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Evaluation of miR-302b-5p expression and molecular mechanism in hepatocellular carcinoma: Findings based on RT-qPCR and in silico analysis

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ABSTRACT

Background and aim: Extensive research has revealed that microRNAs (miRNAs) play a principle role in cancer, and miRNAs associated with specific cancers have also been identified. The role of microRNA (miR)-302b-5p, which is one of the miRNAs reported in association with cancer, in hepatocellular carcinoma (HCC) is still unclear. Thus, the present study aimed to reveal the expression and potential molecule mechanism of miR-302b-5p in HCC.

Methods: An extensive meta-analysis of data from real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR), Gene Expression Omnibus and ArrayExpress microarrays was used to determine the expression of miR-302b-5p in HCC tissue samples and non-cancerous liver tissue samples. The sensitivity and specificity of miR-302b-5p as an indicator of HCC was estimated by plotting the receiver operating characteristic (ROC) and summarized ROC (sROC). Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses were employed to unravel the molecular mechanisms and biological functions of miR-302b-5p in HCC. Further, the putative target genes of miR-302b-5p were harvested based on the predicted genes and differentially expressed genes in HCC. Finally, the protein-protein interaction (PPI) network was built to determine the hub genes.

Results: According to the RT-qPCR results, the expression of miR-302b-5p was pronouncedly decreased in 39 HCC tissue samples as compared to 39 non-cancerous liver tissue samples. The standard mean difference (SMD) values of all the samples used in the meta-analysis also indicated lower miR-302b-5p expression in the 558 HCC tissue samples than in the 286 non-cancerous liver tissue samples. ROC and sROC analyses showed that miR-302b-5p had good specificity and sensitivity for distinguishing HCC tissue from non-cancerous liver tissue. Bioinformatics analyses identified 227 putative genes, and these genes were evidently enriched in the processes of organelle fission, chromosome and chromatin binding and were centralized in a “lysosome” pathway. The PPI network indicated that DNA topoisomerase II alpha (TOP2 A) was the most prominent hub gene of miR-302b-5p in HCC. Interestingly, according to the TCGA and Genotype-Tissue Expression databases, the mRNA and protein expression of TOP2 A were both elevated in HCC tissue samples as compared to non-cancerous liver tissue samples, and the overall survival and disease-free survival revealed that a high level of TOP2 A might reflect poor HCC outcome.

Conclusions: Our findings indicate that miR-302b-5p might suppress HCC progression, and TOP2 A might be a potential target of miR-302b-5p in HCC. However, in-depth in vivo and in vitro experiments are required to verify these findings and explore the mechanisms involved.

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1. Introduction

The morbidity and mortality rates of liver cancer are among the highest of all malignant tumors in the world [1]. According to the latest global statistics on the incidence of cancers in men, liver cancer ranks fifth, accounting for 6.3% of all malignant tumors. Further, it ranked second according to its mortality rate, which was second to that of only lung cancer, and it accounted for 10.2% of deaths. Although liver cancer is not among the ten most common cancers in women, it ranks sixth according to its mortality rate and accounts for 5.6% of cancer-related deaths [2]. The corresponding trends for 2019 in the United States are similar to the worldwide trends: 29,480 males and 12,550 females were estimated to have liver cancer and 21,600 males and 10,180 females were estimated to die from liver cancer. Further, with regard to the mortality rankings, liver cancer ranked fifth among males and seventh among females [3].

Hepatocellular carcinoma (HCC) is regarded as the most common type of primary liver cancer that occurs in hepatocytes. The occurrence and development of HCC is a complex process that involves multiple factors and multiple genes [4–10]. The clinical significance and molecular mechanisms of microRNAs (miRNAs) in HCC have been extensively explored. Studies have found that certain miRNAs play a vital part in the process of proliferation, apoptosis, invasion, epithelial-mesenchymal transition, metastasis, angiogenesis, autophagy, and drug resistance in HCC cells [11–16]. However, the expression and mechanisms of certain other miRNAs in HCC are unclear. An example of such a miRNA is microRNA (miR)-302b-5p, which was previously identified as miR-302b*, as miR-302b-3p is previously termed as miR-302b. So far, the miR-302b-5p expression level has only been detected in the male and female avian primordial germ cell line [17]: Lázár et al. found that suppression of miR-302b-5p remarkably enhanced the doubling time of the primordial germ cells lines. However, miR-302b-5p expression has never been studied in human diseases. To fill in this research gap, in the present study, we chose to focus on its expression in HCC.

Our group previously collected and analyzed miRNA microarrays of HCC to investigate the differentially expressed miRNAs in the carcinogenesis. Interestingly, miR-302b-5p exhibited as a prominent miRNA among the aberrant miRNAs in HCC (data not shown). Hence, a real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) was used to evaluate miR-302b-5p expression in HCC tissue samples for the first time. Additionally, we conducted a meta-analysis on data obtained from RT-qPCR, Gene Expression Omnibus (GEO) and ArrayExpress to verify the results of the RT-qPCR in a larger sample size. Additionally, we identified the probable target genes of a protein-protein interaction (PPI) network to determine the hub gene of miR-302b-5p in HCC (Fig. 1). We believe that these findings could shed light on new indicators of the pathogenesis of HCC.

2. Materials and methods

2.1. Collection of clinical samples

A total of 39 HCC formalin-fixed, paraffin-embedded tissue samples and 39 non-cancerous liver tissue samples were collected from the First Affiliated Hospital of Guangxi Medical University (Nanning, China). All the patients provided their signed consent before participating in the study, and the ethics committee of the First Affiliated Hospital of Guangxi Medical University authorized this study. Total RNA was extracted from HCC tissue samples and non-cancerous liver tissue samples utilizing the miRNeasy FFPE kit (QIAGEN, KJ Venlo, the Netherlands), as formerly reported [18–20]. The Takara PrimeScript RT Reagent Kit (Takara, Nanning, China) was used to reverse transcribe RNA into cDNA. Then, RNU48 (Applied Biosystems Cat No. 4427975-001006) and RNU6B (Applied Bio-systems Cat No. 4427975-001093) were used in combination as the internal reference genes as previously verified to be the best internal reference choice by using both NormFinder and geNorm algorithm, and the sequences were listed as follows : GAUGACCCCAGGUAACUCUGAGUGUGUCGCUGAUGCCAUCACCGCAGCGCUCUGACC (RNU48) and CGCAAGGAUGACACGCAAAUUCGUGAAGCGUUCCAUAUUUUU (RNU6B) [21–24]. The sequence of miR-302b-5p was ACUUUAACAUGGAAGUGCUUUC. RT-qPCR of the miRNA was performed using Applied Biosystems PCR7900 with commercial confidential primers, and miR-302b-5p expression was normalized to that of the internal reference. Then, the $2^{-\Delta cq}$ formula was used to estimate miR-302b-5p expression.

