ORIGINAL RESEARCH

High miR-454-3p expression predicts poor prognosis in hepatocellular carcinoma

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Purpose: Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the clinic all over the world, which has become a public health challenge. The T/N/M staging system plays a core role in HCC prognosis. However, it cannot precisely stratify the risk of HCC prognosis. MiR-454-3p plays an important role in the progression of tumors. Therefore, we need to develop more reliable prognostic markers for HCC patients which can focus on miR-454-3p.

Methods: We used Chi-square and Fisher exact tests to assess correlations between miR-454-3p expression and clinical parameters in liver cancer patients from The Cancer Genome Atlas database (TCGA). Then, Cox regression analysis, Kaplan-Meier curve, and log-rank test were used to compare the difference of survival between the high-expression group and low-expression group, and *P* value was included. Finally, we used TCGA data set to carry out gene enrichment analysis.

Results: In this research, the expression of miR-454-3p increased in HCC and was associated with patient survival, G3/G4 staging, III/IV staging and T staging. Higher miR-454-3p expressed patients had shorter survival time. Besides, mitotic spindle, G2M checkpoint, and E2F targets were differentially enriched in miR-454-3p high-expression phenotype by Gene set enrichment analysis.

Conclusion: Overexpression of miR-454-3p may be a significant and independent predictor of poor prognosis in HCC patients.

Keywords: miR-454-3p, hepatocellular carcinoma (HCC), prognosis, predictor

Introduction

HCC is one of the most common malignant tumors in clinic worldwide, as well as one of the few carcinomas with constant increasing mortality and incidence.[1] However, the prognosis of HCC remains gloomy even after surgical resection and chemoradiotherapy. Predicting the prognosis of patients can help doctors give patients appropriate treatment in time, which is of great significance to improve the survival rate of patients. Although studies have suggested that using different histologic parameters could predict the prognosis of HCC, nonetheless, it cannot adequately stratify the risk of HCC prognosis. Therefore, we need to further explore a new cancer prognosis marker to identify HCC patients with poor prognosis.

With the development of whole genome and transcriptome sequencing techniques, many non-coding RNAs (ncRNAs) without the potential for protein coding while having the capacity of tumor prognosis were identified. As a representative member of the small RNAs, miRNA has been studied in depth and has the potential to predict the prognosis of patients.[2–5] The miRNA can induce mRNA degradation or

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Table 1 Demographic and clinical characteristics of TCGA-LIHC cohort

Characteristics	Number of pts (%)
Age (years)	
<55	118 (31.89)
≥55	252 (68.11)
Gender	
Female	119 (32.08)
Male	252 (67.92)
Histological type	
Fibrolamellar carcinoma	3 (0.81)
Hepatocellular carcinoma	361 (97.3)
Hepatocholangiocarcinoma (mixed)	7 (1.89)
Histologic grade	
G1	55 (14.82)
G2	174 (46.9)
G3	125 (33.69)
G4	13 (3.5)
NA	4 (1.08)
Stage	
I	172 (46.36)
II	86 (23.18)
III	85 (22.91)
IV	5 (1.35)
NA	23 (6.2)
T classification	
T1	181 (48.79)
T2	94 (25.34)
T3	80 (21.56)
T4	13 (3.5)
TX	1 (0.27)
NA	2 (0.54)
N classification	
N0	254 (68.46)
N1	4 (1.08)
NX	112 (30.19)
NA	1 (0.27)
M classification	
M0	268 (72.24)
M1	4 (1.08)
MX	99 (26.68)
Residual tumor	
R0	326 (87.87)
R1	16 (4.31)

(Continued)

Table 1 (Continued).

Characteristics	Number of pts (%)
R2	1 (0.27)
RX	21 (5.66)
NA	7 (1.89)
Vital status	
Deceased	128 (34.5)
Living	243 (65.5)
Recurrence	
No	178 (55.8)
Yes	141 (44.2)

Abbreviation: NA, not available.

inhibit translation of mRNAs by targeting the 3′-non-translation region (3′-UTR).[6,7] Therefore, we paid attention to the role of miRNAs in HCC.

