

Original Article

Evaluation for prediction of recurrence after radiofrequency ablation in early hepatocellular carcinoma by combination of miR-34a and IVIM-DWI

Xianping Cui¹, Zhiyi Wang¹, Guihua Gao², Zhonghou Rong¹, Mengying Xia², Chengkun Qin¹, Zhenguo Yuan²

¹Department of Hepatobiliary Surgery, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, China; ²Shandong Medical Imaging Research Institute, Shandong University, Jinan, China

Received March 6, 2017; Accepted April 7, 2017; Epub May 15, 2017; Published May 30, 2017

Abstract: The prognosis of hepatocellular carcinoma (HCC) treated by radiofrequency ablation (RFA) is mainly associated with tumor recurrence. We previously demonstrated the expression of microRNA-34a (miR-34a) is involved in oncogenesis and progression of HCC, and suggested that the expression of miR-34a in HCC biopsy specimens has an independent predictive value of early recurrence after RFA. The aim of this study was to investigate the prognostic value of combined tissue miR-34a expression and non-CE MR Perfusion Imaging (IVIM-DWI) in patients with HCC treated with RFA. Patients with early-stage single-nodule HCC treated with RFA were included and underwent IVIM-DWI, and tissue expression of miR-34a was assessed by quantitative reverse-transcription polymerase chain reaction. Main clinical endpoints were overall and early recurrence. The Kaplan-Meier method was used to plot recurrence curves and univariable and multivariable Cox regression analyses were performed to assess independent predictive factors for recurrence. Of 135 patients, recurrence occurred in 73 patients (54.1%) with a median follow-up of 30 months. Forty-nine patients (36.3%) recurred within 2 years after RFA. The median miR-34a level was 0.87 (range 0.06-21.54). Low miR-34a level was associated with larger tumor size ($P=0.033$) and low parameter value of IVIM-DWI ($P=0.043$). The parameters obtained from IVIM-DWI were significantly positively correlated with the microvessel density ($r=0.573$ and 0.526 , $P=0.000$ and 0.0000 , respectively). When analyzed with a Cox regression model, the independent predictive factors for overall recurrence were low parameter value of IVIM-DWI (hazard ratio [HR]=1.26; 95% confidence interval [CI]=1.13-1.46; $P=0.028$), low miR-34a level (HR=1.38; 95% CI=1.22-1.56; $P=0.011$), high microvessel density (HR=1.42; 95% CI=1.03-1.62; $P=0.010$). In the evaluation of early recurrence after RFA, the sensitivity and specificity of miR-34a and IVIM-DWI combined were better than miR-34a or IVIM-DWI alone. Taken together, this study suggests that combination of miR-34a and IVIM-DWI can provide more comprehensive evaluation for the early recurrence after RFA of HCC, and should be recommended as an effective replacement technique.

Keywords: MicroRNA-34a, IVIM-DWI, hepatocellular carcinoma, recurrence, radiofrequency ablation

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most common cause of cancer-related death in the world. According to the International Agency for Research on Cancer, an estimated 748,300 new liver cancer cases and 695,900 cancer deaths occurred worldwide in 2008 [1]. Surgery is the standard treatment option for HCC meeting the Milan criteria, defined as single HCC ≤ 5 cm in maximum diameter or up to three nodules ≤ 3 cm [2]. However, in recent years, radiofrequency ablation (RFA) has emerged as the latest oriented treatment option for HCC because of its

favorable outcomes [3]. Five-year overall survival was about 60% in patients who received RFA as first-line therapy for HCC [4]. RFA is superior to surgical resection in terms of peri-procedural mortality while recurrence rate is higher in RFA than surgical resection [5]. Classically, two types of recurrence are identified. Early recurrence, within 2 years after local treatment, is considered to be related to metastatic spread; while late recurrence, after 2 years, would be related to the emergence of *de novo* HCC [6].