2.2. Selection of GEO and ArrayExpress microarrays

We screened available GEO and ArrayExpress microarrays [25,26], which met the following criteria: (1) specimens from humans, (2) more than three HCC tissue samples and non-cancerous liver tissue samples, and (3) evaluation of miR-302b-5p expression in HCC tissue samples and non-cancerous liver tissue samples. Information about the GSE ID, number and mean (M) ± standard deviation (SD) of HCC tissue samples and non-cancerous liver tissue samples was obtained to determine false positivity (FP), true negativity (TN), true positivity (TP) and false negativity (FN).

2.3. Target genes, differentially expressed genes and transcription factors

The predicted target genes of miR-302b-5p were achieved from miRWalk v.2.0, which was composed by twelve online prediction tools. The threshold criteria for possible predicted target genes was that they were demanded to appear more than four of twelve. The differentially expressed genes (DEGs) associated with HCC were obtained using Gene Expression Profiling Interactive Analysis (GEPIA, [27,28]), a database that provides differential expression analysis. Only DEGs with a P value < 0.05 and $\log_2 FC > 1$ were selected. Then, Venn diagrams were framed to identify overlapping genes among the predicted genes and DEGs in order to determine which genes were associated with miR-302b-5p in HCC. Simultaneously, the transcription factors (TFs) associated with miR-302b-5p were explored using CircuitsDB, a database that provides the post-transcriptional and genome-wide transcriptional regulatory network of a factor with the use of bioinformatics sequence-analysis. In particular, we plotted the feed-forward regulatory loops (FFLs), which are elementary circuits that combine TFs, miRNAs and genes identified from CircuitsDB.

2.4. GO, KEGG and PPI analyses of the potential targets of miR-302b-5p

To unveil the molecular mechanisms of miR-302b-5p and its target genes in HCC, Gene Ontology (GO) enrichment [29,30] and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway [31,32] analyses were accomplished using the Database for Annotation, Visualization and Integrated Discovery (DAVID). The PPI network [33,34] was drawn to determine the hub genes of miR-302b-5p in HCC. Further, the mRNA and protein expression of the hub gene was verified using the GEPIA and human protein atlas (HPA) databases, respectively.

2.5. Statistical analysis

The mean and SD value of miR-302b-5p expression in HCC tissue samples and non-cancerous liver tissue samples were estimated by SPSS 22.0 (IBM Corp., Armonk, NY, USA), with an independent *t*-test. Receiver operating characteristic (ROC) and summarized ROC (sROC) curves were plotted to determine the specificity and sensitivity of miR-302b-5p as an indicator of HCC. Further, STATA 12.0 (StataCorp, College Station, TX, USA) was used for the meta-analysis of the present study, and the standard mean difference (SMD) with a random-effects model was used to indicate the expression of miR-302b-5p in HCC tissue samples and non-cancerous liver tissue samples. Finally,

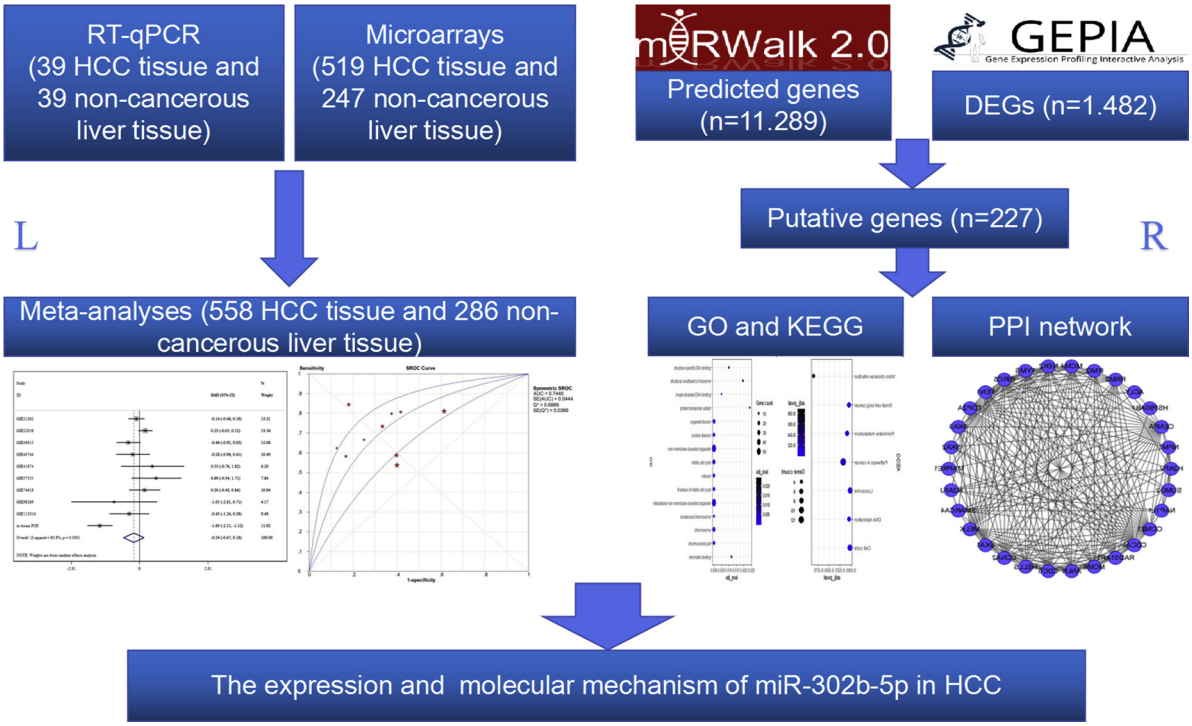

Fig. 1. Flow diagram of the current study protocol. RT-qPCR, real-time reverse transcription quantitative polymerase chain reaction. HCC, hepatocellular carcinoma. DEGs, differentially expressed genes. GO, Gene Ontology. KEGG, Kyoto Encyclopedia of Genes and Genomes. L, Left. R, Right.

sensitivity analysis and Begg's test were used to determine whether there were any discrepancies between the studies used in the meta-analysis and publication biases, respectively. P-value < 0.05 was signified for statistically significance.

3. Results

3.1. miR-302b-5p expression in HCC tissue samples

The RT-qPCR findings showed that miR-302b-5p expression was predominantly decreased in the 39 HCC tissue samples than in the 39 non-cancerous liver tissue samples (1.527 ± 0.764 vs. 3.187 ± 1.217, P < 0.0001, Fig. 2A). ROC curve analysis revealed that miR-302b-5p showed good specificity and sensitivity for distinguishing between HCC tissue and non-cancerous liver tissue (Area under the receiver operating characteristic curve (AUC) = 0.8948, P < 0.0001, Fig. 2B). A total of nine GEO microarrays were screened out for the meta-analysis (Table 1): miR-302b-5p expression was downregulated in HCC tissue samples as compared to non-cancerous liver tissue samples in five of the GEO microarrays, while miR-302b-5p expression was upregulated in HCC tissue samples as compared to non-cancerous liver tissue samples in the other four GEO microarrays (Fig. 3). The difference between the non-cancerous and HCC samples was significant for all microarrays (P > 0.05). As with the previous sample group, ROC analysis of the GEO microarray GSE36915 implied that miR-302b-5p exhibited good specificity and sensitivity for distinguishing between HCC tissue and non-cancerous liver tissue (AUC = 0.6695, P = 0.0194, Fig. 4). Regrettably, none of the ArrayExpress microarrays met the criteria for inclusion in the analysis.

