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High miR-139-3p expression predicts a better prognosis for hepatocellular carcinoma: a pooled analysis

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Abstract

Objective: To observe the expression and clinical significance of micro RNA (miR)-139-3p in liver cancer tissues, and to explore its relationship with miR-139-3p target genes related to the prognosis of hepatocellular carcinoma (HCC).

Methods: A total of 362 patients with HCC were included in the study. Liver hepatocellular carcinoma data were obtained directly from The Cancer Genome Atlas data portal .The bioinformatics analysis tool TargetScan was applied to predict miR-139-3p target genes.

Results: Survival time was significantly higher in patients with high miR-139-3p expression, compared with the low miR-139-3p expression group. Bioinformatics analysis showed that miR-139-3p target genes *ISG20L2*, *RAD54B*, *KIAA0101*, and *PIGS* were significantly negatively correlated with miR-139-3p expression.

Conclusions: High miR-139-3p expression in HCC tissues was indicative of good patient prognosis. miR-139-3p target genes ISG20L2, RAD54B, KIAA0101, and PIGS were related to HCC prognosis.

Keywords

miR-139-3p, hepatocellular carcinoma, The Cancer Genome Atlas, target genes, bioinformatics, prognosis

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Introduction

Liver cancer is the fifth most common cancer in the world.[1] It is particularly common in China, where the incidence is 5–10 times higher than that of developed

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countries.[2] Approximately 110,000 people die annually from liver cancer in China, accounting for 45% of liver cancer deaths worldwide. At present, surgery is the most effective liver cancer treatment; however, 60%–70% of patients suffer metastasis and recurrence within 5 years.[3] Therefore, determining molecular targets for new therapies is imperative.

Several micro (mi)RNAs have been found to be directly involved in the development and progression of hepatocellular carcinoma (HCC), with miRNA expression profiles known to be related to diagnoses, staging, progression, and prognoses.[4,5] miR-139-5P, an important non-coding miRNA, is involved in the evolution of tumors where it mainly functions as a tumor suppressor gene.[6,7] It has also been reported to be involved in adrenocortical carcinoma, bladder carcinoma *in situ*, and adrenal pheochromocytoma in which miR-139-3p tissue dysregulation was reported.[8–10] However, to date there is little research addressing the role of miR-139-3p in liver cancer.

This study, based on The Cancer Genome Atlas (TCGA) data, explores the expression and importance of miR-139-3p in HCC tissues using survival analysis and bioinformatics analysis.

Materials and methods

Data collection

Liver hepatocellular carcinoma (LIHC) data were obtained directly from the TCGA data portal (https://tcga-data.nci.nih.gov/docs/publications/tcga) via bulk download (LIHC cancer type). RNASeqV2 liver cancer data (level 3) were downloaded from the TCGA database. Only cases with complete clinical data and survival information were included, which resulted in a total of 362. This study was approved by the Ethics Committee at Zhaoqing Medical College.

miR-139-3p expression in liver cancer tissue and patient prognosis

TCGA RNA sequencing data from the 362 HCC patients were assessed to divide patients into low expression and high expression groups according to the median of the relative miR-139-3p expression level (50%). The relationship between miR-139-3p expression and patient clinicopathologic parameters, including age, sex, race, tumor stage, tumor–node–metastasis (TNM) stage, tumor grade, and recurrence, was analyzed, and survival times for patients with different miR-139-3p expression levels were compared. Univariate and multivariate survival analyses were conducted for patients with different miR-139-3p expression levels to determine whether miR-139-3p is an independent prognostic factor of liver cancer.

The bioinformatics analysis tool TargetScan (www.targetscan.org) was applied to predict miR-139-3p target genes, and to analyze the correlation between the expression of these target genes and that of miR-139-3p. The top 60 genes were selected according to their scores, and 5-year survival analysis was then conducted on their expression levels.

IBM SPSS Statistics for Windows, Version 22.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Differences in the miR-139-3p levels of patients with various clinical features were assessed using an independent Student's t-test. Correlation analysis was conducted with the Spearman's rank correlation coefficient. Survival curves were drawn with a Kaplan–Meier estimator, then assessed with the log-rank test and Cox regression analysis. $P<0.05$ was considered statistically significant.

Results

Correlation between miR-139-3p expression levels and clinicopathologic parameters in liver cancer patients

Among the 362 liver cancer patients identified from the TCGA database, 181 had high miR-139-3p expression and 181 had low expression. miR-139-3p expression levels did not significantly correlate with sex, age, race, or N or M of TNM staging. However, they correlated significantly with tumor grade, tumor stage, and T of TNM staging ($P<0.05$), as shown in Table 1.

