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Original Article

Down-regulation and clinical role of miR-30a-5p in hepatocellular carcinoma: a study based on public high-throughput datasets

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Abstract: Although some recent studies have shown the aberrant level of miR-30a-5p in a number of classes of malignancies, the level and clinical role of miR-30a-5p in hepatocellular carcinoma (HCC) have not been fully elucidated. Accordingly, the study was conducted to investigate the clinicopathological contribution of miR-30a-5p expression in HCC through The Cancer Genome Atlas (TCGA) database and NCBI Gene Expression Omnibus (GEO) database. The TCGA database was used to explore the expression of miR-30a-5p in HCC-related miRNA-HiSeq datasets, as well as relevance between miR-30a-5p and clinicopathological features in HCC. Receiver operating characteristic (ROC) curve was performed to prove the diagnostic value of miR-30a-5p in HCC patients. Furthermore, meta-analysis was performed based on HCC-related miRNA microarray datasets collected from the GEO database. Quality assessment was carried out with Limma package and ExiMiR package in R. Standardized mean difference (SMD) with 95% confidence intervals (CIs) was calculated and pooled with STATA 13.0. Decreased expression of miR-30a-5p in HCC tissues was confirmed via TCGA data (16.30 ± 1.28 in HCC versus 17.36 ± 0.50 in normal liver, P<0.001). Expression of miR-30a-5p was negatively correlated to advanced grade (r=-0.109, P=0.039). The area under curve (AUC) of miR-30a-5p was 0.795 (P<0.001). Besides, in the meta-analysis, nine miRNA microarray datasets including 304 HCC samples and 321 non-cancerous liver tissues were employed. The pooled results calculated by fixed-effect model indicated that miR-30a-5p was also evidently downregulated in HCC tissues (SMD=-0.495; 95% CI, -0.656 to -0.335; P<0.001) with little heterogeneity (P=0.152, I^2=33.3%). In conclusion, miR-30a-5p is a tumor-suppressive miRNA that plays a pivotal part in the carcinogenesis and deterioration of HCC.

Keywords: MiR-30a-5p, HCC, TCGA, GEO, meta-analysis, microarray datasets

Introduction

Globally, hepatocellular carcinoma (HCC) is a widespread malignant tumor, which presently poses a main hazard to the health of the human race. All over the whole world, HCC ranks the fifth of the most prevalentcancers in males, and the seventh in females [1]. It was confirmed that the development of HCC is a multi-step process involving numerous factors, among which hepatitis B or C virus and cirrhosis are the main risk factors [2, 3]. Nonetheless, a large part of HCC victimsis diagnosed at an advanced stage, thus failing to seize the best treatment time. Despite divergent therapeutic regimes such as surgery, chemotherapy, radiation and molecular targeted therapies, the overall 5-year survival rate for HCC remains only 5%-6% [4]. Therefore, it is an urgent need to analyze and identify the molecular aspects of HCC to improve its control.

MicroRNAs (miRNAs), small non-protein-coding RNAs, possesses approximately 20-24 nucleotides. MiRNAs can modulate the expression of genes post-transcriptionally by restraining translation or fostering degradation of target mRNAs [5]. Accumulating evidence has shown

that miRNAs control wide physiological and pathological processes, including carcinogenesis and tumor progression [6]. Deregulation of miRNA expression has been inferred in many cancers including HCC. Many studies have unveiled aberrant level of a large number of miRNAs between HCC tissues and non-cancerous liver tissues, indicating that miRNAs can behave as both tumor suppressive genes and oncogenes in the biological processes of HCC [7]. Today, with the development of high-throughput technologies, large amounts of cancer data are available through public database, which provide rich information on comparison of aberrant miRNA level between HCC tissues and non-cancerous liver tissues and on relationship between miRNA levels and the corresponding clinical parameters of the HCC patients. The Cancer Genome Atlas (TCGA) database is a collaborative program that features massive data in public describing tumor tissues and corresponding normal tissues from patients on a large scale, which has been used widely by the research community. Meanwhile, the NCBI Gene Expression Omnibus (GEO) database [8] is an international public repository that provides a robust and versatile database, storing high-throughput functional genomic data submitted by the research community all over the world. Thus, we cantake full advantage of this information to conduct an investigation into the expression and clinical role of an interested miRNA in HCC.

Previous studies have reported the aberrant status of miR-30a-5p in osteosarcoma [9], renal carcinoma [10] and lung cancer [11]. As for HCC, only two studies reported the clinical role of miR-30a-5p. Liu et al. [12] described that miR-30a-5p was notably under-expressed in HCC tissues compared with adjacent non-cancerous tissues. Li et al. [13] pointed out that the miR-30a-5p level decreased in HCC tissues based on the detection of 16 matched pairs of HCC tissues and their adjacent non-cancerous hepatic tissues. As for the link between miR-30a-5p expression and clinicopathological aspects in HCC, only one study has been available so far. Liu et al. [12] reported a relation between decreased level of miR-30a-5p and deterioration of HCC based on 63 cases HCC tissues. However, the small sample size may limit the reliability of the conclusion. Thus, the level and clinical role of miR-30a-5p in HCC tissues needs further investigation. Hence, by using TCGA datasets, we investigated the expression level of miR-30a-5p in HCC tissues and non-cancerous liver tissues, and evaluated the correlation between miR-30a-5p expression and clinicopathological features. Furthermore, by using GEO miRNA microarray datasets, we also validated the result of the TCGA.

Materials and methods

Downloading and processing TCGA dataset

In order to probe the association between miR-30a-5p expression and clinical parameters, a level three miRNA expression profile generated from 50 adjacent non-cancerous tissues and 361 HCC tissues was download from The Cancer Genome Atlas (www.cancergenome.nih.gov) on 10th April 2016. The miRNA expression value was generated by using Illumina HiSeq 2000 miRNA sequencing platforms (Illumina Inc, San Diego, CA) and established as reads per million miRNA mapped (RPM) data. The values of miR-30a-5p expression were extracted and log2-transformed for further analysis. In addition, the corresponding clinical information was also obtained from TCGA directly. Further, the gene alterations including mutation detection and amplification of miR-30a-5p across HCC samples was performed by cBioPortal [14] (http://cbioportal.org).

Microarray datasets searching

In the present study, the HCC-related miRNA microarray datasets were collected from GEO (http://www.ncbi.nlm.nih.gov/geo/) which was published before 10th March 2016. The keywords used in the searching strategies were: HCC, liver, hepatocellular, hepatic; malignant*, cancer, tumor, tumor, neoplasm*, carcinoma; miRNAs and non-coding RNAs.