The identification of biomarkers correlating with the outcome of patients with HCC may help

to determine the prognosis, identify patients most likely to benefit from specific treatments, monitor response to treatment, and therefore guide clinicians in designing personalized treatment strategies. Predictive factors of recurrence have been mainly assessed in patients treated by resection. Up to now, besides baseline serum alpha-fetoprotein (AFP) level, multinodularity, and tumor size, the main known predictive factors of recurrence after resection are histological parameters retrospectively assessed with the full pathological specimen, such as poor degree of differentiation [7], presence of satellite nodules [8, 9], and presence of microvascular invasion [6, 9, 10]. However, in patients treated with RFA, the only tumor sample potentially available is a fine needle biopsy embedded in paraffin, and the main histological predictive factors of recurrence-satellite nodules and microvascular invasion-can rarely be assessed. The assessment of prognosis of early HCC using pretherapeutic paraffin biopsy samples could help select a group of patients that will benefit from adjuvant therapy after radiofrequency or refine criteria for transplantation.

Studies have shown that aberrant microRNAs (miRNAs) expression is correlated with the development and progression of cancer, thus miRNAs could be used as biomarkers for diagnosis and prognosis of cancer. On the other hand, the miRNAs can have oncogenic or tumor suppressor activities, so miRNAs are emerging as vital targets for cancer molecular therapies [11]. Recently, miR-34a, known to induce senescence and/or apoptosis in vitro, has been demonstrated to be a direct transcriptional target of p53 and it is commonly deleted in various types of cancers [12]. A previous study has shown that miR-34a expression is linked to disease progression of HCC [13]. A more recent study has demonstrated that miR-34a is a tumor suppressor miRNA that plays a vital role in the oncogenesis and progression of HCC, by targeting multiple pathways (e.g., c-Met signaling pathway) [14]. In an experimental model of liver carcinogenesis, the development of HCC is characterized by prominent early changes in expression of miRNA genes, specifically by inhibition of miR-34a expression [15].

non-CE MR Perfusion Imaging (IVIM-DWI) is a promise technique for evaluation of early recur-

rence after RFA. Therefore, aiming to identify some potential intratumor markers of tumor progression and recurrence in HCC, we assessed the combination of expression of miR-34a and IVIM-DWI in fine needle biopsy samples obtained just before RFA and its predictive value of recurrence in patients with compensated cirrhosis and an early-stage (BCLC 0/A) single-nodule HCC treated by RFA.

Materials and methods

Patients

Between January 2009 and April 2015, all consecutive patients with HCC, treated with RFA in the Department of Hepatobiliary Surgery, Shandong Provincial Hospital Affiliated to Shandong University, were retrospectively selected from a prospective database if fulfilling all the following inclusion criteria: (1) compensated cirrhosis (Child-Pugh A or B), (2) single HCC ≤ 5 cm without detectable portal extension or at distant metastasis (BCLC 0/A), (3) histological confirmation of the diagnosis of HCC and available tumor biopsy sample for RNA extraction, (4) no previous treatment for HCC, (5) prospective follow-up for at least 3 months, (6) complete ablation defined by the absence of enhancing tissue at tumor site, and (7) written informed consent of the patient for the study. The study protocol was approved by the local Ethical Committee of Shandong Provincial Hospital Affiliated to Shandong University, and all patients were required to provide written informed consent.

RFA procedures and follow-up

All procedures were performed percutaneously under general anesthesia and ultrasonography guidance by the same operator (Chengkun Qin, with 7 years of percutaneous treatment experience at the time the study began). The procedure was performed using internally cooled electrodes with an exposed tip (Cool-tip RFA system, Valleylab, Boulder, CO, USA; or RFG-3C RF Lesion Generator System, Radionics, Burlington, MA, USA). Based on the manufacturer's instructions for each device, impedance-based control of the generator was adjusted to transfer the radiofrequency energy. If the sonic window for the target was poor or the tumor was

adjacent to the colon or the diaphragm, an infusion to create an artificial ascites was performed. Dynamic abdominal CT with a section thickness of 0.5 cm was performed to evaluate treatment efficacy within 2 h after RFA procedure. All patients had complete ablation with technical success for the treated lesions by RFA. Complete ablation was defined as hypoattenuation of the whole lesion together with the surrounding liver parenchyma as a safety margin. To achieve an adequate ablation margin, multiple overlapping ablations were performed if needed. After the ablation of the target tumor, the electrode was retracted cautiously to prevent tumor seeding or bleeding.