Analysis of factors affecting liver cancer prognosis

Kaplan–Meier analysis showed that miR-139-3p expression, tumor stage, and T and M of TNM staging significantly affected patient prognosis (χ^2 10.959, 32.310, 39.164, and 12.820, respectively; $P<0.05$). High miR-139-3p expression predicted a higher survival rate, as shown in Figure 1. These prognostic factors were included in multivariate Cox analysis, with miR-139-3p expression, clinical staging, and T and M of TNM staging as concomitant variables. This showed that only miR-139-3p expression, and T and M of TNM staging were significantly associated with prognosis, as shown in Table 2. Multivariate survival curves for miR-139-3p with T and M of TNM staging as concomitant variables suggested that high miR-139-3p expression was an independent prognosis factor that predicted longer survival time in liver cancer, as shown in Figure 2.

Predicting miR-139-3p targeting genes

Because microRNAs mainly operate in combination with their target genes, we next used the bioinformatics tool TargetScan to predict miR-139-3p target genes. We selected the top 60 genes according to their scores, and conducted survival analysis on their expression. Six target genes were shown to be associated with liver cancer prognosis, with the high expression of five genes and the low expression of one indicating poor prognosis (Table 3). Correlation analysis between the six target genes and miR-139-3p expression showed that *ISG20L2* (rs–0.172, P=0.001), *RAD54B* (rs=0.288, $P<0.0001$), *KIAA0101* (rs=–0.325, $P<0.0001$), and *PIGS* (rs=–0.259, $P<0.0001$) were negatively correlated with miR-139-3p expression, with higher expression associated with worse prognosis.

Discussion

miRNAs are closely associated with cancer, so it is important to understand their relationship with tumor development and progression to ultimately reveal the mechanism of carcinogenesis. miR-139-5p is a newly discovered miRNA that is abnormally expressed in tumors. Its expression decreases in a variety of malignancies, where it performs a function similar to tumor suppressor genes. miR-139-5p inhibits the proliferation and metastasis of colorectal cancer cells by regulating the target gene insulin-like growth factor-1 receptor.[11] Further studies have shown that miR-139-5p levels are associated with lymph node metastasis in esophageal cancer, where it inhibits the invasive ability of esophageal cancer cells by targeting human gene receptor hB1F (nr5a2).[12] However, few studies have addressed its role in HCC.

This study compared the relationship between miR-139-3p expression levels and clinicopathological parameters in HCC patients, as well as comparing expression levels with survival times. Neither sex, age, race, nor N or M of TNM staging were related to miR-139-3p expression in HCC tissues. However, low miR-139-3p

Table 1. Distribution of miR-139-3p expression and clinical variables in 362 liver cancer patients.

Clinical variable	All patients (n=362)	Low miR-139-3p expression (n=181)	High miR-139-3p expression (n=181)	P-value
Age (years)				0.058
≤60	174	78	96	
>60	188	103	85	
Sex				0.175
Male	248	118	130	
Female	114	63	51	
Race				0.265
White	177	92	85	
Asian	158	80	78	
Other	18	5	13	
Unknown	9	4	5	
Tumor stage				0.004
1	171	70	100	
2	83	53	30	
3	83	48	35	
4	4	2	2	
Unknown	21	7	14	
T stage				0.004
T1	181	74	107	
T2	89	57	32	
T3	78	42	36	
T4	13	8	5	
Unknown	1	0	1	
N stage				0.784
N0	249	126	123	
N1	4	2	2	
NX	108	53	55	
Unknown	1	0	1	
M stage				0.370
M0	264	137	127	
M1	3	2	1	
MX	95	42	53	
Tumor grade				0.010
1	55	17	38	
2	174	87	87	
3	120	69	51	
4	13	8	5	
Recurrence				0.114
Yes	171	93	78	
No	191	88	103	

expression appeared to associate with early tumor stage and poor prognosis of HCC, independent of the development and/or progression of the tumor.