3.2. Meta-analysis of miR-302b-5p expression

A heterogeneity test indicated obvious heterogeneity across the included studies. Therefore, we used a random-effects model to estimate the pooled SMD, which was found to be -0.24 (-0.67 to -0.18). This SMD value indicates that miR-302b-5p expression was downregulated in the

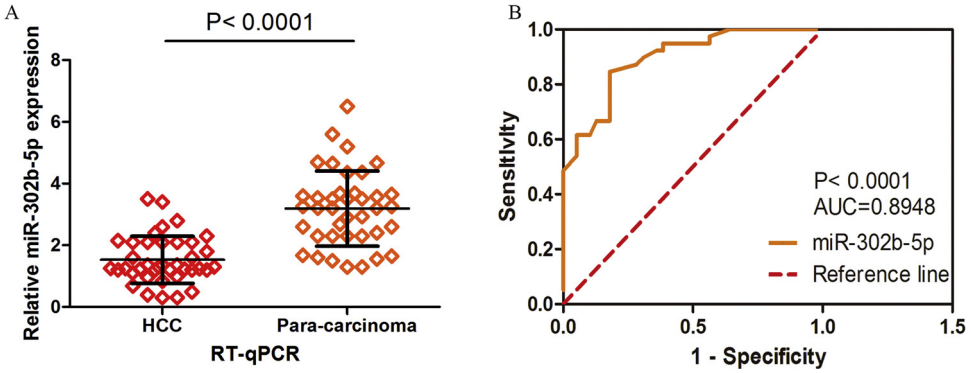

Fig. 2. The clinical significance of miR-302b-5p in HCC based on RT-qPCR findings.
A.The expression of miR-302b-5p was noticeably downregulated in the 39 HCC tissue samples as compared to the 39 non-cancerous liver tissue samples. B. ROC analysis of miR-302b-5p in HCC.

Table 1
Information of miR-302b-5p expression levels from the microarrays.

Dataset	First author	Country	Year	HCC tissue (N = 558)			Non-cancerous liver tissue (N = 286)			SD	AUC	TP	FP	FN	TN
				N	M	SD	N	M							
GSE21362	Sato F	Japan	2012	73	1.34	1.25	73	1.50		1.02	0.5752	43	29	30	44
GSE22058	Burchard J	USA	2013	96	−2.05	0.20	96	−2.10		0.24	0.5679	78	59	18	37
GSE36916	Menet JS	USA	2019	68	8.80	1.34	21	9.41		1.33	0.6695	50	7	18	14
GSE40744	Diaz G	USA	2017	26	1.69	0.14	12	1.74		0.25	0.5978	21	5	5	7
GSE41874	Morita K	Japan	2016	6	1.57	1.65	4	0.88		0.11	0.6667	4	1	2	3
GSE57555	Taguchi Y	Japan	2018	5	−0.04	0.01	16	−0.05		0.02	0.6875	4	6	1	10
GSE74819	Uyhelji HA	USA	2016	230	1.37	0.21	10	1.32		0.20	0.5428	124	4	106	6
GSE98269	Xie Z	China	2018	3	4.89	0.04	3	4.93		0.03	0.7778	2	0	1	3
GSE115016	Ye G	China	2018	12	1.01	0.14	12	1.07		0.14	0.7778	7	2	5	10

HCC, hepatocellular carcinoma. N, number. M, mean. SD, standard deviation. AUC, Area under the receiver operating characteristic curve. TP, true positivity. FP, false positivity. FN, false negativity. TN, true negativity.

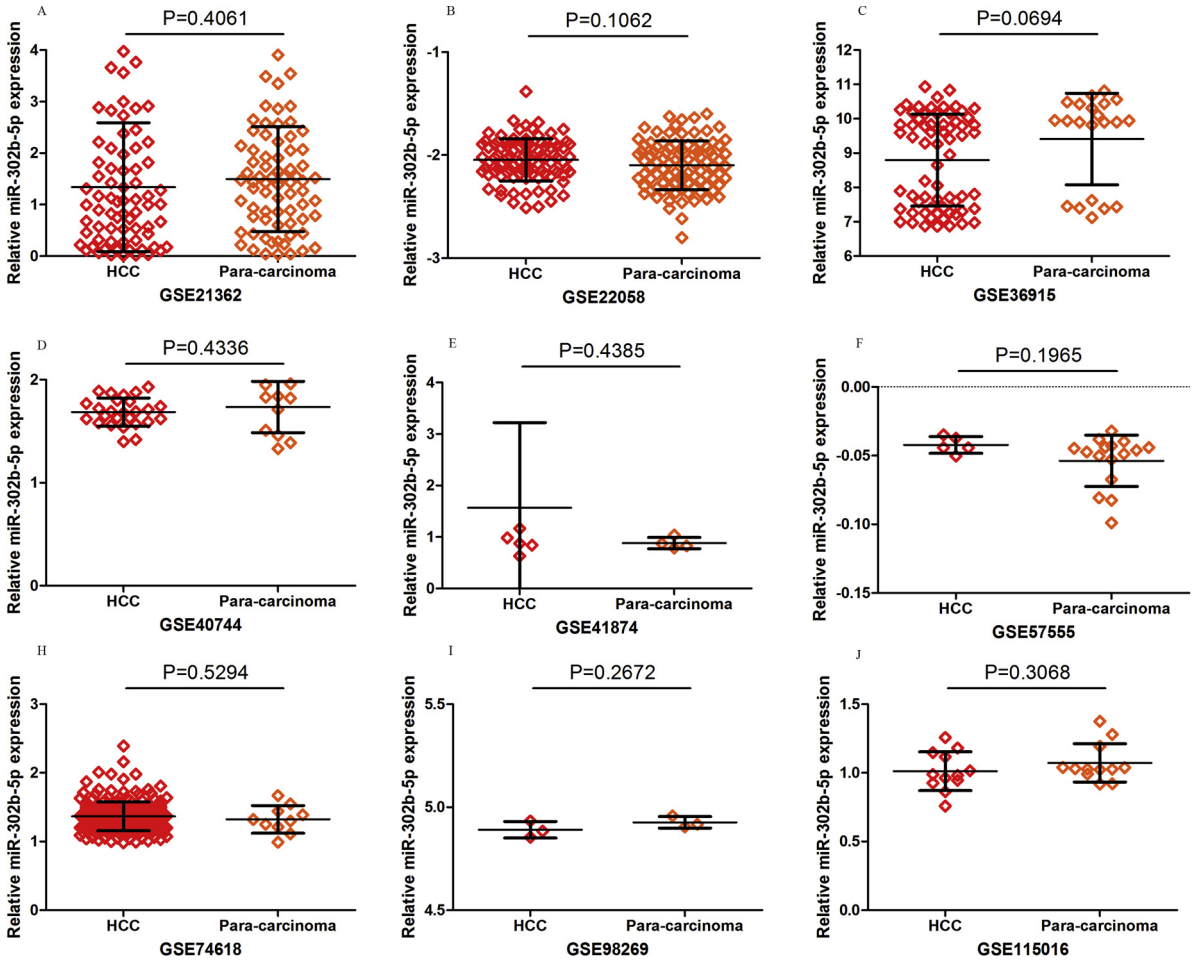

Fig. 3. miR-302b-5p expression in HCC based on the GEO microarray database.
A.GSE21362. B. GSE22058. C. GSE36916. D. GSE40744. E. GSE41874. F. GSE57555. H, GSE74819. I. GSE98269. *J*. GSE115016.

