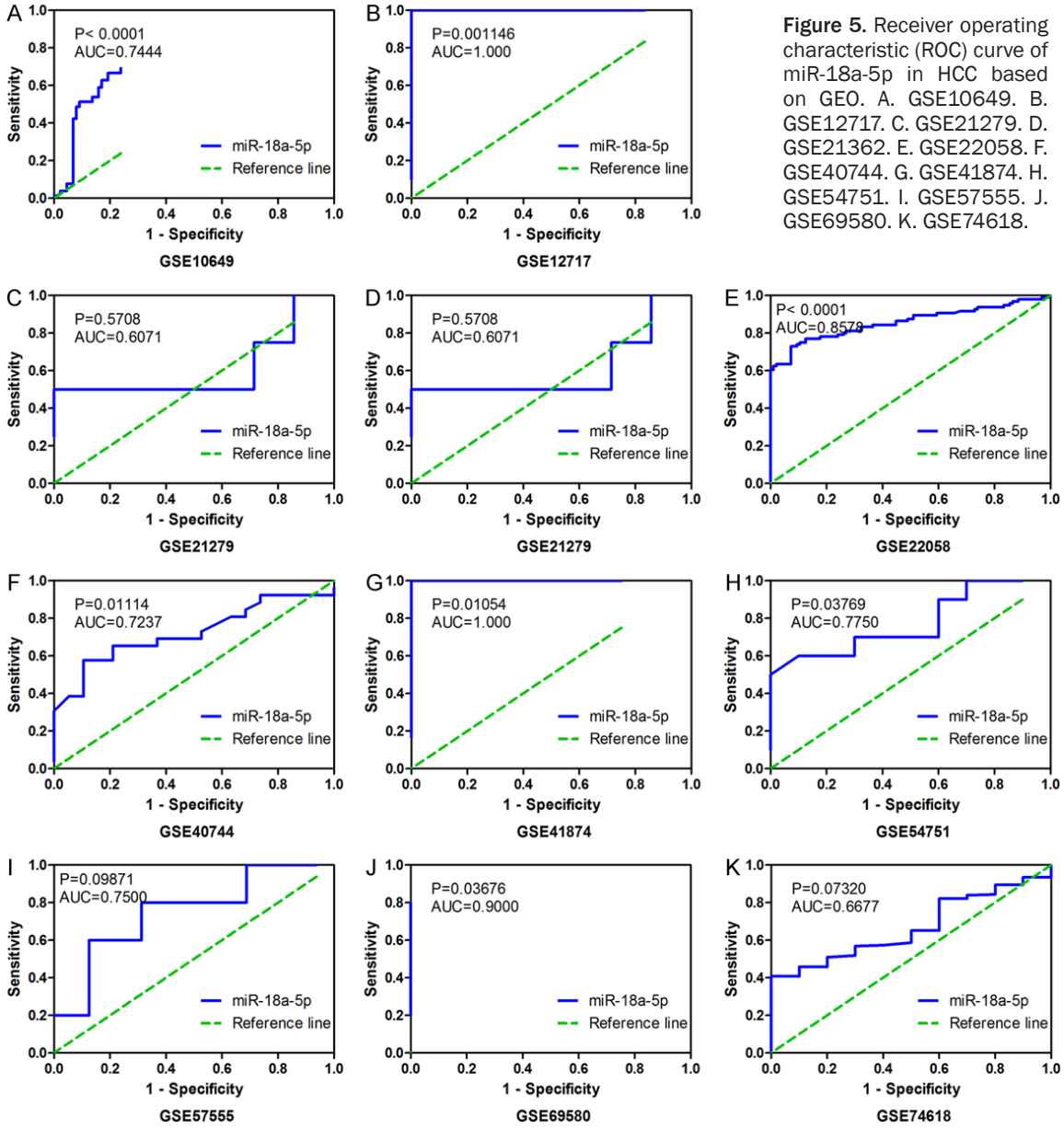

Figure 5. Receiver operating characteristic (ROC) curve of miR-18a-5p in HCC based on GEO. A. GSE10649. B. GSE12717. C. GSE21279. D. GSE21362. E. GSE22058. F. GSE40744. G. GSE41874. H. GSE54751. I. GSE57555. J. GSE69580. K. GSE74618.

following twelve online prediction tools: miRWalk2.0, MicroT4, miRanda, miRBridge, miRDB, miRMap, miRNAMap, PICTAR2, PITA, RNA22, RNAhybrid, and Targetscan. Furthermore, this study selected predicted genes appearing at least four times in the above-mentioned tools as predicted target genes of miR-18a-5p.

Bioinformatics analyses

This study determined the intersection of three groups of genes together, namely predicted genes, DEGs from TCGA, and DEGs from GEO, using Venn diagrams. GO and KEGG pathway enrichment analyses was performed using the bioinformatics tool DAVID, identifying possible pathways related to miR-18a-5p in HCC [49-52]. Additionally, hub target genes were acquired using the Search Tool for Retrieval of Interacting Genes/Proteins (STRING) version 9.1 database (http://string-db.org/). PPI network was conducted using Cytoscape software version 3.3.3 online tool [53]. Finally, expression levels of several potential targets were calculated based on TCGA data.

