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ORIGINAL ARTICLE

MicroRNA-200c expression is decreased in hepatocellular carcinoma and associated with poor prognosis

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Summary Accumulating evidences have shown that microRNA-200c (miR-200c) expression is associated with the prognosis of many types of human cancer. However, the prognostic value of miR-200c in hepatocellular carcinoma (HCC) is still unknown. In the present study, the expression of miR-200c in paired HCC and adjacent non-tumor tissues from 148 patients was determined by quantitative real-time PCR (qRT-PCR), and we analyzed its association with clinicopathological characteristics and prognosis of HCC patients. Our results showed that the expression of miR-200c was significantly decreased in HCC tissues compared with adjacent non-tumor tissues ($P < 0.0001$). Correlation analysis showed that miR-200c expression was significantly associated with tumor size ($P = 0.021$), serum AFP level ($P = 0.016$), TNM stage ($P = 0.019$) and vein invasion ($P = 0.026$). Patients with lower miR-200c expression had significantly worse overall survival (OS, $P = 0.023$) and recurrence-free survival (RFS, $P = 0.002$). The multivariate Cox regression analysis revealed that miR-200c expression was an independent prognostic factor for OS (hazard ratio (HR) [95% CI] = 2.226 [1.235—4.012], $P = 0.008$) and RFS (HR [95% CI] = 2.662 [1.618—4.38], $P < 0.001$). In conclusion, our results suggest that the miR-200c expression was significantly down-regulated and associated with poor prognosis in HCC.

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Introduction

According to the most recent cancer statistics in China, liver cancer is the most commonly diagnosed cancer and the leading cause of cancer death in men under 60 years old [1]. Despite remarkable advances in early diagnosis and treatment, hepatocellular carcinoma (HCC), which accounts for 85%—90% of all primary liver cancers, is still often diagnosed at an advanced stage and has a poor prognosis, because of its characteristics of rapid progression, strong invasiveness, and the high recurrence rate [2,3]. It has been reported that HCC patients have a relatively lower 5-year overall survival (OS) rate, which is approximately 5—9% [4,5]. Currently, hepatic resection is still widely accepted as the first-line therapy for patients with localized tumor and well-preserved liver function. Unfortunately, fatal recurrence or metastasis of HCC could reach an incidence of more than 70% at 5 years after surgery [6]. Since the prediction of the prognosis is crucial for optimizing personalized treatment, various factors such as tumor size, multifocal disease, histological differentiation and vascular invasion have been investigated and proved to be associated with recurrence and survival in patients after surgical resection for HCC [7—9]. However, the prognostic value of these clinicopathological risk factors is still not enough to fulfill a clinical need, additional new biomarkers are required for improving the current prognostic assessment of HCC patients.

MicroRNAs (miRNAs) are a class of highly conserved small non-coding RNAs, about 22—24 bases long, that suppress gene expression by inducing translational repression or mRNA degradation [10,11]. Many studies have reported that miRNAs act as oncogenes or tumor suppressor genes in tumor growth, invasion, metastasis and apoptosis, depending on the functions of their target mRNAs [12]. In recent years, accumulating evidences have suggested that miRNAs play a critical role in HCC development and progression [13—15]. And there are also studies have demonstrated that several miRNAs in tumor tissues have great potential for helping prognostic assessment of HCC patients, such as miR-25, miR-130a, miR-221 and miR-339 [16—19].

MiR-200c, which located on chromosome 12p13, is the most representative and highly studied miRNA among the five members of the miR-200 family [20]. It has been demonstrated that miR-200c plays a critical role in the regulation of epithelial-mesenchymal transition (EMT), which is a crucial step in the invasion and metastasis of tumor cells [21,22]. Thus, miR-200c is supposed to be used as a diagnostic or prognostic biomarker for cancer patients. Up to now, numerous studies have investigated the association between the expression levels of miR-200c and the prognosis of various human cancers, including lung cancer, gastric cancer, bladder cancer, prostate cancer, and so on [23]. But, the results are conflicting on this issue in different types of cancer or populations [23]. It was reported by Guo et al. that reduced miR-200c expression could induce abnormal hepatic lipid accumulation by stimulating JUN expression and activating the transcription of srebp1 [24], and another in vitro study by Li et al. has demonstrated that miR-200c could inhibit the proliferation, migration and invasion of HCC cells by suppressing MAD2L1 [25]. However, to the best of our knowledge, the clinical significance of miR-200c in HCC has not been studied yet.