Bioinformatics analysis revealed that high expression of miR-139-3p target genes *KIAA0101*, *ISG20L2*, *FAM161A*, *PIGS*, and *RAD54B* was associated with

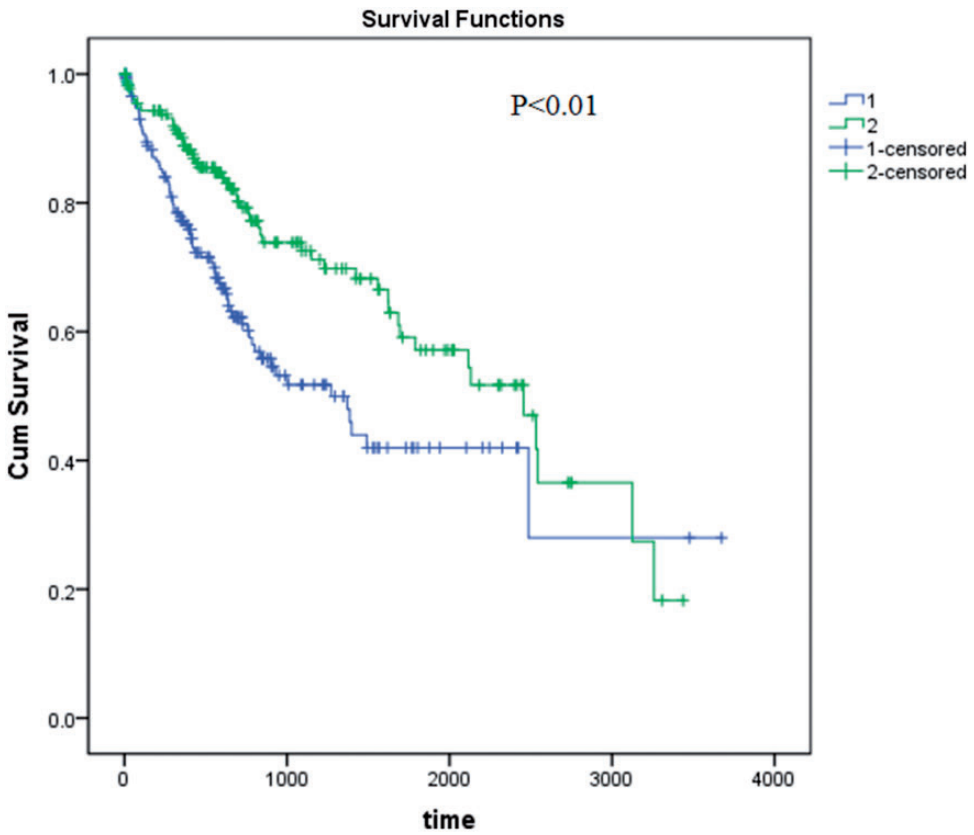

Figure 1. Kaplan–Meier survival analysis of miR-139-3p expression in liver cancer patients ($P<0.01$). Blue line indicates low miR-139-3p expression; green line indicates high miR-139-3p expression.

poor prognosis, while low *AGPAT3* expression was also associated with poor prognosis. The expression of *ISG20L2*, *RAD54B*, *KIAA0101*, and *PIGS* was negatively correlated with the expression of miR-139-3p. The function of these genes has been investigated in previous studies. *KIAA0101*-positive tumor cells were shown to grow more aggressively and to be more tolerant to tissue hypoxia. Moreover, their overexpression was associated with tumor grade, staging, early recurrence, early infiltration, and distant metastasis.[13] In another study, the reduction of *KIAA0101* expression by small interfering RNA inhibited the growth of thyroid cancer cell lines.[14] However, the overexpression of *KIAA0101* in human embryonic kidney cells did not affect their growth characteristics.[15] *PIG11*, another example of *PIGS*, is a downstream target gene of p53 that may be a human liver cancer tumor suppressor gene.[16] HCT116 cells in which *RAD54B* had been knocked-out were used to generate xenografts. After treatment by either oxaliplatin or fluorouracil, these xenografts grew slower in nude mice than xenografts from wild-type HCT116 cells.[17] Finally, *ISG20L1* in peripheral blood lymphocytes exposed to radiation was reported to be significantly up-regulated in healthy humans.[18]

Table 2. Multivariate Cox proportional hazard analysis of overall liver cancer survival rates.

							95.0% CI for Exp (B)	
Variable	B	SE	Wald	df	Sig.	Exp (B)	Lower	Upper
Stage	0.118	0.102	1.326	1	0.250	1.125	.921	1.374
T	0.377	0.121	9.733	1	0.002	1.457	1.150	1.846
M	0.231	0.106	4.801	1	0.028	1.260	1.025	1.550
miR-139-3p	–0.574	0.184	9.726	1	0.002	0.563	0.393	0.808

B, regression coefficient; SE, standard error; Wald, chi-square value; df, degrees of freedom; Sig., P-value; Exp (B), relative risk (hazard ratio); CI, confidence interval.