Inclusion criteria

Eligible datasets were included by the following criteria: (i) the subjects should be humans; (ii) the samples of case group and the control group were HCC tissues versus non-cancerous liver tissues; (iii) both case group and control group contained more than 2 samples; (iv) the expression profiling data of miRNA were available or calculable. In the present study, non-cancerous liver tissues included liver tissues

Table 1. Relationship between miR-30a-5p level and clinicopathological features in HCC (TCGA data)

			Mean ± SD	t	*P*-value
Tissues	Normal liver	50	17.36 ± 0.50	10.51	<0.001***[a]
	Non-HCC liver cancers	10	17.38 ± 1.81	-0.03	0.98[b]
	HCC	361	16.30 ± 1.28	10.84	<0.001***[c]
	Liver cancer	371	16.33 ± 1.30	2.60	<0.01**[d]
Age	<60	166	16.27 ± 1.29	-0.55	0.58
	≥60	194	16.34 ± 1.26		
Gender	Male	246	16.33 ± 1.16	0.62	0.54
	Female	115	16.23 ± 1.50		
Race	Asian	159	16.14 ± 1.34	F=1.56	0.20
	White	173	16.40 ± 1.24		
	Black or African American	17	16.48 ± 1.30		
	American Indian or Alaska native	2	17.20 ± 0.05		
AJCC Pathologic T	T1	177	16.29 ± 1.23	F=0.66	0.62
	T2	89	16.37 ± 1.22		
	T3	79	16.14 ± 1.45		
	T4	13	16.64 ± 1.24		
AJCC Pathologic N	N0	247	16.26 ± 1.23	F=1.16	0.32
	N1	3	15.43 ± 1.07		
	NX	110	16.40 ± 1.36		
AJCC Pathologic M	M0	262	16.27 ± 1.23	F=0.22	0.80
	M1	4	16.55 ± 0.27		
	MX	95	16.36 ± 1.43		
Pathological grading	G1-G2	222	16.41 ± 1.21	2.42	0.02*
	G3-G4	135	16.08 ± 1.32		
Stage	Stage I-II	249	16.29 ± 1.20	1.03	0.30
	Stage III-IV	86	16.13 ± 1.44		
Vascular invasion	No	199	16.41 ± 1.21	1.59	0.11
	Yes	107	16.17 ± 1.37		
Metastasis	None	199	16.41 ± 1.21	F=1.69	0.19
	Micro	90	16.22 ± 1.42		
	Macro	17	15.91 ± 1.05		
Recurrence	No	166	16.27 ± 1.23	-0.34	0.74
	Yes	94	16.32 ± 1.16		
Smoking	-	326	16.32 ± 1.27	0.93	0.35
	+	17	16.03 ± 1.12		
HBV	-	237	16.39 ± 1.31	1.86	0.06
	+	106	16.12 ± 1.14		
HCV	-	289	16.25 ± 1.24	-1.78	0.08
	+	54	16.59 ± 1.36		
Alcohol consumption	-	226	16.30 ± 1.25	-0.05	0.96
	+	117	16.31 ± 1.30		
Alcoholic Fatty Liver Disease	-	324	16.28 ± 1.28	-1.58	0.12
	+	19	16.75 ± 0.86		
Cirrhosis	-	337	16.33 ± 1.24	1.36	0.23
	+	6	15.17 ± 2.07		
Ishak fibrosis score	0 - No Fibrosis	73	16.40 ± 1.17	F=0.07	0.99
	1, 2 - Portal Fibrosis	30	16.31 ± 1.42		
	3, 4 - Fibrous Speta	30	16.36 ± 1.04		
	5 - Nodular Formation and Incomplete Cirrhosis	8	16.23 ± 1.60		
	6 - Established Cirrhosis	71	16.40 ± 1.10		
Child Pugh classification grade	A	206	16.35 ± 1.23	F=0.81	0.45
	B	19	15.97 ± 1.19		
	C	1	16.29		

N number, *SD* stander deviation, *T* AJCC Pathologic Tumor, *N* AJCC Pathologic Node, *M* AJCC Pathologic metastasis, *HBV* hepatitis B virus, *HCV* hepatitis C virus. **P*<0.05, ***P*<0.01, ****P*<0.001. [a]Normal liver vs liver cancer (including HCC and non-HCC liver cancers). [b]Normal liver vs non-HCC liver cancers. [c]Normal liver vs HCC. [d]Non-HCC liver cancers vs HCC.

Table 2. Characteristics of miR-30a-5p level profiling datasets included in the meta-analysis

Study information				Sample				Array and annotation information
Series	Country	Citation (ref.)	Year	Case tissues	Samples of case group	Control tissues	Samples of control group	Platform
GSE69580	China (Taiwan)	Hung et al.	2015	HCC	5	Healthy liver tissues	5	GPL10850
GSE57555	Japan	Murakami et al.	2015	HCC#	5	Adjacent non-cancerous liver	5	GPL18044
GSE54751	USA	Shen et al.	2014	HCC	10	Adjacent non-cancerous liver	10	GPL18262
GSE41874	Japan	Morita et al.	2013	HCC	3	Adjacent non-cancerous liver	3	GPL7722
GSE40744	USA	Diaz et al.	2013	HCC	26	Non-cancerous liver tissues	37	GPL14613
GSE21362	Japan	Sato et al.	2011	HCC	73	Adjacent non-cancerous liver	73	GPL10312
GSE22058	USA	Burchard et al.	2010	HCC	96	Adjacent non-cancerous liver	96	GPL10457
GSE12717	China	Su et al.	2008	HCC	10	Healthy liver tissues	6	GPL7274
GSE10694	China	Li et al.	2008	HCC	78	Non-cancerous liver tissues*	88	GPL6542
Total					547		564	

#Only HCC and its adjacent non-cancerous liver tissues were included. *Non-cancerous liver tissues included adjacent non-cancerous liver and healthy liver tissues.

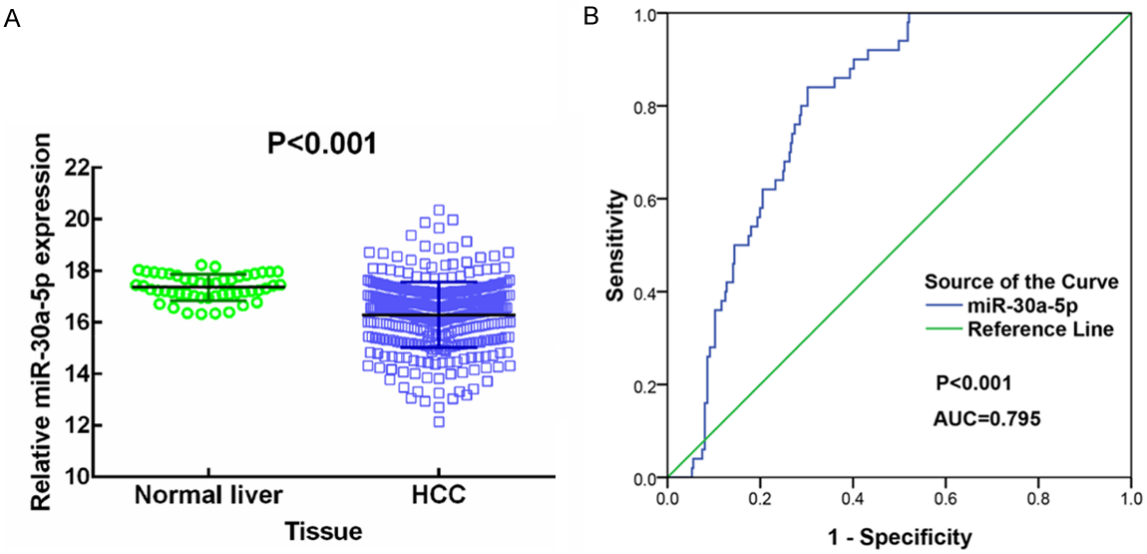

Figure 1. Level of miR-30a-5p in HCC tissues and adjacent non-cancerous tissues. Level of miR-30a-5p in HCC tissues (361 cases) and adjacent non-cancerous tissues (50 cases) were derived from TCGA database. Significance of difference between two groups was analyzed by student's t test. Error bars represented SD. A. The different expression of miR-30a-5p in HCC and adjacent non-cancerous tissues (P<0.001); B. ROC curve of miR-30a-5p expression to distinguish HCC from adjacent non-cancerous tissues. The AUC was 0.795 (95% CI, 0.747 to 0.844, P<0.001).

adjacent to HCC and liver tissues from healthy donors.