Previous studies have examined that miR-454-3p participated in the progression of cancer including non-small cell lung cancer,[8] prostate cancer[9] and breast cancer.[10] However, the role of miR-454-3p in cancer progression and the prognostic meaning of the miR-454-3p expression in HCC remain unclear. In the present study, we found that miR-454-3p expression increased in HCC and it was associated with patient survival, G3/G4 staging, III/IV staging and T staging. Overexpression of miR–454-3p may be a significant independent predictor of poor prognosis in HCC patients.

Materials and methods
RNA-sequence data of hepatocellular carcinoma from TCGA

The RNA-sequence data of 371 liver cancer patients and corresponding clinical data were obtained from the public cancer genome map data portal. Clinicopathological parameters including age, sex, and T/N/M staging were also evaluated. The sequence of miR-454-3p is UAGUGCAAUAUUGCUUAGGGU (http://www.mirbase.org/cgi-bin/mature.pl?mature_acc=MIMAT0003885).

Statistical analysis

The box-plot was obtained to describe the expression difference of discrete variables by ggplot2[11] in R.[12] The correlation between miR-454-3p expression and clinicopathological features was tested by chi-square test. The expression of miR-454-3p determined by ROC threshold value was used to define the high and low expression of
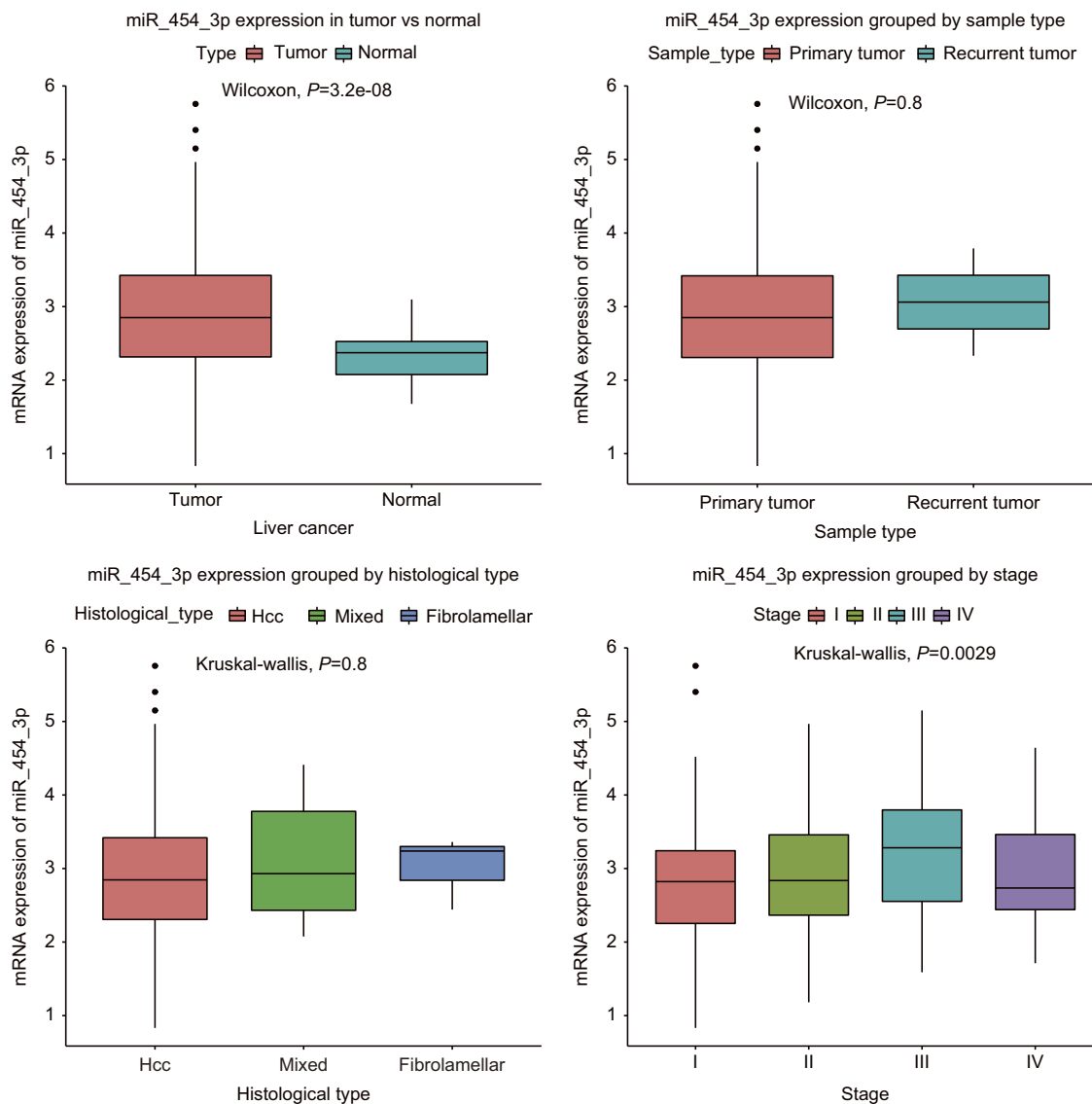
Figure 1 The different miR-454-3p expressions in the boxplot. Boxplots showing the expression differences of miR-454-3p according to histological type, sample type, tumor vs normal clinical and stage.