558 HCC tissue samples as compared to the 286 non-cancerous liver tissue samples (Fig. 5A). The sensitivity analysis showed that no study was distinctly different from the others (Fig. 5B), and Begg's test signified that there was no publication bias (Fig. 5C). Based on the results of FP, FN, TP and TN of each microarrays shown in Table 1, we conducted a sROC analysis and revealed a summarized AUC of 0.7445 (Fig. 5D), with a pooled sensitivity of 0.66 (0.61—0.70) and a pooled specificity of 0.58 (0.52—0.64) (Fig. 5E and F). Thus, miR-302b-5p seemed to have good potential for distinguishing between HCC tissue and non-cancerous liver tissue.

3.3. miRNA-mRNA and miRNA-TF regulatory networks

Initially, 4974 genes were identified over four times with the twelve prediction tools, and they were considered as the predicted genes associated with miR-302b-5p. In addition, 1481 DEGs associated with HCC were identified through the GEPIA database. Finally, 227 putative target genes were identified with a Venn diagram of the predicted genes and DEGs (Fig. 6). In addition, we discovered that miR-302b-5p might be regulated by two TFs, namely, AML1 and SOX-5. Finally, 14 target gene combinations were identified, and 14 FFLs were plotted based on the TFs, miRNAs and target gene combinations (Fig. 7).

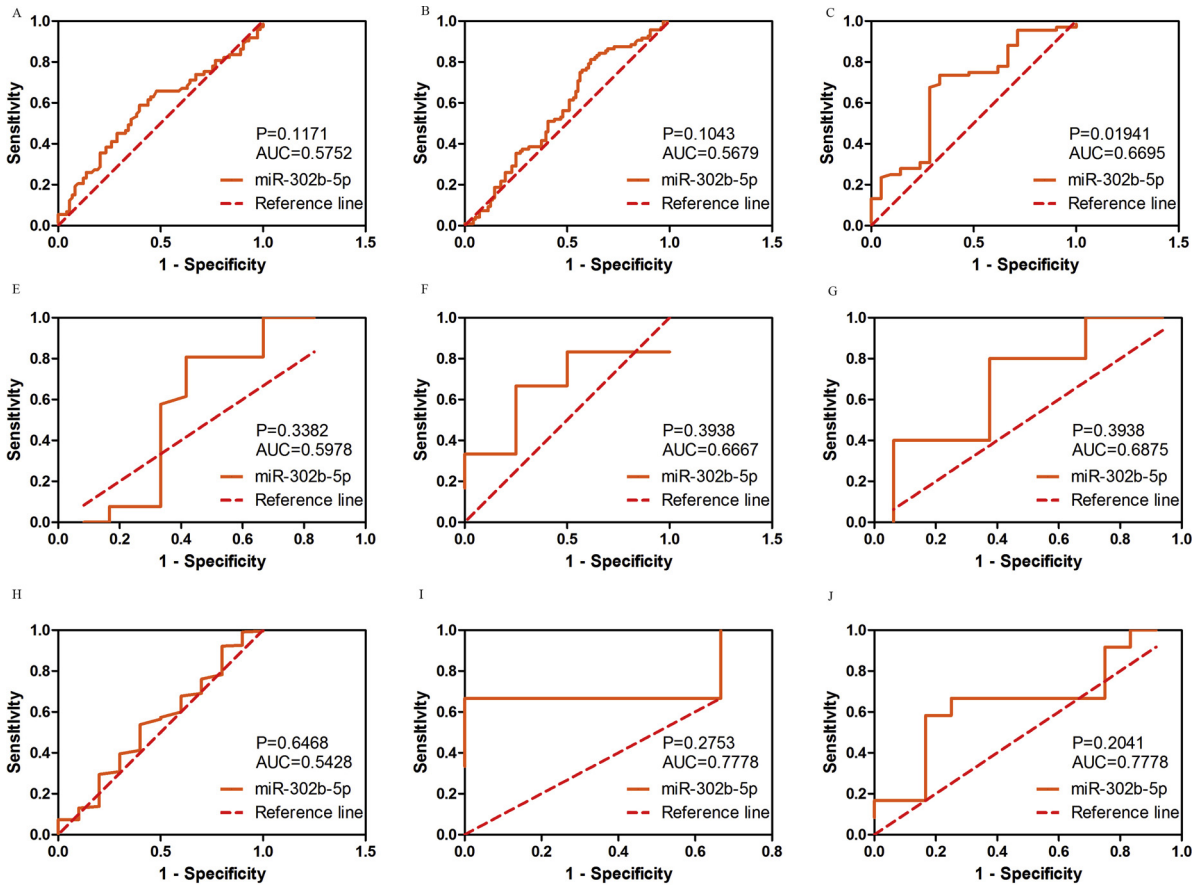

Fig. 4. ROC analysis of miR-302b-5p expression in HCC based on the GEO microarray database.
A.GSE21362. B, GSE21362. C. GSE22058. D. GSE36916. E. GSE40744. F. GSE41874. G. GSE57555. H, GSE74819. I. GSE98269. J. GSE115016.

3.4. GO and KEGG analyses

The 227 putative genes were found to be markedly prominent in organelle fission in biological process (BP), obviously centralized in the chromosome in cellular component (CC), and significantly enriched in chromatin binding in molecular function (MF) (Fig. 8A). Moreover, the "lysosome" was regarded as the most prominent pathway, and we identified that nine target genes (i.e., GNS, LAMP2, AP1S1, GM2 A, ATP6V1H, CTSA, CTSC, CTSS and AP3B1; Fig. 8B) were enriched in this pathway. The expression of these genes was overtly increased in HCC tissue samples as compared to non-cancerous liver tissue samples based on the GEPIA database (Fig. 8C-K). Thus, miR-302b-5p might affect lysosome-related pathways in HCC by targeting these nine genes. According to information obtained from the PPI network, DNA topoisomerase II alpha (TOP2 A) was identified as the hub gene with the highest degree of association with miR-302b-5p (Fig. 9A), and according to the GEPIA database, TOP2 A mRNA expression was observably elevated in HCC tissue samples as compared to non-cancerous liver tissue samples (Fig. 9B), and a high level of TOP2 A might be indicative of poor outcome of HCC patients, based on the overall survival and disease-free survival data from the GEPIA database (Fig. 9C and D). Further, using the HPA database, we also discovered that TOP2 A protein expression was obviously higher in HCC tissue than in non-cancerous liver tissue, with the help of three antibodies (HPA006458, HPA026773 and CAB002448). These findings support the evidence that TOP2 A is a crucial target gene of miR-302b-5p in HCC (Fig. 10).