Quality control and data extraction

Three investigators (Hanlin Wang, Haiwei Liang and Gang Chen) extracted the data independently. The raw CEL files were downloaded directly from the GEO website. Quality assessment was carried out with Limma package and ExiMiR package in R, including background correction and normalization processing [15, 16]. If multiple probes were applied to detect one miRNA, the average value of probes was considered as the relevant level of the miRNA. The following data were extracted: (i) the expression values of miR-30a-5p; (ii) the sample size in both case groups and control groups; (iii) the clinical parameters; (iv) year of publication; (v) the country of the microarray dataset.

Statistical analysis

For TCGA or GEO data, the statistical analysis was carried out by SPSS 22.0. Quantitative variable was summarized as means ± standard

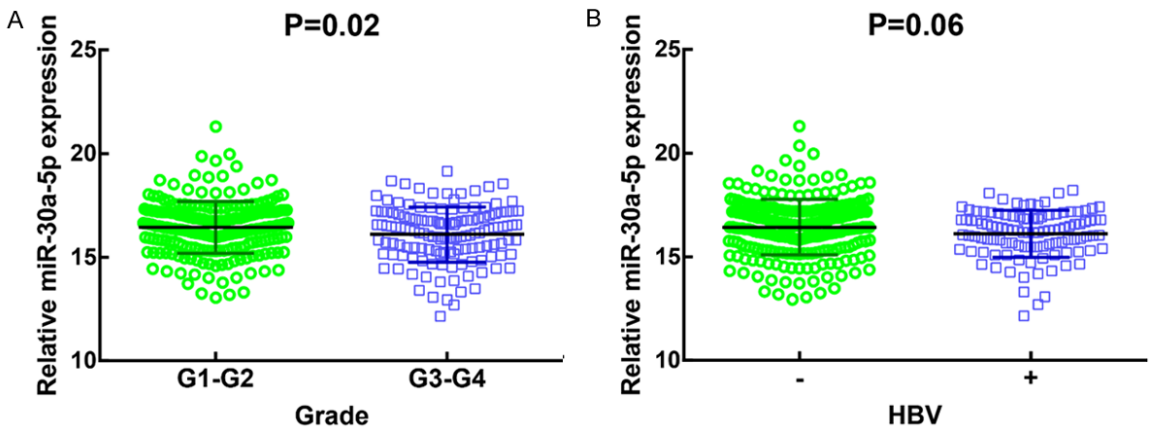

Figure 2. The relationship between the expression of miR-30a-5p and clinicopathological features. The expression data of miR-30a-5p in 361 cases HCC patients was derived from TCGA dataset. The corresponding clinical information was obtained through the cBioPortal. Data was showed as mean ± SD. Significance of difference between two groups was analyzed by student's *t* test. A. Grade of HCC patients; B. HBV infection of HCC patients.

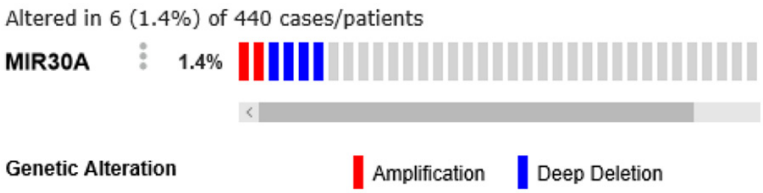

Figure 3. A visual summary of miR-30a mutation in HCC cases.The OncoPrint tab in cBioPortal was used to summarize genomic alterations in miR-30a across HCC sample set. The row represents miR-30a, and each column represents a carcinoma sample. Red bars indicate gene amplifications, blue bars are deep deletions, and gray squares are non-mutations.

deviation (SD). The student's *t* test was employed to analyze the difference between two independent variables. The evaluation of the effectiveness of miR-30a-5p was performed by using receiver operating characteristic (ROC), which helps to distinguish HCC from non-cancerous liver tissues. Youden's Index was used to identify the cut-off values. The Spearman correlation test was employed to detect the relationship between miR-30a-5p level and clinicopathological features. A value of $P<0.05$ calculated by two-tailed test was taken into considerationto be significant.

For meta-analysis, Stata Statistical software version 13.0 (StataCorp, College Station, TX, USA) was applied for the statistical analysis. Standard mean difference (SMD) with 95% confidence interval (CI) was evaluated for the continuous outcomes. Chi-square test of Q and the I^2 statistic was used to assess the heterogeneity across the datasets [17, 18]. If heterogeneity existed ($P<0.05$ or $I^2>50\%$), the SMD was pooled by using random-effect models (DerSimonian-Laird method). Otherwise, the fixed-effect model (Mantel-Haenszel method) was chosen [19]. To further explore the cause of heterogeneity, subgroup analysis was conducted based on the type of the control samples which included healthy hepatic tissues and adjacent non-cancerous liver tissues. Additionally, in order to investigate the relationship between miR-30a-5p expression and clinical parameters, we regrouped HCC samples according to the clinical parameters shared by more than two microarray datasets. In the age group, HCC patients were classified into younger group (<50 years old) and older group (≥50 years old). In the groups of gender, HBV condition and state of cirrhosis, HCC patients were divided according to the actual information. The SMD with CI of miR-30a-5p level in each group was calculated and pooled as was stated above. Finally, sensitivity analysis was conducted by dropping one study at a time to determine the robustness of the pooled result and Begg's and Egger's tests were applied to gauge the potential publication bias. The results of meta-analysis were displayed with forest plots. The possible publication bias was also shown as Begg's and Egger's funnel plots. A two-sided *P* value less than 0.05 was regarded to be statistical significance.