Patients were prospectively followed until June 2014. Serum biological tests including AFP level measurement and imaging examinations of the liver (CT or MRI) were performed 1 month after RFA, then every 3 months during 2 years, then every 6 months. Recurrences were defined as the emergence of one or several liver enhancing foci at CT or MRI and classified as early when recurrence occurred within 2 years after RFA or late when recurrence occurred more than 2 years after complete ablation by RFA. Recurrences were also classified according to their site: distant if separated from the initial tumor site by normal parenchyma and local if adjacent to the ablation site. Whatever the type of recurrence, additional RFA treatment was considered according to the same criteria as those used at the time of the initial RFA. In other cases, patients were considered, when possible, for liver transplantation (LT), resection, transarterial chemoembolization, or other.

IVIM-DWI

Patients underwent IVIM-DWI. Six b factors (0, 50, 100, 150, 200, 600 sec/mm²) were used in IVIM-DWI. Parameters values and maps were calculated by a 2 segment linear fitting algorithms written in Matlab. The parameters included Apparent Diffusion Coefficient (ADC), pure diffusion coefficient (D), perfusion-related diffusion coefficient (D*) and perfusion fraction (PF). CD34 was used as a marker of HCC microvessel density calculation.

RNA isolation and qRT-PCR

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was used to deter-

mine the miR-34a expression level. Briefly, total RNA was extracted from the patients' liver biopsies using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol, and then polyadenylated by poly(A) polymerase (Ambion, Austin, TX, USA). Fifty microliters polyadenylation reaction was set up with 10 µg total RNA and 1 µl (2 U) poly(A) polymerase according to the manufacturer's protocol. The reaction was incubated at 37°C for 60 min. After incubation, poly(A)-tailed total RNA was recovered by phenol/chloroform extraction and ethanol precipitation. Reverse transcription was performed using 1 µg poly(A)-tailed total RNA and 1 µg RT primer (GCGAGCACAGAATTAA TACGACTCACTATAGG(T)18VN) with 1 µl Improm-II TM Reverse Transcriptase according to the manufacturer's protocol; qPCR was performed as described in the method of Quantitect SYBR Green PCR Kit (Qiagen, Valencia, CA, USA) with Mx3000p (Stratagene, La Jolla, CA, USA) supplied with analytical software. One of miR-34a amplification primers is a specific primer (TGGCAGTGTCTTAGCTGGTTGT), and the other is a universal primer (GCGAGCACAGAATTAA-TACGAC). U6 snRNA levels were used for normalization (forward 5'-CGCTTCGGCAGCACATA-TACTA-3', reverse 5'-CGCTTCACGAATTTGCGTG-TCA-3').

Statistical analysis

Baseline continuous variables were presented as means ± standard deviations (SD). Categorical variables were expressed as frequencies (percentages). The primary endpoints were overall and early recurrence. The secondary endpoints were local and distant recurrences and overall and tumor-free survivals. At reference date (June 30, 2015), patients still alive were censored at their last consultation, patients who died were censored at their death date, and patients who underwent liver transplantation were considered as dead and censored at the date of their liver transplantation. The association of risk factors with each endpoint was identified by univariate analysis with hazard ratios (HR) and 95% confidence intervals (CI), and variables with a *P* value less than 0.05 in the univariate analysis were then entered into stepwise Cox regression multivariate models. The Kaplan-Meier method was used to plot the recurrence curves, and the log-

Table 1. Baseline characteristics of patients with early-stage single hepatocellular carcinoma according to the expression of miR-34a