miR-454-3p. The Kaplan-Meier curve was used to compare the overall survival difference between the low and high expression groups, and the survival package in R was used to calculate the *P*-value through the logarithmic rank test. Cox regression analysis was used to evaluate the survival rate of patients with given clinical, pathological factors and the expression of miRNA-454-3p. Multivariate Cox analysis was used to study the effect of miRNA-454-3p expression on survival rate and clinical parameters (gender, grade, clinical stage, T/N/M stage, etc.)

GSEA

GSEA was used to assess the distribution trend of pre-defined gene sets in gene lists sorted by phenotype

correlation and to determine the contribution of genes phenotype.[13] In this study, GSEA was performed by using the GSEA 3.0 software, and the gene set of "h. all.v6.2.symbols.gmt" from the Molecular Signatures Database. We obtained standardized enrichment fraction (NES) by using permutation analysis 1,000 times.

Results
The characteristics of liver cancer patients

According to The Cancer Genome Atlas Liver Hepatocellular Carcinoma data (TCGA-LIHC), 371 clinical patients and miR-454-3p expression data of liver cancer were studied.

Table 2 Correlation between the expression of miR-454-3p and the clinicopathologic characteristics in liver cancer

Characteristics	High expression (%)	Low expression (%)	P
Age (years)			
<55	31	87	0.3231
≥55	53	199	
Gender			
Female	37	82	0.0145
Male	48	204	
Histological type			
Fibrolamellar carcinoma	0	3	0.6005
Hepatocellular carcinoma	83	278	
Hepatocholangiocarcinoma (mixed)	2	5	
Histologic grade			
G1	7	48	0.0529
G2	36	138	
G3	38	87	
G4	3	10	
Stage			
I	25	147	0
II	21	65	
III	35	50	
IV	2	3	
T classification			
T1	27	154	0.0003
T2	23	71	
T3	31	49	
T4	3	10	
TX	1	0	
N classification	61	193	0.0247
N0	3	1	
N1	21	91	
NX			
M classification			
M0	70	198	0.0137
M1	2	2	
MX	13	86	
Residual tumor			
R0	73	253	0.8043
R1	5	11	
R2	0	1	

(Continued)

Table 2 (Continued).

Characteristics	High expression (%)	Low expression (%)	P
RX	5	16	
Vital status			
Deceased	39	89	0.0171
Living	46	197	
Recurrence			
No	32	146	0.0537
Yes	39	102	

Table 1 describes the demographic and clinical characteristics of the liver cancer patients, including age, gender, histological type, T/N/M staging, etc.

The relationship between the expression of miRNA-454-3p and the clinicopathological parameters of liver cancer

In this study, HCC is the largest sample of patients with liver cancer, and the expression of miR-454-3p was not statistical significance with histological type. The expression of miR-454-3p in liver cancer (n=371) were higher than that in normal tissues (n=50, *P*<0.05). Besides, the expression of miR-454-3p was significantly different in the clinical staging of cancer (Figure 1). To assess the relationship between miR-454-3p expression and the clinicopathological parameters of liver cancer, patients were divided into the high expression group of miR-454-3p and the low expression group of miR-454-3p according to the optic cutoff (threshold value in ROC) of miR-454-3p expression (Table 2). Chi-square and Fisher exact tests shows that the high expression group of miR-454-3p with recurrence (*P*=0.0537), survival status (*P*=0.0171), T classification (*P*=0.0003), N classification (*P* =0.0247), M classification (*P*=0.0137), clinical period (*P*=0.048) and gender (*P*=0.0145) are obviously correlated.