Table 3. Standard mean difference (SMD) of miR-30a-5p expression between HCC tissues and non-cancerous liver tissues (a fixed-effect model)

Study	Experimental			Control			Weight (%)	SMD fixed [95% CI]
	Mean	SD	Total	Mean	SD	Total		
GSE69580	6.2495	7.0515	5	19.5400	6.4331	5	1.04	-1.969 [-3.540, -0.398]
GSE57555	0.0508	0.1063	5	0.0288	0.0237	5	1.66	0.286 [-0.962, 1.533]
GSE54751	0.1205	0.0751	10	0.1246	0.0499	10	3.35	-0.064 [-0.941, 0.812]
GSE41874	0.9458	0.3240	6	1.3017	0.4349	4	1.41	-0.963 [-2.313, 0.387]
GSE40744	9.7127	0.9808	26	9.8789	0.4182	37	10.18	-0.236 [-0.739, 0.268]
GSE21362	10.4827	0.6561	73	10.8113	0.3523	73	23.35	-0.624 [-0.956, -0.292]
GSE22058	1.1648	0.2474	96	1.3109	0.1651	96	30.37	-0.695 [-0.986, -0.403]
GSE12717	12.8177	0.6439	5	13.2653	0.2221	3	1.14	-0.827 [-2.333, 0.679]
GSE10694	12.7272	0.7315	78	12.9040	0.5912	88	27.50	-0.268 [-0.574, 0.039]
Total [95% CI]			304			321	100.00	-0.495 [-0.656, -0.335]

SD stander deviation, *SMD* Standard mean difference. Heterogeneity: Tau^2=0.0349; Chi^2=11.99; df=8 (P=0.152); I^2=33.3%. Test for overall effect: Z=4.09 (P<0.001).

Results

Expression of miR-30a-5p by using TCGA database

To examine the expression level of miR-30a-5p of HCC, we derived a dataset from TCGA database which included the miR-30a-5p values in 50 adjacent non-cancerous tissues and in 361 HCC tissues. The level of miR-30a-5p was remarkably lower in the HCC tissues than in the adjacent non-cancerous tissues (16.30 ± 1.28 versus 17.36 ± 0.50, P<0.001) (**Table 1**; **Figure 1A**). Additionally, the area under the curve (AUC) of ROC was 0.795 and the cut-off diagnostic value for miR-30a-5p was 16.96 (**Figure 1B**).

The clinicopathological value of miR-30a-5p based on TCGA database

Meanwhile, we evaluated the potential role and clinicopathological value of miR-30a-5p in the progression of HCC. The level of miR-30a-5p was remarkably down-regulated in HCC with high histological differentiation grade (III and IV) than in those with low histological differentiation grader (I and II) (16.08 ± 1.32 versus 16.41 ± 1.21, P=0.02) (**Figure 2A**). With regard to the status of HBV infection, miR-30a-5p was down-regulated in the group with HBV infection compared to those without HBV infection (16.12 ± 1.14 versus 16.39 ± 1.31, P=0.06) (**Figure 2B**). Simultaneously, the association between miR-30a-5p and clinicopathological features was evaluated with Spearman correlation test. Negative correlation was observed between miR-30a-5p level and differentiation grade (r=-0.109, P=0.039). However, no statistically significant relationships were achieved between the miR-30a-5p and the following parameters: gender, age, clinical TNM stage, cirrhosis, metastasis, vascular invasion, HCV infection state and recurrence. Further, the gene alteration of miR-30a-5p across HCC samples was detected according to cBioPortal. From the OncoPrint, six (1.4%) of 440 cases had alterations, and four of the mutations were deep deletions while two were homozygous amplifications (**Figure 3**).

Characteristics of the included datasets from GEO

According to our inclusion criteria, nine eligible miRNA microarray datasets were included in the meta-analysis (**Table 2**). Among the nine microarray datasets, three were from China, three from Japan, and three from USA. And the publication year ranged from 2008 to 2015. The case groups of the nine microarrays were all HCC tissues, while the control groups contained two types ofnon-cancerous liver tissues. The control groups including healthy liver tissues were GSE57555 (Japan), GSE41874 (Japan), GSE40744 (USA), GSE12717 (China) and GSE10694 (China), while adjacent non-cancerous hepatic tissues were GSE69580 (China), GSE57555 (Japan), GSE54751 (USA), GSE41874 (Japan), GSE22058 (USA), GSE213-

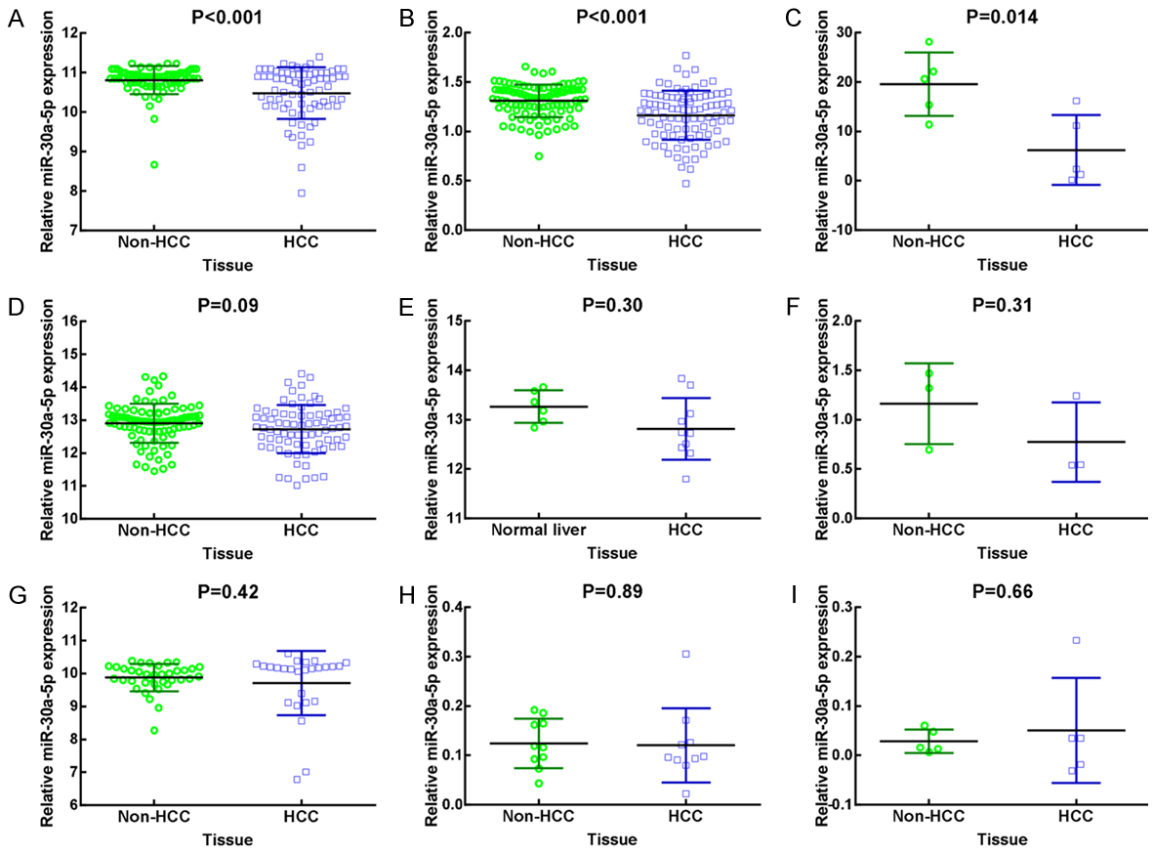

Figure 4. Expression of miR-30a-5p in HCC tissues and non-cancerous tissues based on GEO datasets. Significance of difference between two groups was analyzed by student's t test. Error bars represented SD. A. GSE21362 (P<0.001); B. GSE22058 (P<0.001); C. GSE69580 (P=0.014); D. GSE10694 (P=0.09); E. GSE12717 (P=0.30); F. GSE41874 (P=0.31); G. GSE40744 (P=0.42); H. GSE54751 (P=0.89); I. GSE57555 (P=0.66).