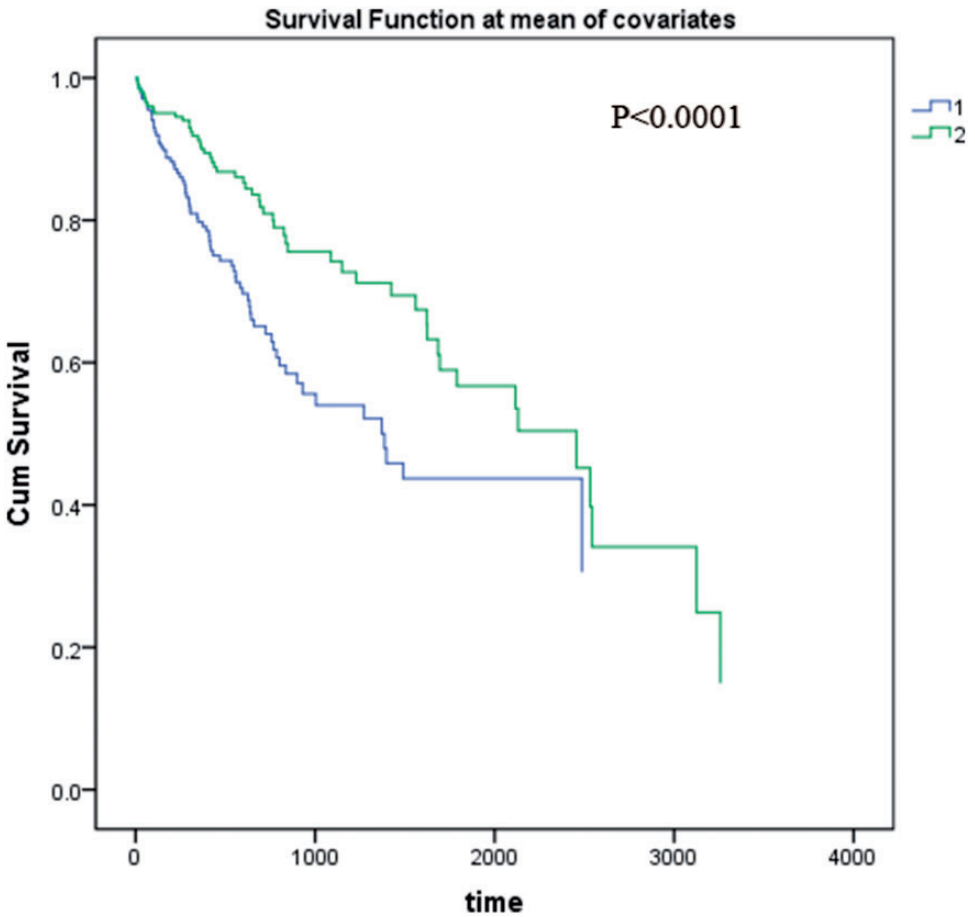

Figure 2. Multivariate Cox survival analysis of miR-139-3p expression in liver cancer patients (covariates: miR-139-3p, T, M). Blue line indicates low miR-139-3p expression; green line indicates high miR-139-3p expression.

Table 3. Predicted target genes related to the overall survival rates of liver cancer patients.

Target gene symbol	Gene name	P-value
AGPAT3	1-acylglycerol-3-phosphate O-acyltransferase 3	0.0299
RAD54B	RAD54 homolog B (*Saccharomyces cerevisiae*)	0.00377
PIGS	phosphatidylinositol glycan anchor biosynthesis, class S	0.0445
FAM161A	family with sequence similarity 161, member A	0.0026
KIAA0101	KIAA0101	0.000151
ISG20L2	Interferon-stimulated 20kDa exonuclease-like 2	0.0326

HCC patients with hepatitis B virus and hepatitis C virus (HCV) infections have higher recurrence rates after surgery than patients with non-hepatitis virus infection.[19] However, the tumor recurrence rate for patients infected with HCV was also higher than in patients without viral infections. This suggests that the HCV infection likely reduces the differentiation of HCC, causing the tumor to recur.[20] Studies have also shown that miR-139 expression is correlated with chronic HCV infection,[21] indicating that miR-139 may influence liver cancer through virus infection.

In summary, the high expression of miR-139-3p seen in HCC patient TCGA data was associated with better prognosis and a longer survival time, suggesting that miR-139-3p may be a prognosis factor for liver cancer. The potential target genes of miR-139-3p, *ISG20L2*, *RAD54B*, *KIAA0101*, and *PIGS*, may play a role in the development and progression of liver cancer.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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