Statistical analyses for meta-analysis

Regarding meta-analyses, all statistics were conducted using STATA 12.0 with both stan-

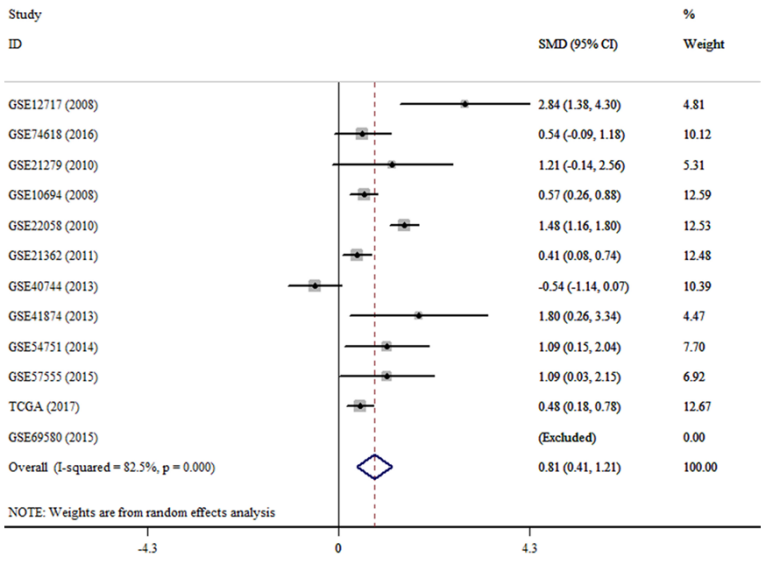

Figure 6. Forest plot evaluating expression of miR-18a-5p in HCC and non-cancerous liver tissues. Expression of miR-18a-5p was higher in HCC compared to non-cancerous controls.

dard mean difference (SMD) and summary receiver operating characteristic (sROC) approaches. Heterogeneity testing was used to assess the heterogeneity of included studies. I^2 > 50% indicated significant heterogeneity. Begg's and Deek's funnel plot asymmetry tests were used to estimate publication bias of included studies. *P*-values > 0.05 were considered to have no publication bias. Additionally, the method of sROC was applied to examine the prospective distinguishing value of miR-18a-5p in HCC.

Results

Clinical expression levels of precursor miR-18a expression in HCC

Comparison of expression of precursor miR-18a in HCC samples and non-cancerous samples revealed that there was a significantly higher expression level (6.2620±1.46955) in HCC tissues than in non-cancerous liver tissues (5.880±0.6416, P=0.0015, **Figure 1A**). Results of ROC analysis indicated that the AUC with 95% CI was 0.6468 (95% CI: 0.590-0.704, P=0.0007502, **Figure 1B**) for miR-18a in HCC. Elevated expression levels of precursor miR-18a were discovered in groups < 60 years old, female, and no smoking as compared to their matched groups (all P < 0.05, **Table 1**, **Figure 1C-E**). Additionally, survival analyses based on OS and DFS revealed that lower expression of precursor miR-18a was associated with better prognosis (OS: P=0.0315, **Figure 2A**; DSF: P=0.0054, **Figure 2B**). HRs were 1.139 (95% CI: 1.027-1.264) and 1.115 (95% CI: 0.985-1.263) for OS and DFS, respectively.

Meta-analysis based on GEO microarrays and TCGA

Ultimately, 11 GEO microarrays, including 531 cases of HCC and 334 controls, were acquired (**Table 4**, **Figure 3**). A total of 7 microarrays revealed a significant upregulated trend of miR-18a-5p in HCC (GSE10649, GSE12717, GSE21362, GSE22058, GSE41874, GSE54751 and GSE57555; all P < 0.05; **Figure 4**). Accordantly, 7 microarrays revealed a certain distinguishing value of miR-18a-5p in HCC (GSE10649, GSE12717, GSE22058, GSE40744, GSE41874, GSE54751, and GSE69580; all P < 0.05, AUC > 0.7, **Figure 5**). SMD of the meta-analysis revealed that miR-18a-5p was clearly increased in HCC, compared to noncancerous liver tissues (**Figure 6**). Results of the heterogeneity test with a random effects model were obvious (I^2=82.5%, P < 0.001). *P*-values of the Begg's and Deek's funnel plots were 0.087 and 0.33, respectively, implying no publication bias (**Figure 7A** and **7B**). In addition, the outcomes of DLR positive and DLR negative indicated that DLR positive was 6.76 (4.20-10.89), while DLR negative was 0.41 (0.30-0.57) (**Figure 8**). The diagnostic score was 2.80 (2.20-3.40) and diagnostic odds ratio was 16.46 (9.00-30.11) (**Figure 9**). Results of the prior and post probability showed that prior probability, post probability positive, and post probability negative were 20%, 63%, and 9%, respectively (**Figure 10**). AUC of sROC reached 0.88 (0.85-0.91), suggesting a certain distinguishing value of miR-18a-5p in HCC (**Figure 11**), and the sensitivity was 0.63 (0.50-0.74) when specificity was 0.91 (0.85-0.95) (**Figure 12**). This study attempted to collect relevant literature from another meta-analysis to confirm clinical findings based on high throughput data, however, no sufficient data could be collected.

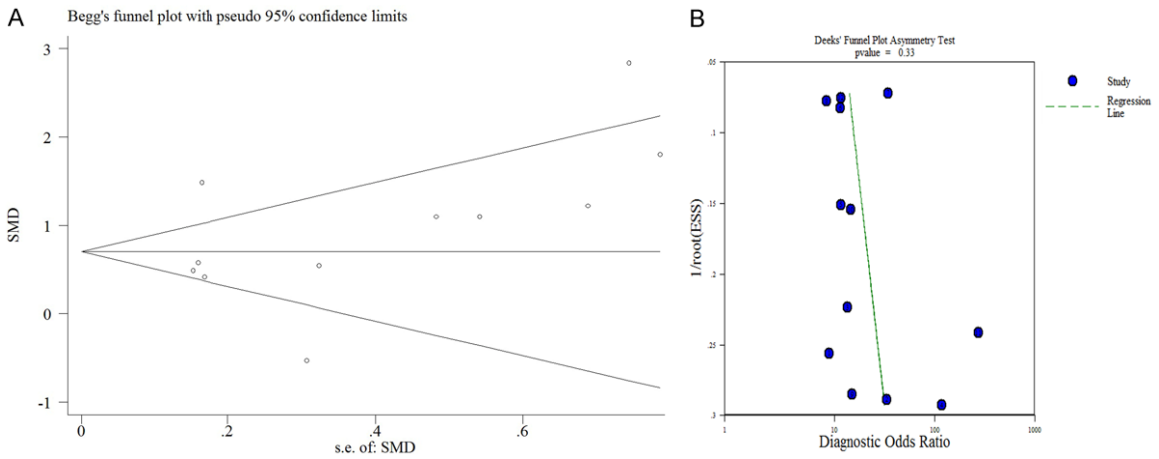

Figure 7. Publication bias of data extracted from TCGA and 11 GEO microarrays. A. Begg's funnel plot implied no publication bias in the present study. B. Deek's funnel plot implied no publication bias.