62 (Japan) and GSE10694 (China). Besides, GSE57555, GSE41874 and GSE10694 included both normal liver and para-cancerous hepatictissues. The total number of HCC tissues was 304 and the number of non-cancerous liver tissues was 321, which were recruited ultimately in the meta-analysis.

MiR-30a-5p was down-regulated in HCC tissues based on GEO data

The expression of miR-30a-5p in HCC tissues and non-cancerous liver tissues were derived from GEO datasets. Out of nine microarray datasets, the results of GSE21362 GSE22058 and GSE69580 indicated the down-regulated miR-30a-5p in HCC (P<0.001, P<0.001, P= 0.014) (**Figure 4A-C**). Other five datasets, namely GSE10694 (P=0.09), GSE12717 (P= 0.30), GSE41874 (P=0.31), GSE40744 (P= 0.42) and GSE54751 (P=0.89), also presented the relatively lower expressed miR-30a-5p in HCC than that in non-cancerous liver tissues (**Figure 4D-H**). However, only GSE57555 showed a reversely trend of higher expressed miR-30a-5p in HCC due to the small account of cases (P=0.66) (**Figure 4I**).

To further investigate the pooled results of GEO datasets, we performed a meta-analysis. Heterogeneity test was conducted to investigate the heterogeneity among the nine microarray datasets. The result showed that heterogeneity scarcely existed (P=0.152, I^2=33.3%), so the fixed-effect models was adopted to calculate the pooled SMD with 95 % CI accordingly. We observed that compared with non-cancerous liver tissues, miR-30a-5p was down-expressed in HCC tissues with statistical significance (SMD=-0.495; 95% CI, -0.656 to -0.335, P<0.001) (**Table 3**; **Figure 5A**).

Since little heterogeneity occurred among the individual datasets, we explored the source of heterogeneity via subgroup analysis. We divided the miRNA microarray datasets into two

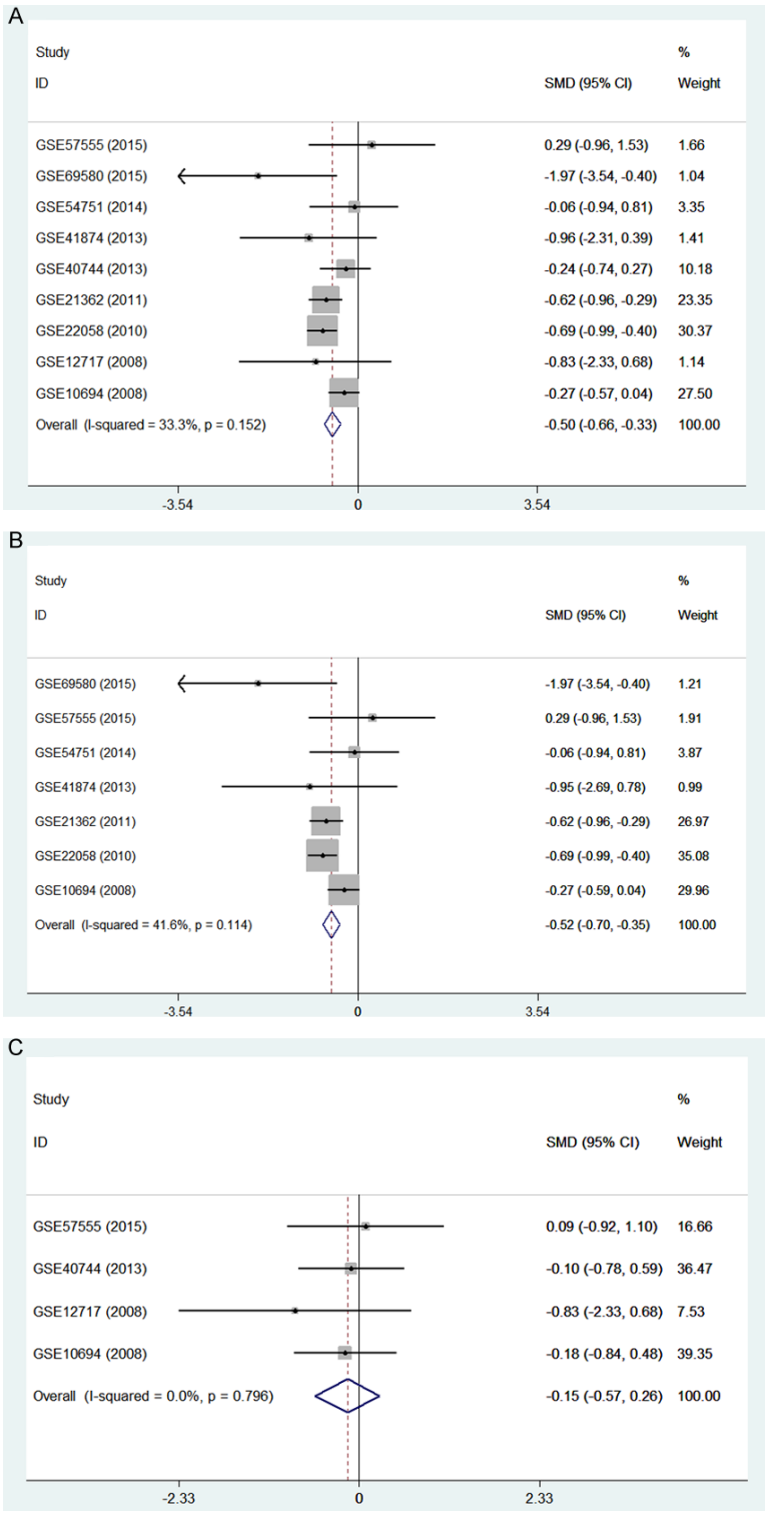

Figure 5. Forest plots of the downregulation of miR-30a-5p in HCC tissues. A. Forest plot for miR-30a-5p expression in HCC tissues and non-cancerous liver tissues; B. Forest plot for miR-30a-5p expression in the subgroup of HCC tissues versus adjacent non-cancerous tissues; C. Forest plot for miR-30a-5p expression in the subgroup of HCC tissues versus healthy liver tissues.

groups according to the type of control samples (adjacent non-cancerous tissues versus healthy liver tissues) and used appropriate model to pool the results according to the heterogeneity in the subgroup. Statistically significant underexpression of miR-30a-5p was observed between HCC tissues and adjacent non-cancerous tissues as we pooled the SMD with the fixed-effect model (SMD=-0.524; 95% CI, -0.696 to -0.351, P<0.001) (**Figure 5B**), since heterogeneity test showed that no significant heterogeneity was achieved (P=0.114, I^2=41.6%). Meanwhile, by comparison between HCC tissues and healthy liver tissues, no heterogeneity existed (P=0.796, I^2=0%). Thus, the fixed-effect model was selected to pool the SMD. However, no significant difference of miR-30a-5p level was found in HCC tissues than that in adjacent non-cancerous tissues (SMD=-0.152; 95% CI, -0.565 to 0.261, P=0.471) (**Figure 5C**).