In the present study, we investigated the expression levels of miR-200c in HCC and adjacent non-tumor tissue samples. And we also analyzed the correlation of miR-200c expression with clinicopathological characteristics and survival rates of the HCC patients after 2 years surgery, by which to determine the prognostic role of miR-200c in HCC.

Material and methods

Patients and tissue samples

A total of 148 HCC patients who underwent surgical resections at affiliated hospital of Youjiang medical college for nationalities were recruited in the study between January 2014 and December 2016. None of the patients had received chemotherapy, radiotherapy or immunotherapy before surgery. The diagnosis of each HCC case was histopathologically confirmed. All tumor and matched adjacent non-tumor liver tissue samples were obtained and well-preserved at -80° until they were used for the experiments. The present study was approved by the ethics committee of affiliated hospital of Youjiang medical college for nationalities. All patients provided written informed consent to approve the use of their tissue samples for research purposes. Patients' clinical information, including age, gender, tumor size, number of tumor nodules, histologic grade, TNM stage, vein invasion, cirrhosis status, Hepatitis B virus (HBV) infection, and serum level of α-fetoprotein (AFP) was collected. The follow-up information was updated at least every 3 months by a telephone call or clinical examination during 2 years of follow-up. OS is defined as the time elapsed from the surgery to the death of the HCC patients.

RNA isolation and quantitative real-time PCR (qRT-PCR)

Total RNA was isolated from all tissue samples using the Trizol reagent (Invitrogen, US) according to the manufacturer's protocol. The purity and concentration of RNA were measured with a Nanodrop™ spectrophotometer (Thermo Scientific, US). The miR-200c and U6-specific cDNAs were synthesized from the total RNA using TaqMan miRNA Reverse Transcription kits (Thermo Scientific, US). Then, the reverse transcription products were amplified and detected by real-time PCR using TaqMan™ MicroRNA Assays specific for miR-200c and U6 (Thermo Scientific, US) on a CFX Connect™ Real-Time PCR system (Bio-Rad, US). Each sample was carried out in triplicate. The cycling conditions were: 95° for 10 minutes, 40 cycles of 95° for 15 seconds and 60° for 1 minute. U6 small nuclear RNA was used as an endogenous control for normalization. The cycle threshold (CT) value was calculated. And relative quantification of miR-200c expression for each sample was calculated by using the $2^{-\Delta\Delta CT}$ method.

Statistical analysis

The statistical analyses were performed using the SPSS version 19.0 software and GraphPad Prism 5.0 software. Continuous variables were expressed as means ± standard

deviations (SD). Paired student's t test was used to compare the miRNA levels between tumor and adjacent non-tumor tissues. Patients were divided into high and low expression groups according to the median relative level of miR-200c. Associations between miR-200c expression and clinicopathological characteristics were analyzed using Chi-square test. The survival curves were estimated using the Kaplan-Meier method, and differences in the survival distributions were analyzed by the log-rank test. Multivariate analysis of the prognostic factors was performed with a Cox proportional hazards regression model. A *P*-value of less than 0.05 was considered to be significant.

Results

Patient characteristics

The clinical characteristics for 148 HCC patients are described in Table 1. The 148 patients included 100 men and 48 women. The median age at diagnosis was 51.7 years (range, 35—70 years). Most of patients had positive HBV infection (85.1%), cirrhosis (86.5%), well differentiated or moderately differentiated HCC (80.4%), and vascular invasion was only present in 24 patients (16.2%). Nearly 40 percent of patients had elevated serum AFP level (> 400 ng/mL) and multiple tumor nodules. The median time of follow-up was 19.5 months (range, 3—24 months). By the end of the last follow-up, eighty patients had experienced tumor recurrence and 55 patients had died. The median recurrence-free survival (RFS) was 17.5 months.