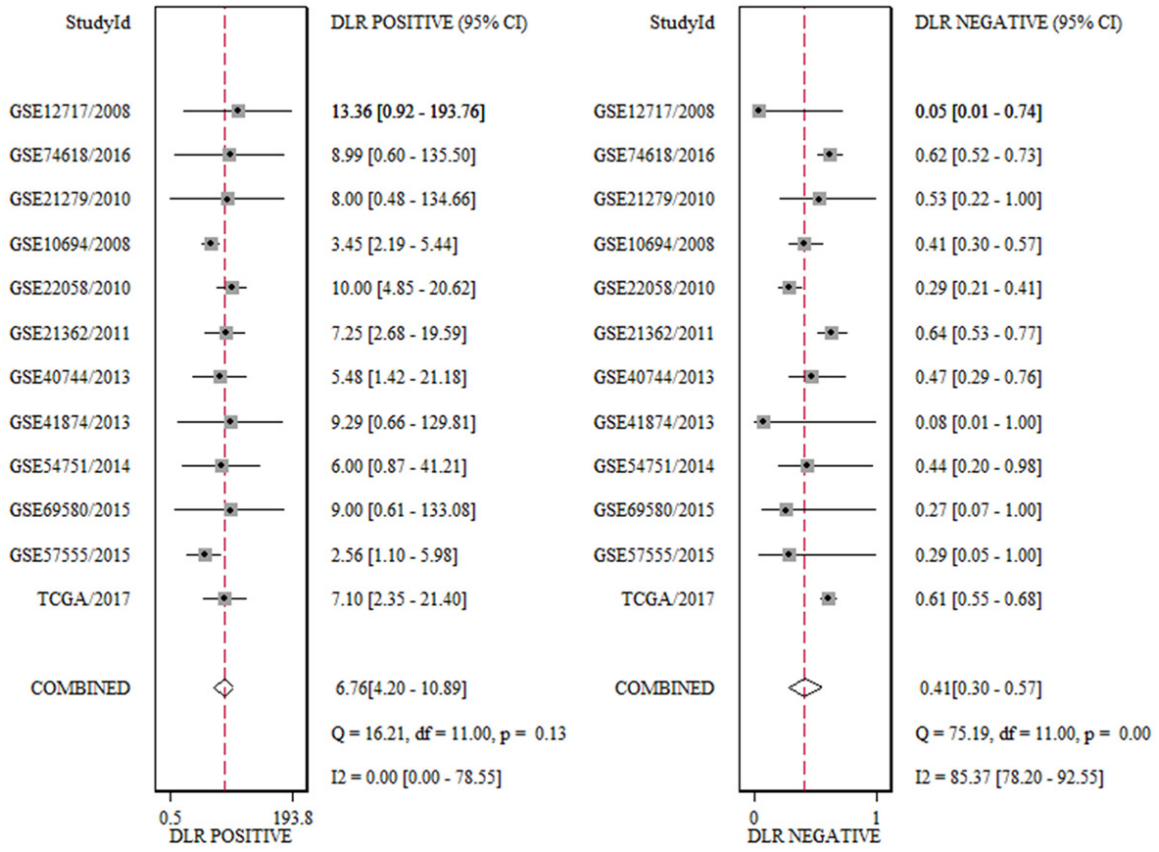

Figure 8. DLR positive and DLR negative of data extracted from TCGA and 11 GEO microarrays. DLR positive was 6.76 (4.20-10.89) and DLR negative was 0.41 (0.30-0.57).

In silico evaluations

Finally, 4,243 genes were obtained as predicted genes of miR-18a-5p, appearing at least four times among the 12 online prediction tools. Subsequently, a total of 733 DEGs of TCGA and 3,898 DEGs of GEO were acquired. Then, 78 overlapping genes were gathered