Relationship between miR-30a-5p and clinicopathological features of HCC based on GEO data

Referring to the relationship between miR-30a-5p and clinicopathological aspects of HCC, we collected the clinical parameters recorded by more than two microarray datasets. Four parameters were collected: age, gender, and status of metastasis and cirrhosis. In the age group, the comparison between younger and older had no statistical significance (SMD=0.066; 95% CI, -0.305 to 0.437, P=0.728) (**Figure 6A**), which was pooled by

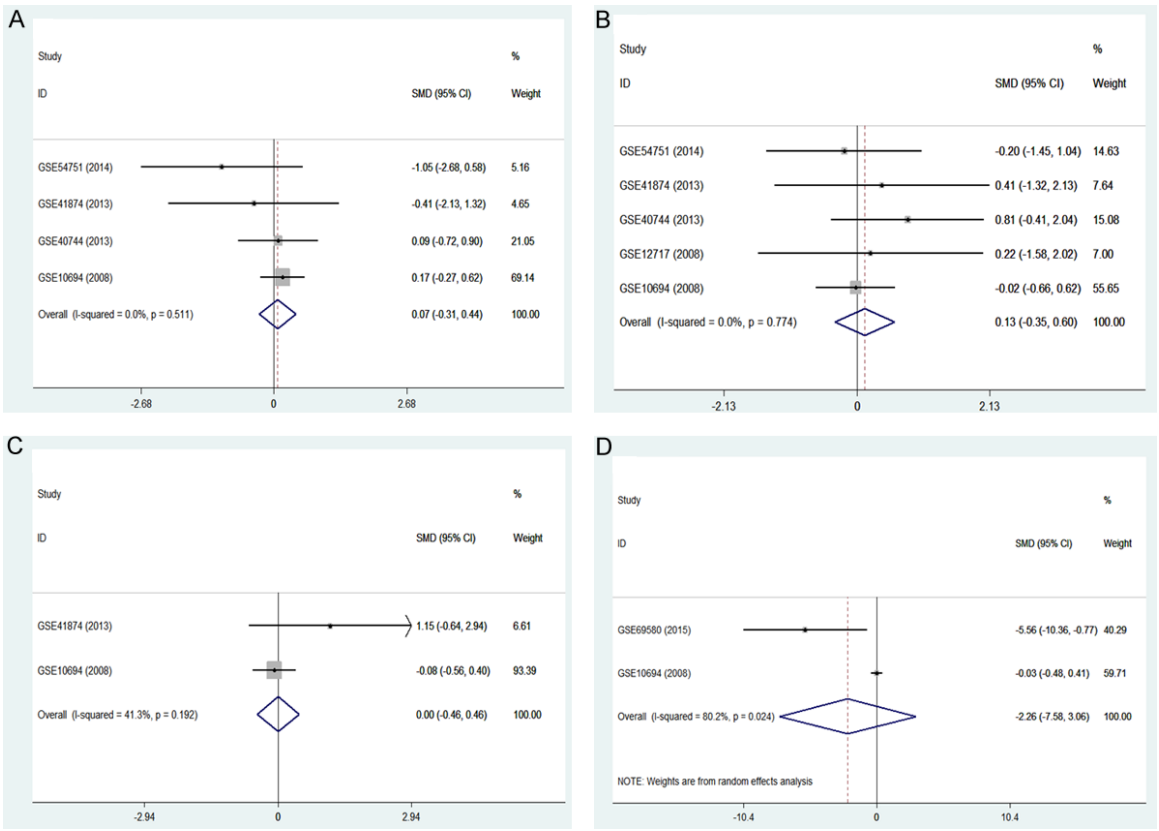

Figure 6. Forest plots of studies evaluating the relationship between miR-30a-5p expression and clinicopathological features. A. Forest plot for miR-30a-5p expression in younger group (<50 years old) and older group (≥50 years old); B. Forest plot for miR-30a-5p expression in male and female; C. Forest plot for miR-30a-5p expression in HCC patients with metastasis and without metastasis; D. Forest plot for miR-30a-5p expression in HCC patients with cirrhosis and without cirrhosis.

fixed-effect model as no significant heterogeneity existed (P=0.511, I^2=0.00%). Besides, no significant difference was found between male and female (SMD=0.128; 95% CI, -0.347 to 0.604, P=0.597) (**Figure 6B**). Two microarray datasets, which contained the values of miR-30a-5p in 28 HCC patients with metastasis and in 56 HCC patients without metastasis in total, were applied to investigate the correlation between miR-30a-5p level and tumor metastasis state. We used the fixed-effect model to calculate the pooled SMD with corresponding 95% CI because heterogeneity test showed that mild between-study heterogeneity was found (P=0.192, I^2=41.3%). However, no statistically significant relationship was detected (SMD=0.002; 95% CI, -0.458 to 0.461, P=0.944) (**Figure 6C**). As for cirrhosis, 43 HCC patients with cirrhosis and 40 HCC patients without cirrhosis were included in the analysis. The result showed that patients with liver cirrhosis did not show a different miR-30a-5p expression compared with those without liver cirrhosis (SMD=-2.261, 95% CI, -7.577 to 3.055, P=0.405) (**Figure 6D**).In conclusion, no significant correlation was discovered between miR-30a-5p expression and age, gender, status of metastasis and cirrhosis in the current meta-analysis.

Sensitivity analysis

The stability of the present meta-analysis was assessed with sensitivity analysis. According to the results, no matter which dataset was omitted, significant down-expression of miR-30a-5p in HCC tissues existed throughout, which pointed out that the result of the current meta-analysis was stable (**Figure 7A**).

Publication bias

The Begg's test showed no statistically significant publication bias in the present meta-analysis (z=0.52, P=0.602), which was confirmed by Egger's tests (t=-0.18, P=0.859). In addi-