MiR-200c expression was down-regulated in HCC

Expression of miR-200c was measured in 148 paired HCC and adjacent non-tumor liver tissues by qRT-PCR. The results showed that the relative expression of miR-200c in HCC tissues was 3 ± 1.28 (mean $\pm$ SD), while the relative expression of miR-200c in adjacent non-tumor liver tissues was 5.99 ± 1.82. The difference was statistically significant ($P < 0.0001$, Fig. 1), indicating that miR-200c expression was down-regulated in HCC.

The association of miR-200c expression with clinicopathological characteristics of HCC patients

To identify the role of miR-200c expression in HCC, we analyzed the correlation between miR-200c expression and clinicopathological characteristics, including gender, age, tumor size, number of tumor nodules, histologic grade, TNM stage, vein invasion, cirrhosis status, HBV infection and serum AFP level. The results showed that low expression of miR-200c was more frequently detected in patients with large tumor ($P = 0.021$), elevated serum AFP level ($P = 0.016$), advanced TNM stage ($P = 0.019$) and vein invasion ($P = 0.026$) (Table 1). However, there were no significant correlations of miR-200c expression with other clinicopathological characteristics.

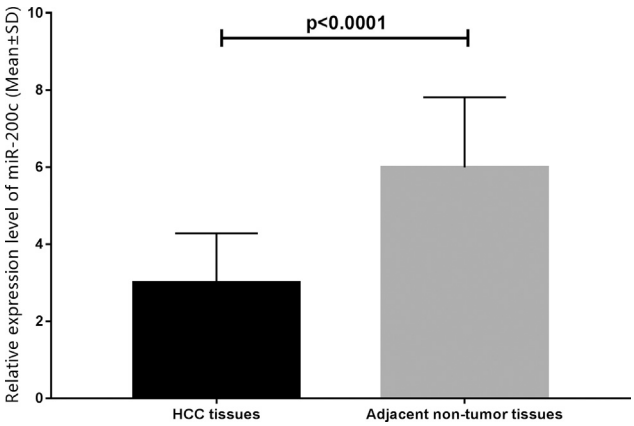

Figure 1 Comparison of miR-200c expression levels between HCC tissues and adjacent non-tumor liver tissues. Analysis using the paired student's *t*-test showed that the relative expression levels of miR-200c in the HCC tissues were significantly lower than those in adjacent non-tumor liver tissues ($P < 0.0001$).

The expression levels of miR-200c correlate with prognosis of patients with HCC

We analyzed the OS and RFS rates to evaluate the prognostic significance of miR-200c expression by using the Kaplan-Meier method and log-rank test. The results showed that HCC patients with low miR-200c expression had shorter OS ($p = 0.023$, Fig. 2A) and RFS ($P = 0.002$, Fig. 2B) than those with high miR-200c expression. The 2-year OS and RFS rates in the low expression group was 54.1% and 33.8% respectively, compared with 71.6% and 58.1% respectively, in the high expression group. Univariate analysis showed that miR-200c expression levels, tumor size, AFP level, TNM stage, vein invasion were significantly correlated with OS and RFS ($P < 0.05$, Table 2 and Table 3). A multivariate analysis was performed on all variables which were significant in the univariate analysis, by using the Cox proportional hazards model. The results showed that miR-200c expression levels ($P = 0.008$), tumor size ($P = 0.006$) and TNM stage ($P = 0.007$) were independent prognostic factors for predicting the 2-year OS of HCC patients (Table 2). Moreover, on analyses of RFS, miR-200c expression levels ($P < 0.001$), tumor size ($P = 0.007$), serum AFP level ($P = 0.018$) and TNM stage ($P = 0.022$) emerged as significant independent prognostic factors (Table 3).