High miR-454-3p expression is an independent prognostic factor for overall survival

Kaplan-Meier curve of overall survival showed that high miR-454-3p expression was associated with poor

survival (*P*=0.0046, Figure 2). The subsequent subgroup analysis demonstrated that high miR-454-3p expression was associated with poor overall survival in G3/G4 stage tumors (*P*=0.026) and advanced (III/IV) liver cancer (*P*=0.016; Figure 2).
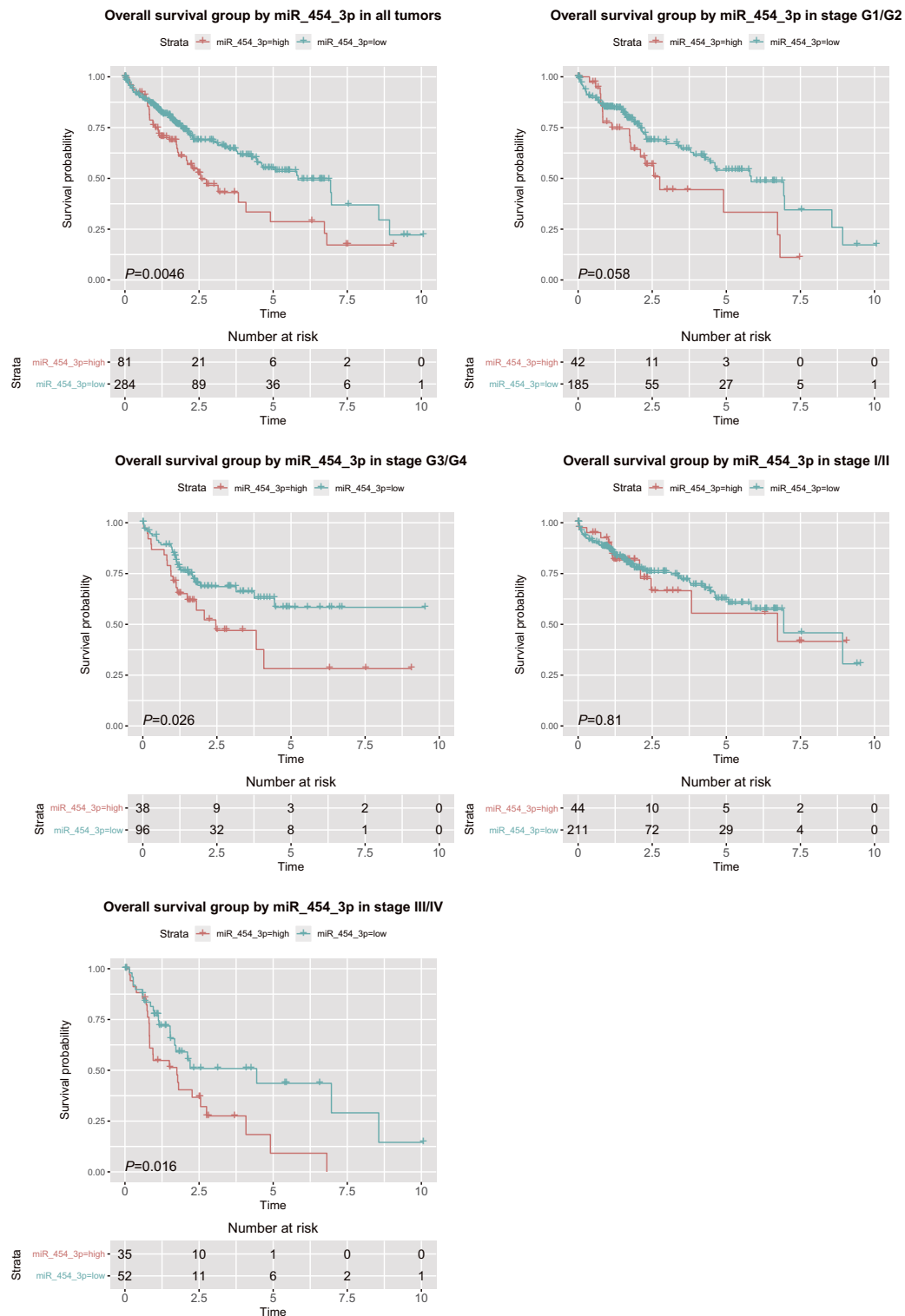
Figure 2 Survival analysis of miR-454-3p expression in terms of overall survival. Kaplan–Meier curves produced survival analysis and subgroup analysis. Subgroup analysis includes histological grade (G1/G2 and G3/G4) and clinical stage (I/II and III/IV).