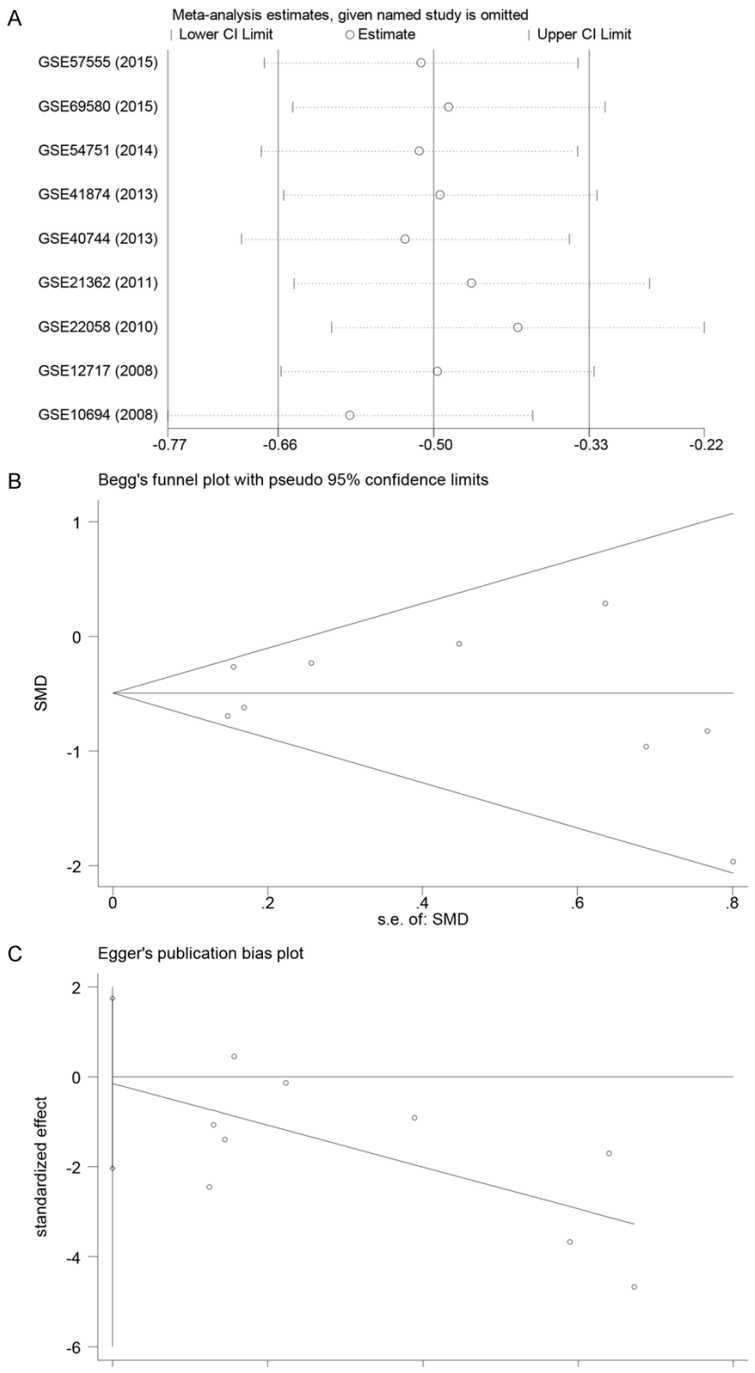

Figure 7. Sensitivity analysis and potential publication bias of blood miR-30a-5p in HCC. A. Sensitivity analysis; B. Begg's funnel plot; C. Egger's funnel plot.

tion, the funnel plot for publication bias was almost symmetrical, which suggested that no publication bias was visible (**Figure 7B** and **7C**).

Discussion

Many studies have demonstrated that aberrantly expressed miRNAs play a critical part in the development and progresses of cancer by functioning as tumor oncogenes or suppressive genes [20-22]. Previous studies also confirmed the essential role of miR-30a-5p in the cell proliferation and apoptosis on HCC cells [23], which aroused our interest in exploring the probable role of miR-30a-5a in HCC patients. Herein, we attempted to investigate the miR-30a-5p level in HCC and further analyze the association between miR-30a-5p and clinical significance of HCC based on public high-throughput datasets.

Downregulation of miR-30a-5p in HCC tissues has been described by several studies. According to Liu et al. [12], miR-30a-5p was confirmed to be pronouncedly down-expressed in 63 cases of HCC tissues. Also, Li et al. [13] confirmed decreased miR-30a-5p level in 16 cases HCC tissues. Adjacent non-cancerous tissues were used as control group in the aforementioned studies. However, the studies mentioned above were based on a small sample size, which may reduce the reliability of the conclusions. In present study, a total of 361 HCC tissues and 50 adjacent non-cancerous tissues were derived from TCGA database, based on which we investigated the level of miR-30a-5p in HCC tissues. The results showed that significant down-regulation of miR-30a-5p level was noted between HCC tissues and non-canceroushepatic tissues (16.30 ± 1.28 in HCC versus 17.36 ± 0.50 in normal liver, P<0.001) (**Table 1**), which was in line with previous studies. The ROC curve indicated a moderate diagnostic significance of miR-30a-5p in HCC with the AUC being 0.795. Furthermore, we validated the level of miR-

30a-5p in HCC tissues with the approach of meta-analysis, which enrolled nine miRNA microarray datasets including 304 HCC tissues and 321 non-cancerous liver tissues. Consistent decreased miR-30a-5p levelin HCC was also achieved in the pooled SMD (SMD=-0.495; 95% CI, -0.656 to -0.335, P<0.001). Moreover, sensitivity analysis revealedthat the pooled result was stable, as none of the results were reversed when the included individual studies were removed one at a time. Significant heterogeneity was observed among the datasets in which control groups were adjacent non-cancerous tissues. The results of TCGA database and validation based on meta-analysis, together with previous studies, indicatedthat miR-30a-5p play a pivotal part in hepatocarcinogenesis by functioning as a tumor suppressor.

Concerning the association between miR-30a-5p level and HCC clinicopathological features, only one study has been available until now. Liu et al. [12] determined the level of miR-30a-5p in 63 cases of HCC tissues and para-cancerous tissues by using real-time quantitative polymerase chain reaction (qPCR) and reported a correlation between decreased miR-30a-5p and intrahepatic metastasis, advanced TNM stage, high Edmonson pathological classification and shorter DFS (disease-free survival). In present study, we studied the correlation between miR-30a-5p expression and different clinicopathological parameters in TCGA database. The results revealed a negative association between miR-30a-5p level and differentiation grade (r=-0.109, P=0.039), which indicated that miR-30a-5p play a crucial part in the differentiation process of HCC. However, no statistically significant correlation was observed between miR-30a-5p level and other prognosis related clinicopathological characteristics, for example, TNM stage and metastasis. As for the meta-analysis, we mainly focused on the correlation between miR-30a-5p level and four clinical parameters: age, gender, state of cirrhosis and HBV infection, due to the limited information recorded in the microarray datasets. However, none of the pooled results showed statistically significance. Since the number of datasets enrolled in a single meta-analysis was small, the reliability of the conclusions was limited. Liu et al. documented that miR-30a-5p was remarkably down-regulated in the patients with advanced TNM stage. In this study, a lower miR-30a-5p expression was also found in the subgroup with advanced TNM stage, which corresponded to the result of Liu et al. Thus, a larger cohort is needed to determine the relationship between miR-30a-5p and the progression of HCC in the future.

Since lower miR-30a-5p level was related with poor differentiation in HCC patients, the potential role of miR-30a-5p in differentiation of HCC aroused our interest. With respect to differentiation of HCC, however, the specific mechanism was unclear. The level of miR-30a-5p was revealed to be associated with hepatic differentiation. Increased level of miR-30a-5p was found during hepatic differentiation [24]. And ectopic expression of miR-30a-5p with miR-122 can activate hMSC conversion into mature hepatocytes [25]. Thus, it was notsurprising to observe the relationship between the down-expression of miR-30a-5p and poor differentiation in HCC, as miR-30a-5p presented a pro-differentiation property. Further studies will be needed to inspect the precise molecular mechanisms of miR-30a-5p involving in the differentiation process of HCC.