Discussion

Despite the success of HBV vaccine to prevent liver cancer in children in China, there are approximately 466,100 new cases of liver cancer reported per year, which account for one-third to one-half of the global incidence [1]. And death rates are still increasing rapidly for liver cancer, while the overall cancer death rates are decreasing continuously over the past 2 decades [1,26]. Thus, novel biomarkers for HCC diagnosis and prognosis are required, as well as new therapies. Numerous studies have shown that miRNAs hold great promise as diagnostic and prognostic markers for cancer, because they are stable and easy to detect. Up to now,

Table 1 The miR-200c expression and clinicopathological characteristics of HCC patients.

Variable	No.	Tissue miR-200c relevant expression level		
		Low, *n* (%)	High, *n* (%)	*P*-value
Gender				
Male	100	52 (35.1)	48 (32.4)	0.482
Female	48	22 (14.9)	26 (17.6)	
Age				
< 50 years	63	30 (20.3)	33 (22.3)	0.618
≥ 50 years	85	44 (29.7)	41 (27.7)	
HBV infection				
Negative	22	15 (10.1)	7 (4.7)	0.065
Positive	126	59 (39.9)	67 (45.3)	
Cirrhosis				
Negative	20	8 (5.4)	12 (8.1)	0.336
Positive	128	66 (44.6)	62 (41.9)	
Tumor size (cm)				
≤ 5	82	34 (23)	48 (32.4)	0.021[a]
> 5	66	40 (27)	26 (17.6)	
Tumor number				
Solitary	92	44 (29.7)	48 (32.4)	0.498
Multiple	56	30 (20.3)	26 (17.6)	
Serum AFP level (ng/mL)				
≤ 400	96	41 (27.7)	55 (37.2)	0.016[a]
> 400	52	33 (22.3)	19 (12.8)	
TNM stage				
I—II	88	37 (25)	51 (34.5)	0.019[a]
III—IV	60	37 (25)	23 (15.5)	
Vein invasion				
Absent	124	57 (38.5)	67 (45.3)	0.026[a]
Present	24	17 (11.5)	7 (4.7)	
Histologic grade				
Well + moderate	119	58 (39.2)	61 (41.2)	0.534
Poor	29	16 (10.8)	13 (8.8)	

HBV: hepatitis B virus; AFP: α-fetoprotein.
[a] Means *P*-value < 0.05.

Table 2 Univariate and multivariate Cox regression analyses of overall survival in 148 HCC patients.

	Univariate analysis		Multivariate analysis	
Variable	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
miR-200c (low)	1.88 (1.091—3.24)	0.023[a]	2.226 (1.235—4.012)	0.008[a]
Gender (female)	0.97 (0.548—1.719)	0.917		
Age (≥ 50 years)	1.401 (0.825—2.377)	0.212		
HBV infection (positive)	1.254 (0.567-2.771)	0.576		
Cirrhosis (positive)	1.129 (0.533-2.389)	0.751		
Tumor size (> 5 cm)	1.872 (1.098-3.193)	0.021[a]	2.192 (1.257—3.823)	0.006[a]
Tumor number (multiple)	1.25 (0.732-2.137)	0.414		
AFP (> 400 ng/mL)	2.411 (1.419-4.098)	0.001[a]	1.624 (0.858—3.073)	0.136
TNM stage (III—IV)	2.789 (1.622-4.795)	< 0.001[a]	2.205 (1.246—3.902)	0.007[a]
Vein invasion (present)	3.058 (1.699-5.505)	< 0.001[a]	1.872 (0.929—3.769)	0.079
Histologic grade (poor)	1.502 (0.709-3.179)	0.288		

HBV: hepatitis B virus; AFP: α-fetoprotein; HR: hazard ratio; CI: confidence interval.
[a] Means *P*-value < 0.05.

Table 3 Univariate and multivariate Cox regression analyses of recurrence-free survival in 148 HCC patients.