Univariate Cox regression analysis showed that poor overall survival was correlated with clinical stage (*P*=0.001), T classification (*P*=0), residual tumor (*P*=0.002), and miR-454-3p expression (*P*=0.005; Table 3). A further multivariate analysis confirmed overexpression of miR-454-3p was an independent prognostic factor for poor overall survival (hazard ratio: 1.58, 95% confidence interval: 1.06–2.34, *P*=0.024; Table 3).

GSEA identifies a miR-454-3p-related signaling pathway

To identify the signaling pathways activated in HCC, GSEA was performed between the low miR-454-3p expression data set and the high miR-454-3p expression data set.[13] As shown in Table 4, GSEA reveals significant differences in the enrichment of MSigDB Collection (FDR <0.25, NOM *P*-value<0.05; h.all.v6.2. symbols.gmt). We chose the most significant signaling pathways based on NES (Figure 3; Table 4). Figure 3 shows that E2F targets, mitotic spindle, and G2M checkpoint is differentially enriched in miR-454-3p high-expression phenotype.

Discussion

Analysis of TCGA-LIHC data showed that miR-454-3p expression was increased in liver cancer, and the miR-454-3p expression was relevant to the patient's gender, clinical stage of liver cancer, T/N/M stage and survival status. Survival analysis showed that high expression of miRNA-454-3p was associated with poor overall survival, especially

Table 3 Summary of univariate and multivariate Cox regression analyses of overall survival duration

	Parameters	Univariate analysis			Multivariate analysis		
		HR	95%CI	P	HR	95%CI	P
1	Age						
	≥55/<55	1.04	0.71–1.51	0.836			
2	Gender						
	Male/female	0.81	0.57–1.17	0.261			
3	Histological type						
	Hepatocholangiocarcinoma/hepatocellular/fibrolamellar	0.99	0.27–3.69	0.99			
4	Histologic grade						
	G4/G3/G2/G1	1.05	0.84–1.3	0.671			
5	Stage						
	IV/III/II/I	1.38	1.15–1.66	0.001	0.82	0.66–1.03	0.088
6	T classification						
	T4/T3/T2/T1/NX	1.67	1.39–2	0.00	1.87	1.48–2.35	0.00
7	N classification						
	N1/N0/NX	0.72	0.5–1.04	0.083			
8	M classification						
	M1/M0/MX	0.73	0.5–1.06	0.097			
9	Radiation therapy						
	Yes/no	0.51	0.25–1.02	0.056			
10	Residual tumor						
	Yes/no	1.44	1.14–1.83	0.002	1.43	1.12–1.83	0.005
11	miR-454-3p						
	High/low	1.72	1.18–2.51	0.005	1.58	1.06–2.34	0.024

Table 4 Gene sets enriched in phenotype high

MSigDB collection	Gene set name	NES	NOM *P*-value	FDR q-value
h.all.v6.2.symbols.gmt	HALLMARK_MITOTIC_SPINDLE	1.905323	0.00	0.074578
h.all.v6.2.symbols.gmt	HALLMARK_G2M_CHECKPOINT	1.801321	0.002079	0.084591
h.all.v6.2.symbols.gmt	HALLMARK_E2F_TARGETS	1.691148	0.010309	0.12907