4. Discussion

The role of miRNAs in tumorigenesis and development has been extensively studied in the last decade. Dozens of miRNAs have been discovered to play a regulating part in the development and progression of HCC. A former study has shown that miR-302b-3p (previous also termed as miR-302b) expression is markedly decreased in HCC tissues and cells compared with their corresponding control group [35–37]. However, miR-302b-5p expression in HCC has never been reported. In this study, RT-qPCR was applied to evaluate miR-302b-5p expression in HCC tissue samples and non-cancerous liver tissue samples obtained from clinical procedures. The findings indicate that the expression of miR-302b-5p is significantly decreased in HCC, and this finding was verified by microarray data. We further predicted the likely target genes of miR-302b-5p in HCC and found that these potential target genes were enriched in the lysosome pathways. Among the target genes identified, TOP2 A was at the top of the list of hub genes, and its mRNA and protein levels were high in HCC tissues. However, the pathways that link miR-302b-5p and TOP2 A are unclear, and this needs to be investigated in the future. Overall, the findings of this study imply that low expression or loss of expression of miR-302b-5p may play a role in promoting HCC by targeting specific genes and signaling pathways.

Using RT-qPCR, we first found that miR-302b-5p expression in the HCC tissues was approximately only half that in the non-cancerous control tissues (1.53 vs 3.19). Furthermore, miR-302b-5p was found to have good sensitivity and specificity for discriminating HCCs from non-cancerous liver tissues (AUC = 0.89). However, since only a small sample (n = 39) was employed for the RT-qPCR analysis, the finding needs to be validated in other cohorts and through other analyses. Next, we collected miRNA microarray and miRNA sequencing data to validate the RT-qPCR findings. Unfortunately, miR-302b-5p expression data could not be obtained from The Cancer Genome Atlas, which is one of the most authoritative cancer sequencing datasets. The final

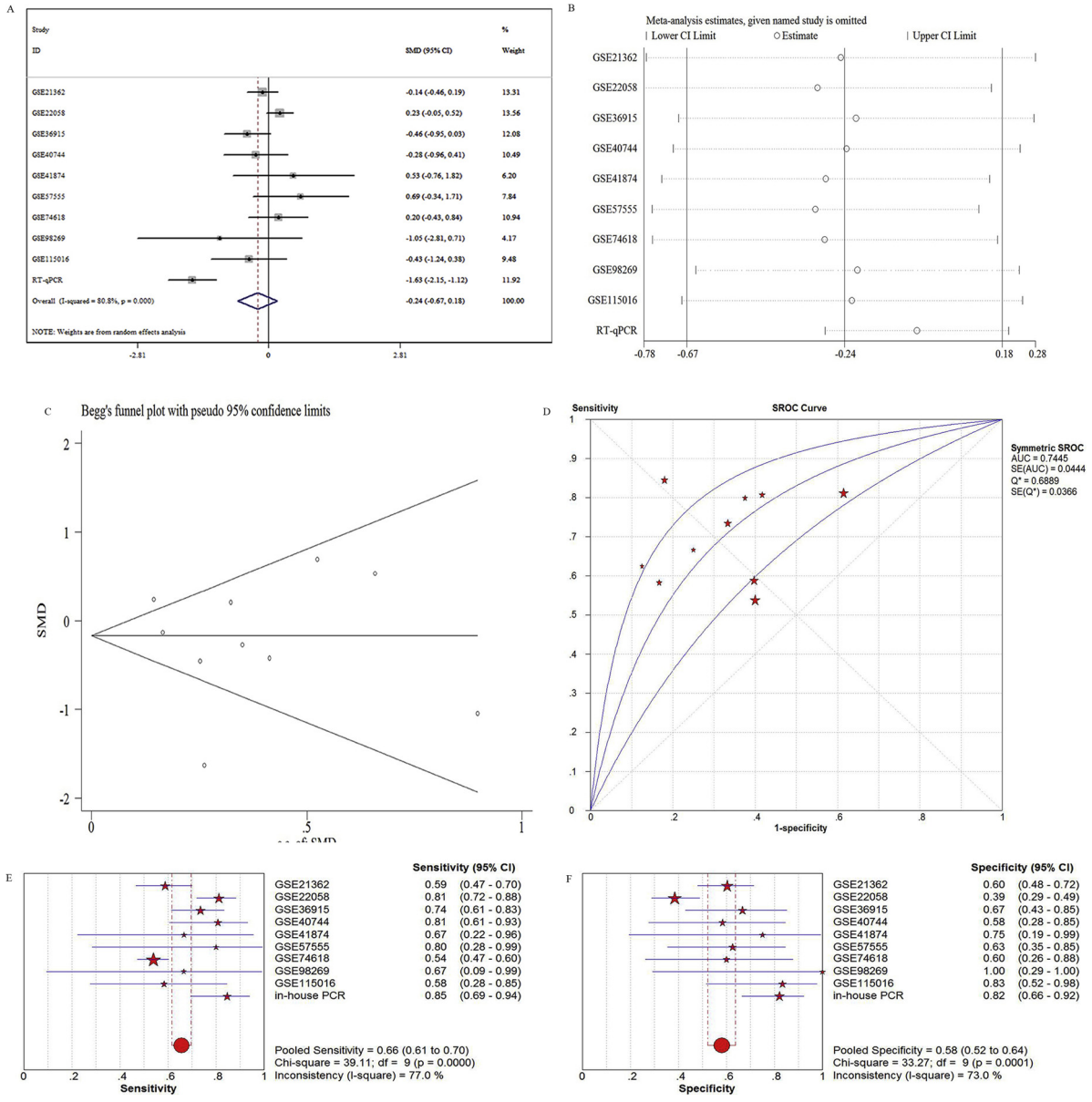

Fig. 5. Meta-analysis estimates of miR-302b-5p expression levels.
A.The pooled SMD was -0.24 (-0.67 to -0.18), which indicated that the expression of miR-302b-5p was reduced in the 558 HCC tissue samples as compared to the 286 non-cancerous liver tissue samples. B. The sensitivity analysis showed that no study was distinctly different from the other studies. C. Begg's funnel plot showed that no publication bias was shown in the studies included in the meta-analysis. D. The AUC of sROC was 0.7445, which indicates that miR-302b-5p had good sensitivity and specificity to distinguish HCC tissue from non-cancerous liver tissue. E. The pooled sensitivity was 0.66 (0.61–0.70). F. The pooled specificity was 0.58 (0.52 − 0.64).

verification data were obtained from GEO, as the ArrayExpress microarrays did not meet the study's inclusion criteria. Among nine GEO microarrays that were used, five showed a consistent decrease in miR-302b-5p expression in HCC tissues as compared to non-cancerous liver tissues. To gain an extensive view of the expression level of miR-302b-5p, an extensive meta-analysis was conducted of all the relevant data. The pooled SMD reached -0.24, which was indicative of lower miR-302b-5p expression in the 558 cases of HCC than in the 286 non-cancerous liver tissue samples. Combined sROC analysis also yielded an AUC of 0.74, which is indicative of a moderate distinguishing capacity between cancer and non-cancer. Hence, through a combination of RT-qPCR and microarray data and meta-analysis, we were able to confirm that the miR-302b-5p level is decreased in HCC tissues. This could mean that miR-302b-5p plays a protective role in the process of hepatocellular carcinogenesis. It is possible that in normal liver cells, a certain level of miR-302b-5p expression is required to prevent malignant transformation of liver cells. A decline in or loss of miR-302b-5p expression may lead to the induction of the tumorigenesis process. This hypothesis needs to be investigated in the future, along with the fundamental molecular mechanisms of this miRNA in HCC.