	Univariate analysis		Multivariate analysis	
Variable	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
miR-200c (low)	2.019 (1.286—3.17)	0.002[a]	2.662 (1.618-4.38)	< 0.001[a]
Gender (female)	1.082 (0.681—1.72)	0.74		
Age (≥ 50 years)	1.343 (0.866—2.083)	0.188		
HBV infection (positive)	1.726 (0.831—3.585)	0.143		
Cirrhosis (positive)	1.091 (0.577—2.062)	0.788		
Tumor size (> 5 cm)	1.662 (1.071—2.579)	0.024[a]	1.89 (1.187—3.008)	0.007[a]
Tumor number (multiple)	1.495 (0.962—2.323)	0.074		
AFP (> 400 ng/mL)	2.198 (1.413—3.419)	< 0.001[a]	1.936 (1.122—3.342)	0.018[a]
TNM stage (III-IV)	2.221 (1.429—3.452)	< 0.001[a]	1.74 (1.085—2.79)	0.022[a]
Vein invasion (present)	2.217 (1.291—3.806)	0.004[a]	1.209 (0.634—2.306)	0.564
Histologic grade (poor)	1.369 (0.755—2.48)	0.301		

HBV: hepatitis B virus; AFP: α-fetoprotein; HR: hazard ratio; CI: confidence interval.
[a] Means *P*-value < 0.05.

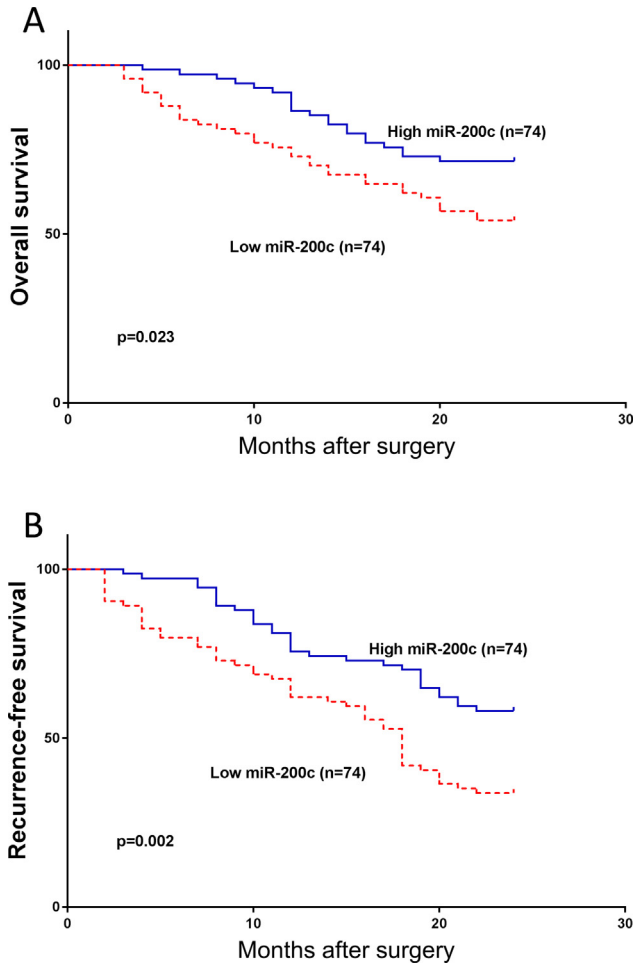

Figure 2 Survival analysis of 148 HCC patients by Kaplan—Meier method. A. Overall survival of patients with high and low expression of miR-200c in HCC tissues. B. Recurrence-free survival of patients with high and low expression of miR-200c in HCC tissues.

approximately 50 miRNAs have been reported to be associated with the prognosis of HCC [27]. For example, the overexpression of miR-25 in HCC tissues, which was significantly correlated with serum AFP level and TNM stage, is of predictive value on poor prognosis [16]. MiR-200 family is composed of two clusters: the miR-200a/b/429 cluster and the miR-200c/141 cluster [20]. Since Hurteau et al. first reported that miR-200c was dysregulated in several cancer cell lines [28], many studies have demonstrated that miR-200c could be used as an intracellular marker in tissues and an extracellular marker in body fluids for diagnosis and prognosis of patients with various types of cancer [23]. To the best of our knowledge, the present study is the first to provide reliable evidence about the association of miR-200c expression in HCC tissues with clinicopathological characteristics, OS and RFS rates of the HCC patients after 2 years surgery, in a Chinese population.