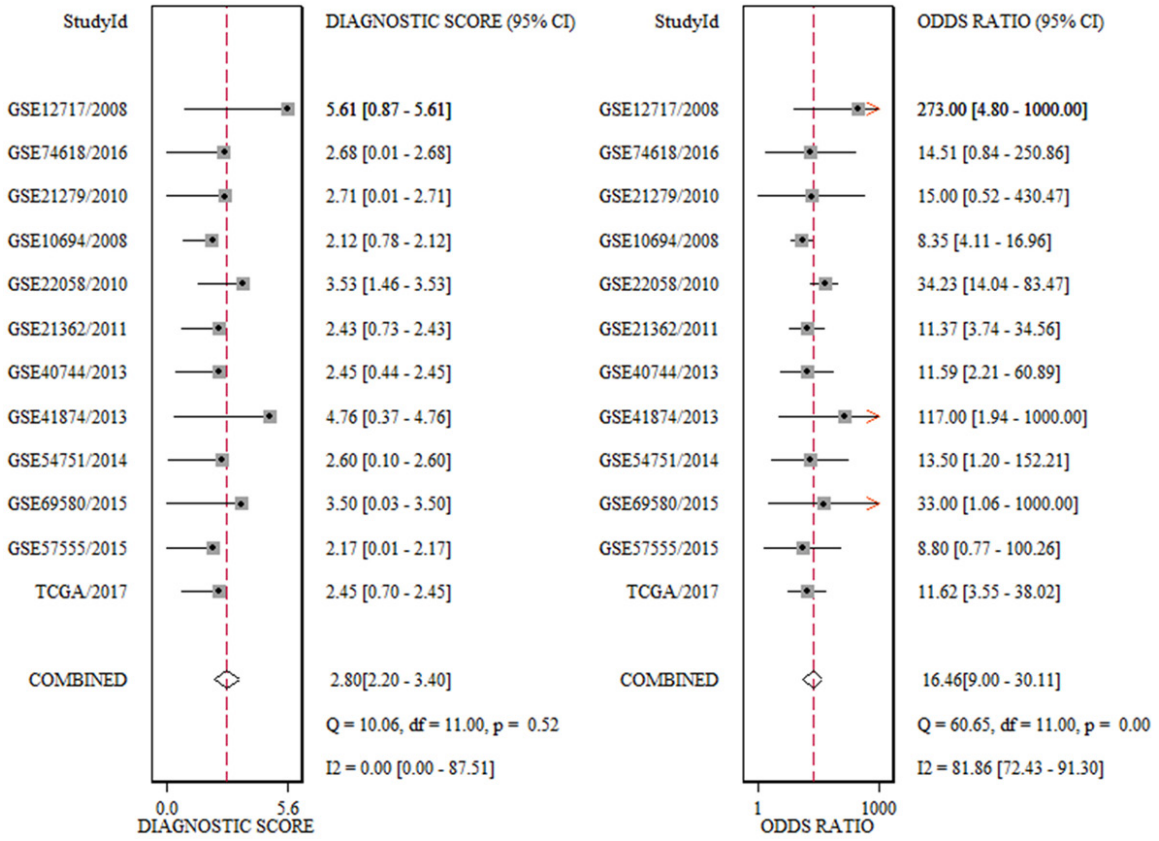

Figure 9. Diagnostic score and diagnostic odds ratio of data extracted from TCGA and 11 GEO microarrays. Diagnostic score was 2.80 (2.20-3.40) while diagnostic odds ratio was 16.46 (9.00-30.11).

from plotting the intersection of predicted genes and DEGs obtained from TCGA and GEO (**Figure 13**). These genes may play important roles in HCC. They were picked out for subsequent GO and KEGG pathway analyses.

GO analysis indicated the following centralized pathways for miR-18a-5p in HCC: striated muscle cell differentiation, cell fraction, and electron carrier activity (**Table 2**, **Figure 14A-C**). As for KEGG pathways, 'retinol metabolism' was the most centralized pathway for miR-18a-5p in HCC and five genes (CYP4A11, CYP2B6, ADH1B, CYP2A6, and CYP1A2) were involved in this pathway (**Table 3**, **Figure 14D**). In addition, expression levels of these genes were discovered to be predominantly lower in HCC than non-cancerous liver tissues, based on data extracted from TCGA (all $P < 0.05$, **Figure 15**). Moreover, a certain distinguishing value of these genes was found in HCC (all $P < 0.05$, AUC > 0.7, **Figure 16**). CXCL12 and IGF1 might be the most crucial target genes among the 78 overlapping genes according to the PPI network (**Figure 17**). Additionally, these two genes were downregulated in HCC ($P < 0.05$, **Figure 18A** and **18B**) and they owned a certain distinguishing value in HCC ($P < 0.05$, AUC > 0.7, **Figure 18C** and **18D**). Therefore, they might be hub target genes of miR-18a-5p in HCC.

Discussion

Upregulation of miR-18a-5p in cancer has been extensively reported in many studies. Elevated miR-18a-5p expression has been reported in various tumors, including ovarian cancer, gastric cancer, esophageal squamous cell carcinoma, breast cancer, prostate cancer, and lung adenocarcinoma [54-59]. Regarding expression levels of miR-18a-5p in HCC, there have been a few reports. Li CL et al. [60] reported that expression levels of miR-18a-5p were notably upregulated in HBV patients (n=15) with HCC compared with healthy controls (n=15) ($p < 0.01$). Additionally, Wang Y et al. [61] showed that miR-18a-5p was also upregulated in 23 cases of small HCCs infected with

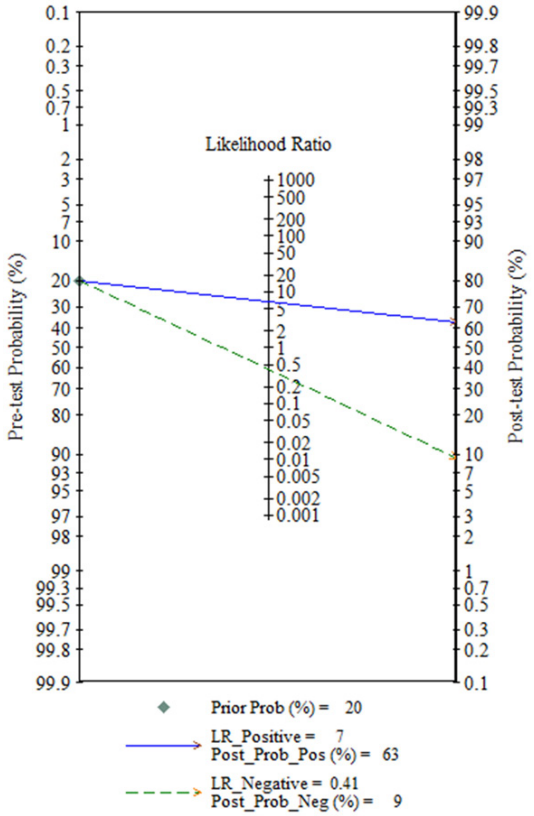

Figure 10. Prior probability and post probability of data extracted from TCGA and 11 GEO microarrays. Prior probability, post probability positive, and post probability negative were 20%, 63%, and 9%, respectively.