It is known that miRNAs and TFs play a regulating role in the biological processes of cancer by modulating post-transcriptional gene modifications and binding to the promoter region of genes, respectively. In addition, miRNAs and TFs may also have regulatory effects on each other. An FFL that comprises the associated miRNAs, TFs and genes may be ideal for examining the role of miRNAs in cancer [38]. In the present study, we identified the regulatory TFs and mutual genes based on CircuitsDB. Eventually, 14 FFLs were plotted by combining miR-302b-5p, two regulatory TFs (AML1 and SOX-5) and 14 mutual genes. AML1, also known as RUNX1, was a crucial TF in the proper

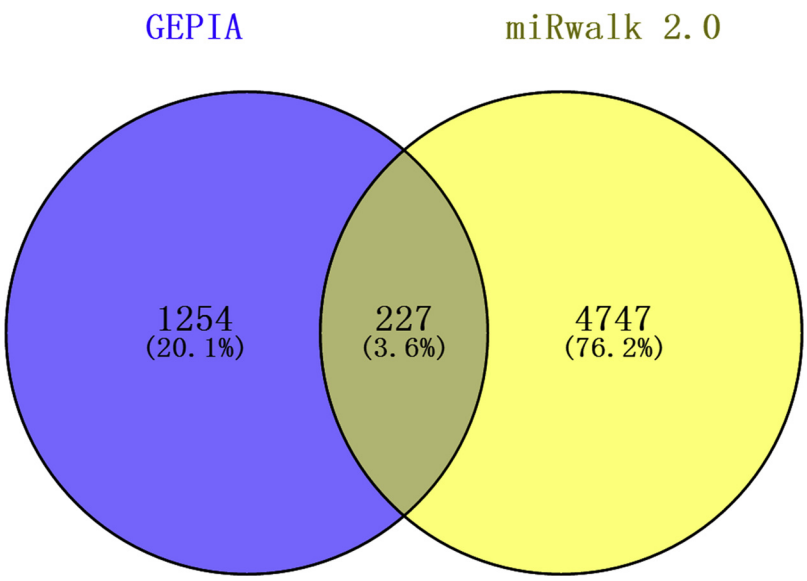

Fig. 6. Venn diagram of the predicted genes from miRwalk 2.0 and DEGs from GEPIA.
In total, 227 putative genes were identified.

development of some cell lineages and tissues, including cartilage, mammary glands, blood, hair follicles and bone, through regulating various gene expression [39]. About the role of RUNX1 in HCC, Lu et al. suggested that RUNX1 was one of the most markedly mutated genes, indicating that RUNX1 plays a key role in the liver carcinogenesis [40]. Precious study also verified that SOX-5 was a pivotal TF in nasopharyngeal carcinoma (NPC). SOX-5 could transcriptionally suppress expression of secreted protein, acidic, cysteine-rich and might affect NPC progression. In addition, the elevated SOX-5 expression was associated with a poorer survival outcome in NPC [41]. Nevertheless, no study focused on the transcriptional effect of AML1 and SOX-5 in HCC. The present study was the first to point out the transcriptional effect of AML1 and SOX-5 by targeting miR-302b-5p in HCC. However, these findings need to be confirmed by in vivo and in vitro experiments in the near future.

As the specific biological role of a miRNA can be understood by identifying its target genes, we predicted the prospective target genes of miR-302b-5p in HCC. Since miR-302b-5p expression is distinctly low in HCC, genes that are highly expressed in HCCs are more likely to be the target genes of miR-302b-5p. Unfortunately, we were unable to obtain the protein expression profiles of HCC, so we used the mRNA expression level to predict the target genes. Although this approach did not yield precise results, it could reveal with a certain level of possibility the target genes of miR-302b-5p. Finally, 227 putative genes were identified and clustered in different pathways. The lysosome pathway appears in multiple enriched pathways. Lysosome-dependent processes play an important modulating role in autophagy and are closely related to a variety of tumors including HCC [42–44]. Therefore, based on the present findings, miR-302b-5p may affect lysosome-related pathways in HCC to regulate tumorigenesis. However, this theory requests to be studied in the future.

We also conducted PPI analysis of the 227 putative genes, because hub gene function is more clear and powerful than that of the other genes. The PPI results showed that TOP2 A was the highest-ranking hub gene. Both TCGA and Genotype-Tissue Expression sequencing data and HPA immunohistochemistry data showed that TOP2 A showed significant overexpression in HCC. Moreover, our previous work also reported the upregulation of TOP2 A in HCC by PCR, tissue microarray,

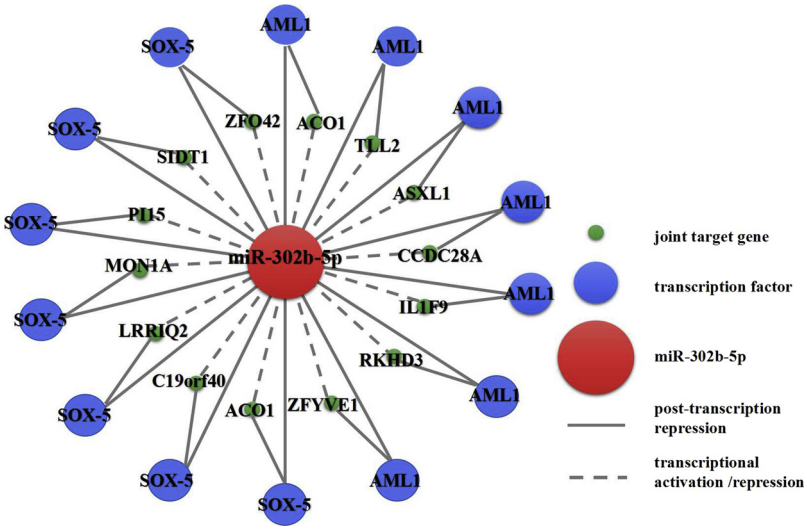

Fig. 7. FFLs of TFs, miR-302b-5p and the target gene combinations.
A total of 14 FFLs that included TFs, miR-302b-5p and target gene combinations were plotted.