As for meta-analysis of miR-30a-5p level in the GEO datasets, the following limitations should be noted in the present study. Firstly, slight heterogeneity, which may occur in different technological platforms, might disturb the strength of the meta-analysis. Secondly, the sample size in the meta-analysis and validation process was still small, which downplayed the conclusion that miR-30a-5p played a role in HCC patients. Thirdly, the data of miR-30a-5p level derived from the public datasets required to be further validated by RT-qPCR, which is now being carried out by our research group. Fourthly, this study was limited to exploring the correlation between miR-30a-5p and its clinicopathological featuresin HCC tissues, not the correlation of miR-30a-5p with the expression profiling of its potential targeting genes. Finally, the miRNA profiles in this study were only from tissues. It is more beneficial to investigate those non-invasive biomarkers for the early detection and screening for HCC patients. Limited number of HCC patients enrolled in each group reduced the reliability of the meta-analysis, so validation of the correlation between miR-30a-5p level and clinicopathological features is needed with more patients involved.

Conclusions

Together with previous studies, the current observation confirms that miR-30a-5p is a tumor-suppressive miRNA that plays an essential role in differentiation process of HCC as well as the tumorigenesis and worsening of HCC. The precise molecular mechanisms of miR-30a-5p in HCC need further investigation.

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Disclosure of conflict of interest

None.

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References

[1] Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.

[2] Mitchell JK, Lemon SM and McGivern DR. How do persistent infections with hepatitis C virus cause liver cancer? Curr Opin Virol 2015; 14: 101-108.

[3] Ren M, Qin D, Li K, Qu J, Wang L, Wang Z, Huang A and Tang H. Correlation between hepatitis B virus protein and microRNA processor Drosha in cells expressing HBV. Antiviral Res 2012; 94: 225-231.

[4] Buonaguro L, Petrizzo A, Tagliamonte M, Tornesello ML and Buonaguro FM. Challenges in cancer vaccine development for hepatocellular carcinoma. J Hepatol 2013; 59: 897-903.

[5] He L and Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004; 5: 522-531.

[6] Tie J and Fan D. Big roles of microRNAs in tumorigenesis and tumor development. Histol Histopathol 2011; 26: 1353-1361.

[7] Hung CH, Chiu YC, Chen CH and Hu TH. MicroRNAs in hepatocellular carcinoma: carcinogenesis, progression, and therapeutic target. Biomed Res Int 2014; 2014: 486407.

[8] Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S and Soboleva A. NCBI GEO: archive for functional genomics data sets--update. Nucleic Acids Res 2013; 41: D991-995.

[9] Zhang R, Yan S, Wang J, Deng F, Guo Y, Li Y, Fan M, Song Q, Liu H, Weng Y and Shi Q. MiR-30a regulates the proliferation, migration, and invasion of human osteosarcoma by targeting Runx2. Tumour Biol 2016; 37: 3479-3488.

[10] Huang QB, Ma X, Zhang X, Liu SW, Ai Q, Shi TP, Zhang Y, Gao Y, Fan Y, Ni D, Wang BJ, Li HZ and Zheng T. Down-Regulated miR-30a in Clear Cell Renal Cell Carcinoma Correlated with Tumor Hematogenous Metastasis by Targeting Angiogenesis-Specific DLL4. PLoS One 2013; 8: e67294.

[11] Tang R, Liang L, Luo D, Feng Z, Huang Q, He R, Gan T, Yang L and Chen G. Downregulation of MiR-30a is Associated with Poor Prognosis in Lung Cancer. Med Sci Monit 2015; 21: 2514-2520.

[12] Liu Z, Tu K and Liu Q. Effects of microRNA-30a on migration, invasion and prognosis of hepatocellular carcinoma. FEBS Lett 2014; 588: 3089-3097.

[13] Li WF, Dai H, Ou Q, Zuo GQ and Liu CA. Overexpression of microRNA-30a-5p inhibits liver cancer cell proliferation and induces apoptosis by targeting MTDH/PTEN/AKT pathway. Tumour Biol 2016; 37: 5885-5895.

[14] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C and Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013; 6: pl1.

[15] Meng X, Xiao C, Zhao Y, Jia L, Tang Y and Li D. Meta-analysis of microarrays: diagnostic value of microRNA-21 as a biomarker for lung cancer. Int J Biol Markers 2015; 30: e282-285.

[16] Sewer A, Gubian S, Kogel U, Veljkovic E, Han W, Hengstermann A, Peitsch MC and Hoeng J. Assessment of a novel multi-array normalization method based on spike-in control probes suitable for microRNA datasets with global decreases in expression. BMC Res Notes 2014; 7: 302.

[17] Lau J, Ioannidis JP and Schmid CH. Quantitative synthesis in systematic reviews. Ann Intern Med 1997; 127: 820-826.

[18] Higgins JP, Thompson SG, Deeks JJ and Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327: 557-560.

[19] Zamora J, Abraira V, Muriel A, Khan K and Coomarasamy A. Meta-DiSc: a software for meta-analysis of test accuracy data. BMC Med Res Methodol 2006; 6: 31.
[20] Liu Y, Ren F, Luo Y, Rong M, Chen G and Dang Y. Down-Regulation of MiR-193a-3p dictates deterioration of HCC: a clinical real-time qRT-PCR study. Med Sci Monit 2015; 21: 2352-2360.
[21] Langsch S, Baumgartner U, Haemmig S, Schlup C, Schafer SC, Berezowska S, Rieger G, Dorn P, Tschan MP and Vassella E. miR-29b mediates NF-kB signaling in KRAS-induced non-small cell lung cancers. Cancer Res 2016; 76: 4160-9.
[22] Weber CE, Luo C, Hotz-Wagenblatt A, Gardyan A, Kordass T, Holland-Letz T, Osen W and Eichmuller SB. miR-339-3p is a tumor suppressor in melanoma. Cancer Res 2016; 76: 3562-71.
[23] He R, Yang L, Lin X, Chen X, Lin X, Wei F, Liang X, Luo Y, Wu Y, Gan T, Dang Y and Chen G. MiR-30a-5p suppresses cell growth and enhances apoptosis of hepatocellular carcinoma cells via targeting AEG-1. Int J Clin Exp Pathol 2015; 8: 15632-15641.
[24] Raut A and Khanna A. Enhanced expression of hepatocyte-specific microRNAs in valproic acid mediated hepatic trans-differentiation of human umbilical cord derived mesenchymal stem cells. Exp Cell Res 2016; 343: 237-247.
[25] Cui L, Shi Y, Zhou X, Wang X, Wang J, Lan Y, Wang M, Zheng L, Li H, Wu Q, Zhang J, Fan D and Han Y. A set of microRNAs mediate direct conversion of human umbilical cord lining-derived mesenchymal stem cells into hepatocytes. Cell Death Dis 2013; 4: e918.