Previous studies have reported that the expression of miR-200c was significantly down-regulated in tumor tissues including high-grade dysplastic nodules (HGDNs), unifocal small HCCs (sHCCs) and advanced HCCs (aHCCs), compared with their corresponding adjacent non-tumor liver tissues [25]. Similar to that, in the present study, we also found that the expression levels of miR-200c were significantly decreased in HCC tissues. CRKL plays an important role in cell proliferation and correlates with the invasion and metastasis of liver tumors [29]. Previous study by Tamura et al. has demonstrated that the miR-200b/200c/429 could inhibit CRKL mRNA and protein expression by directly targeting its 3'-UTR region, and miR-200 family members are direct targets of p53 family. Thus, p53 family could down-regulate the CRKL oncogene through miR-200b/200c/429 transactivation [30]. Since the somatic mutations in the *p53* gene are involved in the molecular pathogenesis of HCC, and *p53* response pathway is frequently defective in HCC [31], these might be the mechanism by which the miR-200c expression was down-regulated in HCC tissues.

Previous study by Karakatsanis et al. has also reported that the expression levels of miR-200c were significantly decreased in HCC tissues, but no significant associations were found between miR-200c expression and clinical fea-

tures including tumor size, differentiation and stage [32]. However, in the search of possible correlations with clinicopathological characteristics, our correlational analyses revealed that the expression of miR-200c was significantly associated with tumor size, serum AFP level, TNM stage and vein invasion. These differences in results might be related to the clinical characteristics of the different study populations, because the proportion of HCC patients with large tumor, poor differentiated tumor and advanced TNM stage was much lower in the present study, when compared with the study by Karakatsanis et al [32]. Our results were consistent with previous in vitro studies, which had shown that up-regulation of miR-200c could suppress the proliferation, migration, invasion and induced apoptosis and cell cycle arrest of HCC cells [25]. Thus, it suggested that miR-200c might represent a novel biomarker of tumor aggressiveness. Considering that circulating miRNAs, which are notably stable in the serum or plasma, have been suggested as promising non-invasive diagnostic markers for various types of cancer, further basic and clinical studies which aim to evaluate the diagnostic value of circulating miR-200c for HCC patients would be of great value in improving detection practices. Moreover, although miR-200c plays a critical role in the regulation of EMT, it was surprising that we did not found a correlation between miR-200c expression and tumor differentiation. It might be deviations in analysis because of the relative small number of poorly differentiated tumors (29/148) in the present study. Further studies are required. In addition, the expression patterns of several miRNAs might be different in benign, malignant and various subtypes of hepatocellular tumors, such as miR-200c, miR-203, miR-10b, miR-21 and miR-222 [33]. It is noteworthy that miR-200c has been reported to be under-expressed in hepatocellular benign tumors when compared to HCC [33], but it is still unknown how and why benign tumors cause changes in the expression levels of miR-200c. We supposed it was related to some specific oncogene and tumor suppressor gene mutations, which might be used for a refined classification of hepatocellular tumors. Further studies are also required.

Accumulating evidences have shown that miR-200c plays an important role in regulating cancer metastasis and acquisition of drug resistance [20]. In a most recent review, 34 eligible studies concerning the miR-200c expression and cancer prognosis, were included in the meta-analysis [23]. Approximately half of these studies demonstrated that the elevated expression levels of miR-200c was associated with the superior prognosis of certain types of cancer including gastric cancer, ovarian cancer, breast cancer, colorectal cancer and so on, indicating that miR-200c served as an anti-oncogene. However, other studies reported opposing results in these cancers and verified the oncogenic function of miR-200c. The results showed that the pooled hazard ratios (HRs) reveal a significant relationship between the up-regulated expression levels of miR-200c and worse overall survival rates (HR = 1.37, 95% confidence interval (CI): 1.01—1.85) [23]. In contrast, our results demonstrated that HCC patients with low miR-200c expression levels had a significantly worse 2-year OS and RFS rates than those with high miR-200c expression levels. Moreover, multivariate analysis identified that miR-200c was a prognostic indicator independent of adjusted well-known prognostic factors for HCC including tumor size, serum AFP level, TNM stage and vein invasion. Thus, miR-200c has great potential to be used as a prognostic biomarker for HCC patients after surgery.