Notes: Gene sets with NOM *P*-value <0.05 and FDR q-value <0.25 are considered as significant.
Abbreviations: FDR, false discovery rate; NES, normalized enrichment score; NOM, nominal.
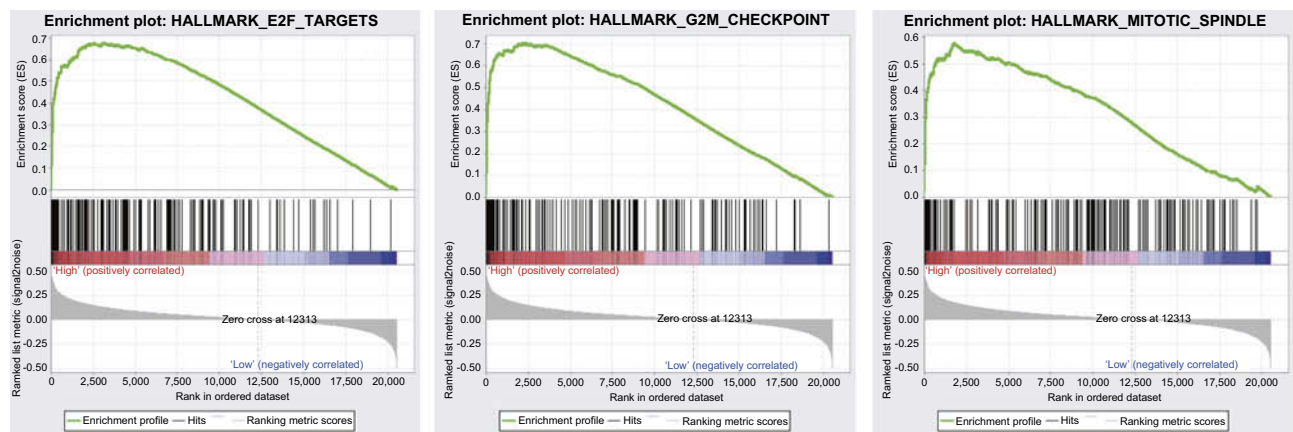
Figure 3 Enrichment plots from GSEA. GSEA results showing E2F targets, G2M checkpoint, and mitotic spindle pathway were differentially expressed in high miR-454-3p expression group and low miR-454-3p expression group.

in III/IV stage of liver cancer. Univariate Cox and multivariate Cox analysis showed that miR-454-3p expression was an independent risk factor for prognosis of HCC patients.

Many former studies have shown that miR-454-3p is highly expressed in a variety of cancers, such as non-small cell lung cancer,[8] prostate cancer[9] and breast cancer.[10] Yue ZD reported that high miR-454 expression was significantly associated with histologic grade, and T/N/M stage.[14] However, we found that miR-454-3p expression levels were not statistically significant in histologic grade, N classification and M classification, while it was associated G3/G4 staging, III/IV staging and T staging in liver cancer. The reason may be that the samples we studied come from many countries, we studied miR-454-3p expression in samples of liver cancer patients from white, yellow and black people in TCGA, whereas the previous study used HCC samples from yellow people only. Also, we researched that the 3′ product (miR-454-3p) of miR-454 may be an independent risk factor for the prognosis of HCC patients.

It is well known that miRNAs deregulation is involved in cell cycle regulation among various diseases.[15–18] In previous studies, miR-454-3p promotes the proliferation of prostate cancer cells by down-regulating N-myc downstream-regulated gene 2 (NDR 2).[19] Based on our findings, miR-454 expression was related to clinical stage and T stage in liver cancer, as well as mitotic spindle and G2M checkpoint were enriched, we postulate that miR-454-3p promotes cellular proliferation and tumor growth due to the failure of cell cycle checkpoint. However, the target gene of miR-454-3p in HCC remains to be further verified. miR-454-3p can promote the progression of

cancer, which implies that its high expression is associated with poor prognosis of patients.

Metastasis of cancer cells is one of the main causes of poor prognosis in HCC patients.[20] Phosphatase and tensin homolog (PTEN), as cancer cell metastasis inhibitors, was confirmed as a direct target of miR-454 by luciferase report analysis in non-small cell lung cancer (NSCLC).[21] Also, we identified that miR-454-3p expression has a statistical difference in the N and M stages of the tumor. Meanwhile, the accurate mechanisms of miR-454-3p metastasis in HCC need further verification.

In conclusion, the present study has demonstrated a universal fact that miR-454-3p may be a significant independent predictor of poor prognosis in HCC patients through TCGA database mining. Besides, mitotic spindle, G2M checkpoint, and E2F may be targets of miR-454-3p. However, we are aware of the limitations of our work – there is no patient tissue sampled. In our future work, the precise mechanism of miR-454-3p regulating cell cycle and metastasis in a large number of HCC samples will be verified.

Disclosure

Yang Li and Jing Su are co-authors. The authors report no conflicts of interest in this work.

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