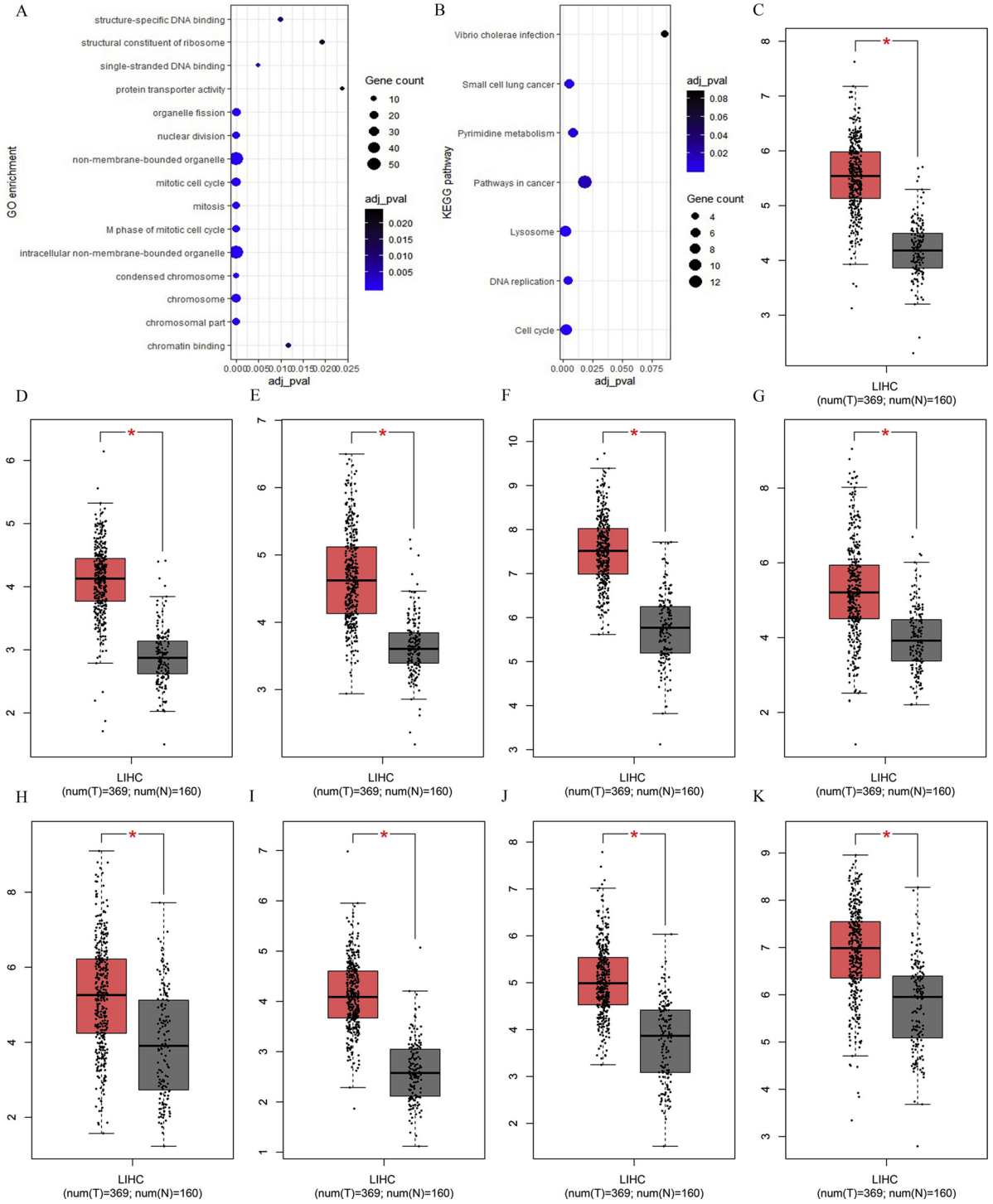

Fig. 8. GO enrichment and KEGG pathway of the 227 putative genes.
A. Organelle fission, chromosome and chromatin binding were the most prominent GO terms that emerged. B. The 'lysosome' was the most prominent pathway. C–K. The expression of 'lysosome' pathway-related genes were all overtly increased in HCC tissue samples as compared with non-cancerous liver tissue samples based on data from the GEPIA database

gene chip and sequencing analyses. We also reported in our previous study that TOP2 A is a clear target of the traditional Chinese medicine nitidine chloride in HCC cells through in vitro and in vivo experiments, which confirmed that after nitidine chloride application, HCC cells grew more slowly and tumor growth slowed down, partly due to the decreased expression of TOP2 A. Additionally, molecular docking experiments confirmed that TOP2 A is a target of nitidine chloride [45]. miR-302b-5p may also target TOP2 A in HCC, but the effects of nitidine chloride on the expression of miR-302b-5p and the resulting effects of miR-302b-5p on the biological behavior of HCC remain to be studied.

With regard to the diagnostic potential of miRNAs in HCC, it has been revealed that serum miR-122 and serum miR-224 are vital diagnosis biomarkers in HCC and they have higher sensitivity, specificity and accuracy than alpha fetoprotein (AFP) [46]. Additionally, Feng et al. reported that the sensitivity and accuracy of serum miR-221 was better than that of AFP in HCC, but combining serum miR-221 and AFP