HBV, compared with that in 20 samples of cirrhosis, 20 samples of liver tissues from chronic hepatitis B infectors, and 16 samples from healthy donors. Liu L et al. [40] revealed similar upregulated patterns of miR-18a-5p in 52 cases of HCC tissues, compared to their matched adjacent tumor tissues. They further observed higher miR-18a-5p expression in several HCC cell lines, including Hep3B, Bel-7402, Huh7, and SK-hep-1 cells, compared to normal liver cell LO2. However, sample sizes in these previous studies were quite small and results require confirmation by further investigation. This present study first profiled the miRNA data of 371 HCC samples and 50 non-cancerous liver samples, evaluating them using the Illumina HiSeq platform and microarrays from GEO database. In line with previous findings, this study detected that expression of both precursor miR-18a and mature miR-18a-5p was markedly higher in HCC compared with non-cancerous liver tissues, based on TCGA and most of the GEO data. More importantly,

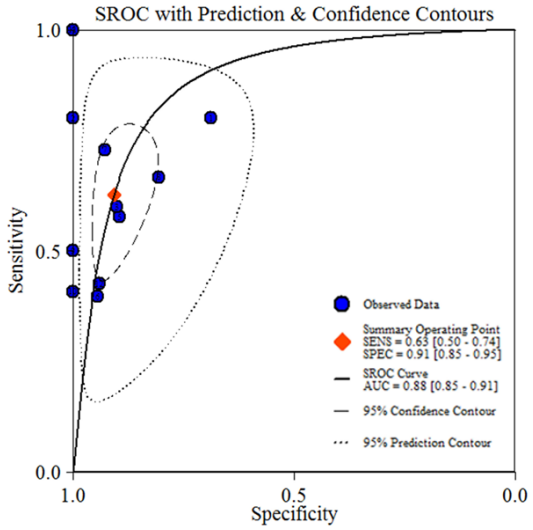

Figure 11. Summary receiver operating characteristic (SROC) curve of miR-18a-5p in HCC. AUC reached 0.88 (0.85-0.91), implying a certain distinguishing value of miR-18a-5p in HCC.

results from this comprehensive meta-analyses, including 902 HCC cases, indicated that levels of miR-18a-5p were obviously elevated in HCC tissues. Additionally, AUC of sROC being 0.88 suggested a certain distinguishing value of miR-18a-5p in HCC. This study is the first to mine high throughput data of miRNA-seq and miRNA microarrays to reveal clinical expression levels and prospective roles of miR-18a-5p in HCC. Together with previous reports, these current findings further support the oncogenic character of miR-18a-5p in the carcinogenesis of HCC.

Referring to the prognostic implication of miR-18a-5p in HCC, there has been only one study. Morita K et al. [42] demonstrated that increased expression of miR-18a-5p was related to a high recurrence rate in HCC patients after living donor liver transplantation (LDLT). Consistent with Morita K et al., the current study also verified the risky role of miR-18a-5p via analyses with both the OS and DSF of HCC patients, suggesting that elevated levels of miR-18a-5p could lead to unfavorable outcomes of HCC patients, independent of therapeutic strategies. Detection of miR-18a-5p in HCC tissues may be applied in clinic to predict the prognosis of HCC patients.

The regulatory function of miR-18a-5p may partially explain its prognostic role in HCC, which requires various potential targets, possible

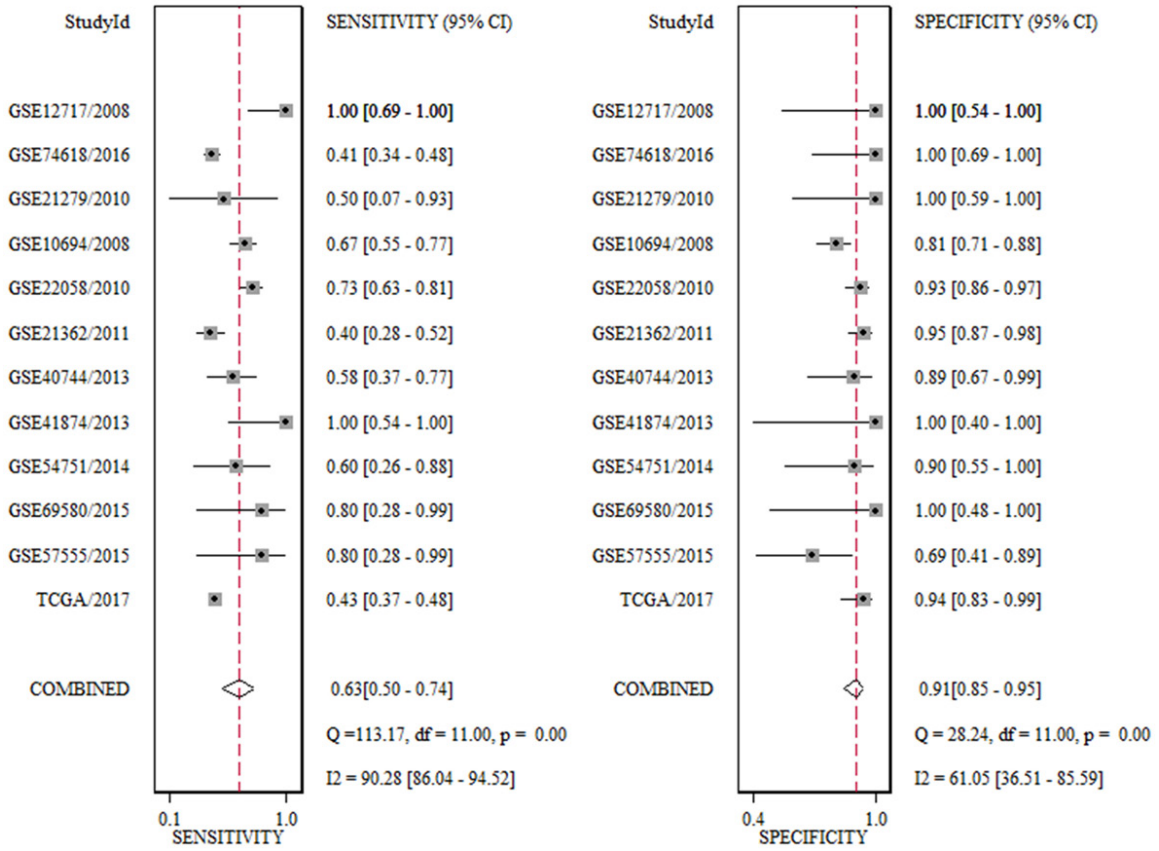

Figure 12. Sensitivity and specificity of data extracted from TCGA and 11 GEO microarrays. Sensitivity was 0.63 (0.50-0.74) and specificity was 0.91 (0.85-0.95).