Several limitations in the present study should be acknowledged. First, all patients were recruited consecutively from a single center in our hospital, which means they may not be representative of the general HCC patients. Second, we still lack of uniformity and reproducibility in the criteria for discriminating between high and low expression of miR-200c in HCC patients, which may lead to its poor transferability to clinical practice, and different results from other studies in the future. Third, the retrospective nature of the present study and the small sample size did not allow us to draw any concrete conclusions regarding the effectiveness of miR-200c in the prediction of the HCC prognosis. Therefore, further prospective studies with larger sample sizes are required to confirm the present findings.

Conclusions

In conclusion, our results demonstrated that miR-200c expression was significantly decreased in HCC tissues and associated with tumor invasion and progression. Moreover, miR-200c may serve as a prognostic biomarker for HCC patients after surgery.

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Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, et al. Cancer statistics in China, 2015. CA Cancer J Clin 2016;66(2):115—32.
[2] Reig M, Darnell A, Forner A, Rimola J, Ayuso C, Bruix J. Systemic therapy for hepatocellular carcinoma: the issue of treatment stage migration and registration of progression using the BCLC-refined RECIST. Semin Liver Dis 2014;34(4):444—55.
[3] Fu J, Wang H. Precision diagnosis and treatment of liver cancer in China. Cancer Lett 2018;412:283—8.
[4] Schwartz M, Roayaie S, Konstadoulakis M. Strategies for the management of hepatocellular carcinoma. Nat Clin Pract Oncol 2007;4(7):424—32.
[5] Poon RT, Fan ST. Hepatectomy for hepatocellular carcinoma: patient selection and postoperative outcome. Liver Transpl 2004;10(2 Suppl 1):S39—45.
[6] Llovet JM, Schwartz M, Mazzaferro V. Resection and liver transplantation for hepatocellular carcinoma. Semin Liver Dis 2005;25(2):181—200.
[7] Forner A, Reig ME, de Lope CR, Bruix J. Current strategy for staging and treatment: the BCLC update and future prospects. Semin Liver Dis 2010;30(1):61—74.
[8] Kamiyama T, Nakanishi K, Yokoo H, Kamachi H, Tahara M, Kakisaka T, et al. Analysis of the risk factors for early death due to disease recurrence or progression within 1 year after hepatec-