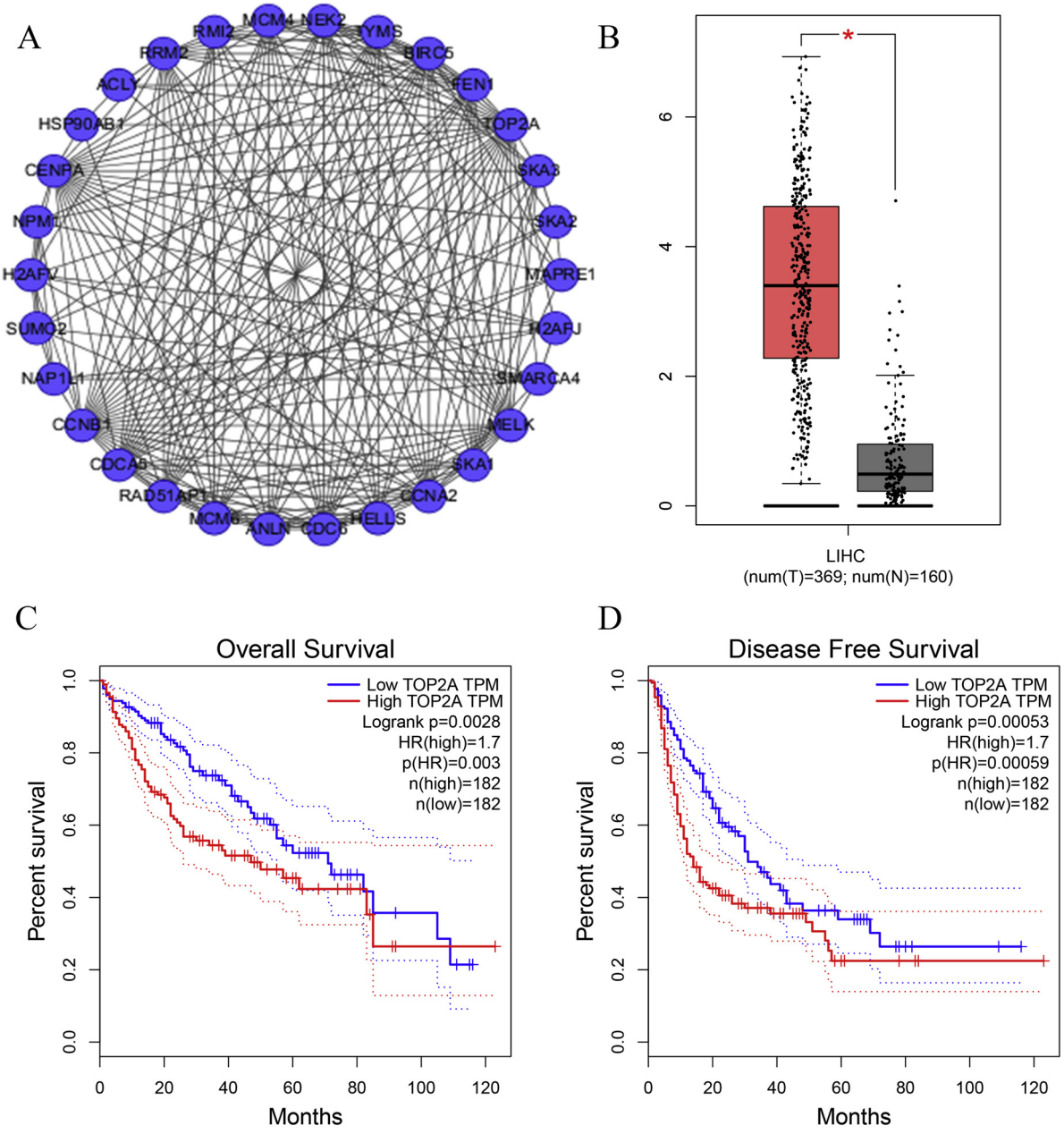

Fig. 9. PPI network was performed to reveal the hub gene of miR-302b-5p in HCC.
A. PPI network indicated that TOP2 A was identified as the hub gene with the highest degree of association with miR-302b-5p. B. Expression of TOP2 A in HCC tissue samples and non-cancerous liver tissue samples based on data from the GEPIA database. C. Overall survival analysis of TOP2 A in HCC based on data from the GEPIA database. D. Disease-free survival analysis of TOP2 A in HCC based on data from the GEPIA database

resulted in better sensitivity, specificity and accuracy [47]. Regrettably, we only could determine the diagnostic ability of miR-302b-5p with ROC analysis on account of the limited data. Therefore, in the future, it is important to focus on the diagnostic capacity of miR-302b-5p in more detail, as this could have important clinical implications.

The present study is not without limitations, and the first of these is the small sample size used for the RT-qPCR and microarray analysis. A larger sample size and more detecting methods would definitely increase the veracity of our results. Further, even though we identified putative target genes of miR-302b-5p, we were unable to examine its prognostic potential or exact functions in HCC. Detailed in vivo and in vitro tests should be conducted later in this direction. Ultimately, even though TOP2 A was identified as a prominent hub gene of miR-302b-5p in HCC, in-depth experiments need to be conducted to validate the relationship between miR-302b-5p and TOP2 A and the mechanisms involved.

5. Conclusion

Our combined data from RT-qPCR, GEO and ArrayExpress microarray analysis indicate that miR-302b-5p might suppress HCC progression. Further, TOP2 A was identified as a hub gene of miR-302b-5p in HCC, and it may have potential as a prognostic marker in HCC.

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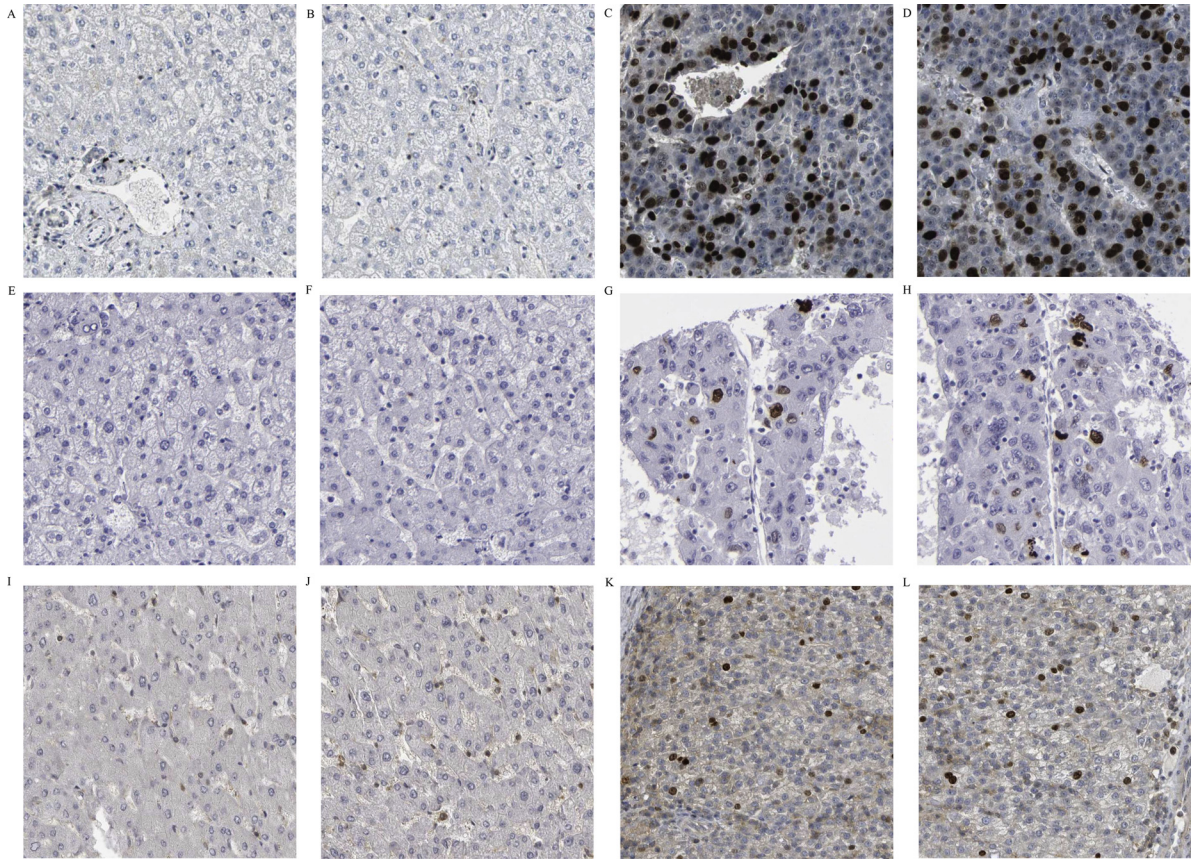

Fig. 10. Protein expression of TOP2 A in HCC tissue and normal tissue.
A.Non-cancerous liver tissue (HPA006458). B. Non-cancerous liver tissue (HPA006458). C. HCC tissue (HPA006458). D. HCC tissue (HPA006458). E. Non-cancerous liver tissue (CAB002448). F. Non-cancerous liver tissue (CAB002448). G. HCC tissue (CAB002448). H. HCC tissue (CAB002448). I. Non-cancerous liver tissue (HPA026773). J. Non-cancerous liver tissue (HPA026773). K. HCC tissue (HPA026773). L. HCC tissue (HPA026773)

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Disclosure statement

No conflict of interests exists.

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