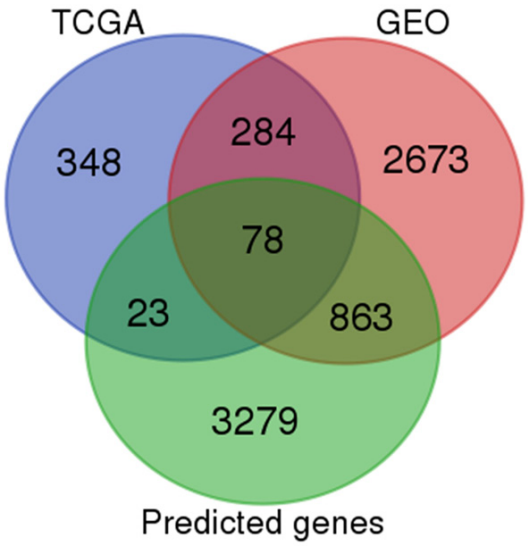

Figure 13. Venn diagram showing the intersection of genes from TCGA, GEO, and predicting target genes.

pathways, and interactions with distinct targets. Liu L et al. [62] reported that KLF4 was downregulated in HCC tissues and miR-18a-5p might play a vital role in promoting metastasis and proliferation of HCC via targeting KLF4. Besides, some target genes of miR-18a-5p have been reported in prior studies, such as TNFAIP3 [42], p53 [60], and K-ras [63]. However, there might be plenty of target genes needing to be excavated. Indeed, 78 possible targets were predicted using online databases and bioinformatics analyses. To further examine the prospective pathways of these genes, KEGG analysis showed that 'retinol metabolism' was the most enriched pathway of miR-18a-5p in HCC, including five genes: CYP4A11, CYP2B6, ADH1B, CYP2A6 and CYP1A2. Interestingly, these five genes were downregulated in HCC tissues, further supporting the probability of their targeting relationships. Modification of retinol metabolism has been well studied in hepatic stellate cell activation and fatty liver disease [64, 65]. However, no studies are available concerning the function of the pathway of 'retinol metabo-

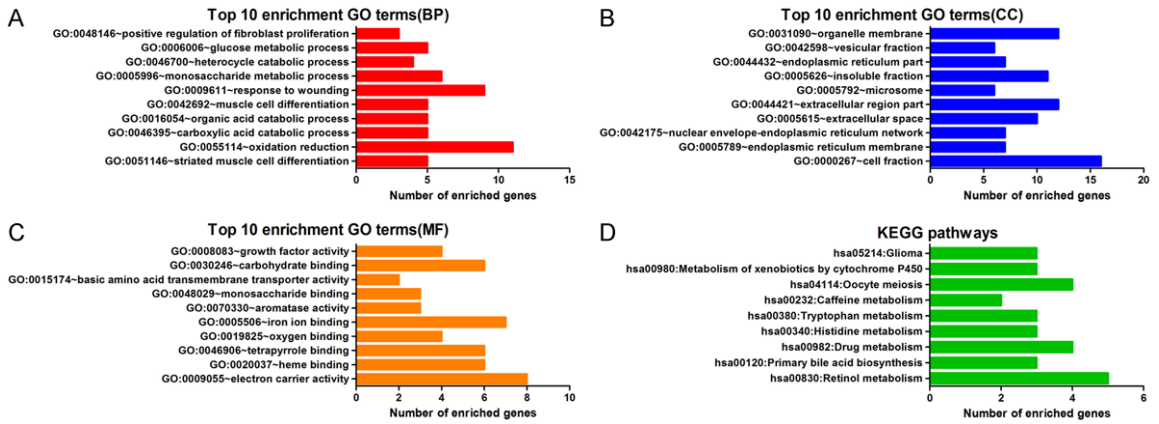

Figure 14. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses of 78 overlapping genes. A. Top 10 enrichment GO terms of biological process (BP). B. Top 10 enrichment GO terms of cellular component (CC). C. Top 10 enrichment GO terms of molecular function (MF). D. KEGG pathway.