tomy in patients with hepatocellular carcinoma. World J Surg Oncol 2012;10:107.
[9] Liu L, Miao R, Yang H, Lu X, Zhao Y, Mao Y, et al. Prognostic factors after liver resection for hepatocellular carcinoma: a single-center experience from China. Am J Surg 2012;203(6):741—50.
[10] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;136:215—33.
[11] Filipowicz W, Bhattacharyya SN, Sonenberg N. Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat Rev Genet 2008;9:102—14.
[12] Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annu Rev Pathol 2014;9:287—314.
[13] Liu Y, Ding Y, Huang J, Wang S, Ni W, Guan J, et al. MiR-141 suppresses the migration and invasion of HCC cells by targeting Tiam1. PLoS One 2014;9(2):e88393.
[14] Chaiteerakij R, Addissie BD, Roberts LR. Update on biomarkers of hepatocellular carcinoma. Clin Gastroenterol Hepatol 2015;13(2):237—45.
[15] Giordano S, Columbano A. MicroRNAs: new tools for diagnosis, prognosis, and therapy in hepatocellular carcinoma? Hepatology 2013;57(2):840—7.
[16] Su ZX, Zhao J, Rong ZH, Geng WM, Wu YG, Qin CK. Upregulation of microRNA-25 associates with prognosis in hepatocellular carcinoma. Diagn Pathol 2014;9:47.
[17] Li B, Huang P, Qiu J, Liao Y, Hong J, Yuan Y. MicroRNA-130a is down-regulated in hepatocellular carcinoma and associates with poor prognosis. Med Oncol 2014;31(10):230.
[18] Chen F, Li XF, Fu DS, Huang JG, Yang SE. Clinical potential of miRNA-221 as a novel prognostic biomarker for hepatocellular carcinoma. Cancer Biomark 2017;18(2):209—14.
[19] Wang YL, Chen CM, Wang XM, Wang L. Effects of miR-339-5p on invasion and prognosis of hepatocellular carcinoma. Clin Res Hepatol Gastroenterol 2016;40(1):51—6.
[20] Mutlu M, Raza U, Saatci Ö, Eyüpoğlu E, Yurdusev E, Şahin Ö. miR-200c: a versatile watchdog in cancer progression, EMT, and drug resistance. J Mol Med (Berl) 2016;94(6):629—44.
[21] Chaffer CL, San Juan BP, Lim E, Weinberg RA. EMT, cell plasticity and metastasis. Cancer Metastasis Rev 2016;35(4):645—54.
[22] Diepenbruck M, Christofori G. Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe? Curr Opin Cell Biol 2016;43:7—13.
[23] Zhang JY, Wang YM, Song LB, Chen C, Wang YC, Song NH. Prognostic significance of microRNA-200c in various types of cancer: An updated meta-analysis of 34 studies. Mol Clin Oncol 2016;4(6):933—41.
[24] Guo J, Fang W, Sun L, Lu Y, Dou L, Huang X, et al. Reduced miR-200b and miR-200c expression contributes to abnormal hepatic lipid accumulation by stimulating JUN expression and activating the transcription of srebp1. Oncotarget 2016;7(24):36207—19.
[25] Li Y, Bai W, Zhang J. MiR-200c-5p suppresses proliferation and metastasis of human hepatocellular carcinoma (HCC) via suppressing MAD2L1. Biomed Pharmacother 2017;92:1038—44.
[26] Siegel RL, Miller KD, Jemal A. Cancer Statistics, 2017. CA Cancer J Clin 2017;67(1):7—30.
[27] He S, Zhang DC, Wei C. MicroRNAs as biomarkers for hepatocellular carcinoma diagnosis and prognosis. Clin Res Hepatol Gastroenterol 2015;39(4):426—34.
[28] Hurteau GJ, Spivack SD, Brock GJ. Potential mRNA degradation targets of hsa-miR-200c, identified using informatics and qRT-PCR. Cell Cycle 2006;5(17):1951—6.
[29] Liu CH, Chen TC, Chau GY, Jan YH, Chen CH, Hsu CN, et al. Analysis of protein-protein interactions in cross-talk pathways reveals CRKL protein as a novel prognostic marker in hepatocellular carcinoma. Mol Cell Proteomics 2013;12(5):1335—49.
[30] Tamura M, Sasaki Y, Kobashi K, Takeda K, Nakagaki T, Idogawa M, et al. CRKL oncogene is downregulated by p53 through miR-200s. Cancer Sci 2015;106(8):1033—40.
[31] Hussain SP, Schwank J, Staib F, Wang XW, Harris CC. TP53 mutations and hepatocellular carcinoma: insights into the etiology and pathogenesis of liver cancer. Oncogene 2007;26(15):2166—76.
[32] Karakatsanis A, Papaconstantinou I, Gazouli M, Lyberopoulou A, Polymeneas G, Voros D. Expression of microRNAs, miR-21, miR-31, miR-122, miR-145, miR-146a, miR-200c, miR-221, miR-222, and miR-223 in patients with hepatocellular carcinoma or intrahepatic cholangiocarcinoma and its prognostic significance. Mol Carcinog 2013;52(4):297—303.
[33] Ladeiro Y, Couchy G, Balabaud C, Bioulac-Sage P, Pelletier L, Rebouissou S, et al. MicroRNA profiling in hepatocellular tumors is associated with clinical features and oncogene/tumor suppressor gene mutations. Hepatology 2008;47(6):1955—63.