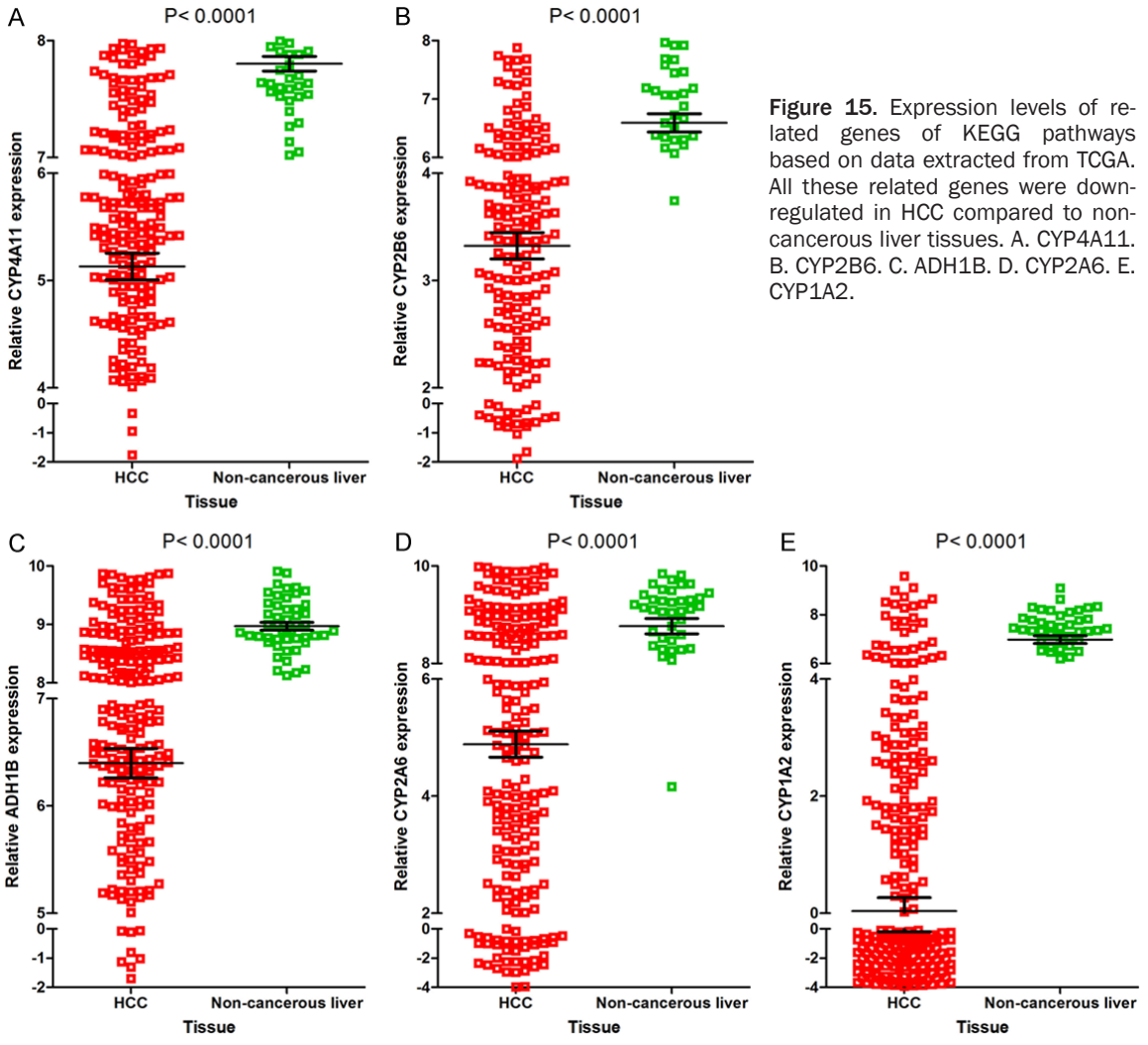

Figure 15. Expression levels of related genes of KEGG pathways based on data extracted from TCGA. All these related genes were down-regulated in HCC compared to non-cancerous liver tissues. A. CYP4A11. B. CYP2B6. C. ADH1B. D. CYP2A6. E. CYP1A2.

lism' in HCC. Hence, the molecular mechanism whereby miR-18a-5p targets the pathways of 'retinol metabolism' requires further in-depth investigation.

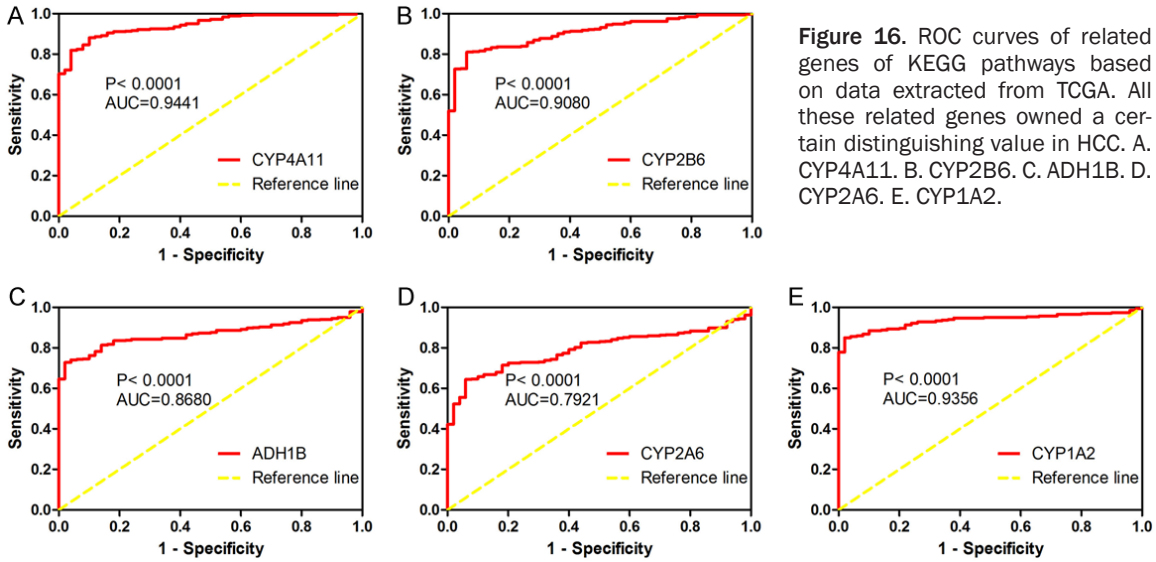

Figure 16. ROC curves of related genes of KEGG pathways based on data extracted from TCGA. All these related genes owned a certain distinguishing value in HCC. A. CYP4A11. B. CYP2B6. C. ADH1B. D. CYP2A6. E. CYP1A2.

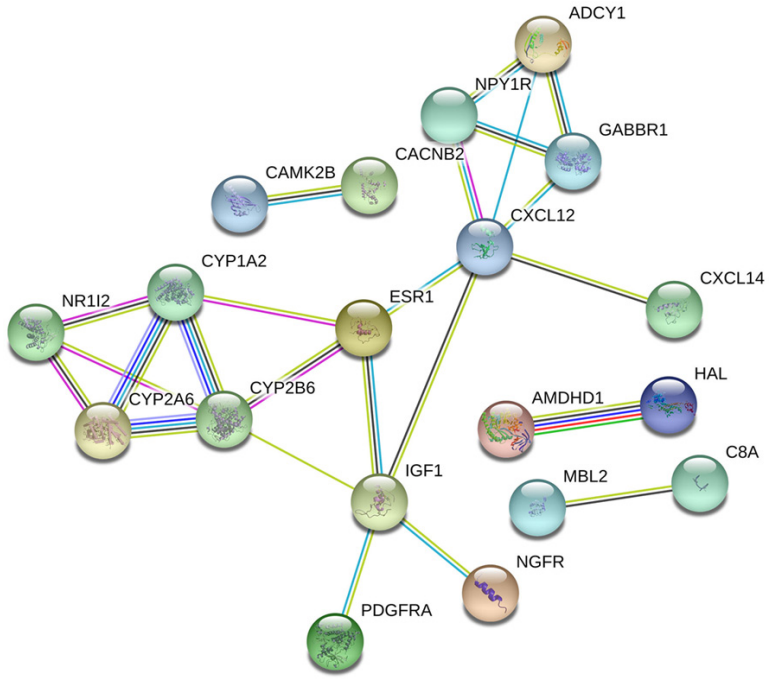

Figure 17. Protein-protein interaction (PPI) network of 65 target genes. Nodes represent target genes. The size and color represent the connectivity. The solid and dashed lines represent interaction with proportional combined score.

Additionally, PPI network analysis was conducted. This study identified 2 hub target genes (CXCL12 and IGF1) for miR-18a-5p in HCC. CXCL12 is also referred as C-X-C motif chemokine ligand 12. CXCL12 encodes a protein which plays a vital role in the growth and metastasis of tumors. With regards to functions of CXCL12 in HCC, it has been demonstrated that Interstitial Fluid Flow (IFF) could promote the invasion of HCC via CXCR4/CXCL12 signaling [66]. Additionally, CXCL12 is crucial in boosting the invasion, progression, growth, and angiogenesis of HCC [67]. IGF1 is also known as insulin like growth factor 1. It encodes a protein owning analogical structure and functions with insulin. Regarding the effects of IGF1 in HCC, it may enhance the proliferation of HCC cells [68]. Simultaneously, IGF1 possesses a key effect in suppressing the invasion and migration of HCC cells. Expression of IGF1 was downregulated in HCC [69] and IGF1 might be a biomarker in HCC. Herein, downregulation of CXCL12 and IGF1 were both found in HCC tissues, compared with non-cancerous liver tissues, based on data extracted from TCGA. Thus, this study hypothesized that miR-18a-5p might feature pivotal functions in HCC through targeting CXCL12 and IGF1, according to reverse correlation between miRNA and potential targets. However, further investigation is surely needed to estimate the molecular mechanisms between CXCL12, IGF1, and miR-18a-5p in HCC.

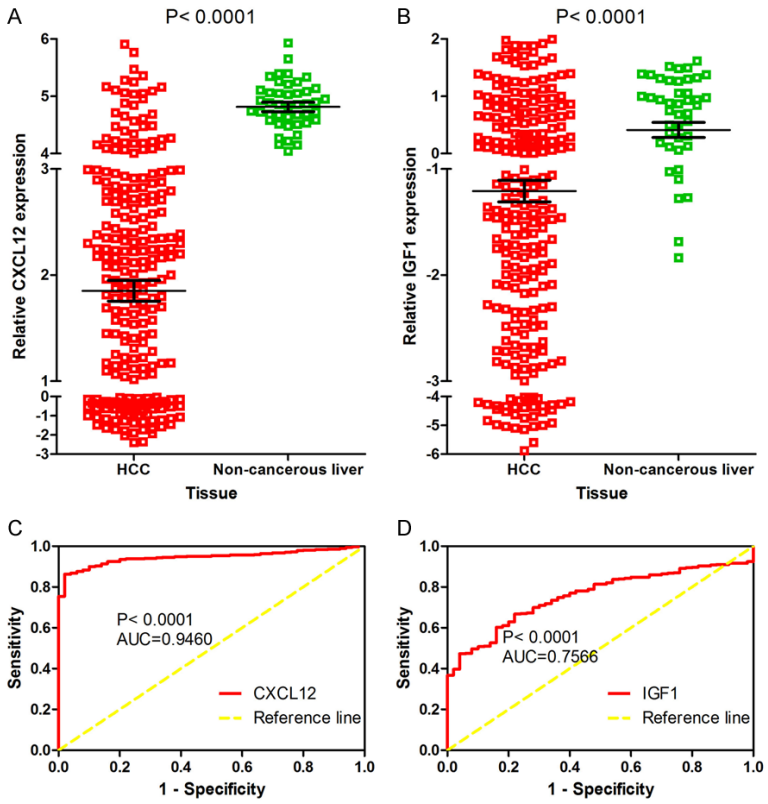

Figure 18. Clinical expression levels and their relevance of two potential targets obtained from PPI network. A. Relative CXCL12 expression. B. Relative IGFA expression. C. ROC of CXCL12. D. ROC of IGF1.

Conclusion

In summary, upregulation of miR-18a-5p may function as a promoting factor in incidence and development of HCC. Higher miR-18a-5p levels may predict worse prognosis for HCC. Furthermore, two hub genes (CXCL12 and IGF1) might be crucial novel target genes of miR-18a-5p in HCC.

Disclosure of conflict of interest

None.

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