	All patients (n=135)	High miR-34a level (n=59)	Low miR-34a level (n=76)	P value
Male sex, n (%)	96 (71.1%)	38 (64.4%)	56 (73.7%)	0.863
Age, yr $\pm$ SD	56.4 $\pm$ 11.8	56.3 $\pm$ 12.4	55.3 $\pm$ 12.6	0.851
Cirrhosis etiology (n)				
HBV/HCV/alcohol/other	111/10/8/6	49/3/6/1	62/6/3/5	0.534
Child-Pugh A/B (n)	116/19	52/7	65/11	0.617
Bilirubin, μ mol/L, mean $\pm$ SD	19.1 $\pm$ 8.4	19.5 $\pm$ 8.3	19.9 $\pm$ 9.1	0.417
Albumin, g/L, mean $\pm$ SD	39.6 $\pm$ 6.3	40.0 $\pm$ 6.8	36.5 $\pm$ 6.1	0.279
Platelets, $\times 1000$ /ml	145.3 $\pm$ 55.1	147.1 $\pm$ 60.5	141.9 $\pm$ 58.6	0.092
HCC median size, mm	27.5 $\pm$ 8.8	27.4 $\pm$ 7.9	32.1 $\pm$ 9.4	0.033
HCC<20	49 (36.3%)	21 (15.6%)	26 (19.3%)	
HCC $\geq$ 20	86 (63.7%)	39 (28.9%)	49 (36.3%)	
IVIM-DWI	1.19 $\pm$ 0.60	1.91 $\pm$ 0.75	0.65 $\pm$ 0.42	0.043
Edmondson grade 3 or 4, n (%)	40 (25.8%)	13 (25.0%)	18 (26.5%)	0.445

HBV hepatitis B virus, HCV hepatitis C virus, HCC hepatocellular carcinoma, SD standard deviation.

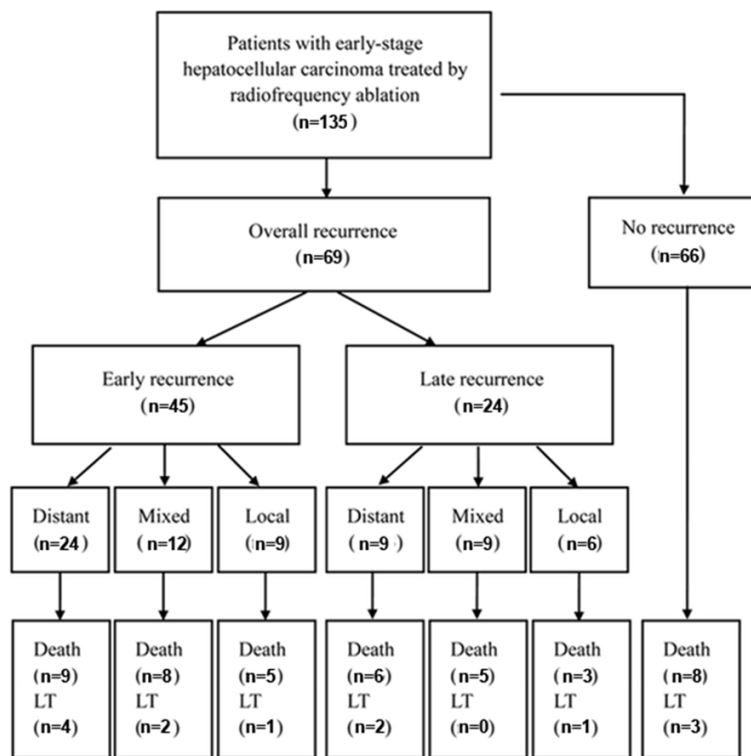


Figure 1. Flow chart of recurrence according to the delay and the site of intrahepatic recurrence. LT liver transplantation.

rank test was used for comparison. Statistical analyses were performed using SPSS software (SPSS 19.0, Chicago, IL, USA). All tests were two-sided and $P < 0.05$ was considered statistically significant.

Results

One hundred and thirty-five consecutive naive patients with BCLC O/A uninodular HCC treated with RFA and available biopsy for the diagnosis of HCC were collected. The baseline characteristics of patients are summarized in **Table 1**. Patients were mainly males (71.1%) with compensated Child-Pugh A hepatitis B-related cirrhosis and small single HCC (median diameter 24 mm). Forty patients (25.8%) had high Edmondson grade (grade 3 or 4). The median miR-34a expression level was 0.87 (range 0.06-21.54). By adopting cut-off value according to the median miR-34a level, patients were sorted into two categories: 59 patients with high miR-34a levels and 76 patients with low miR-34a levels. As shown in **Table 1**, low miR-34a expression level

was associated with larger tumor size ($P = 0.033$) and low parameter value of IVIM-DWI ($P = 0.043$).

Figure 1 represents the flow chart of recurrence according to the delay and the site of

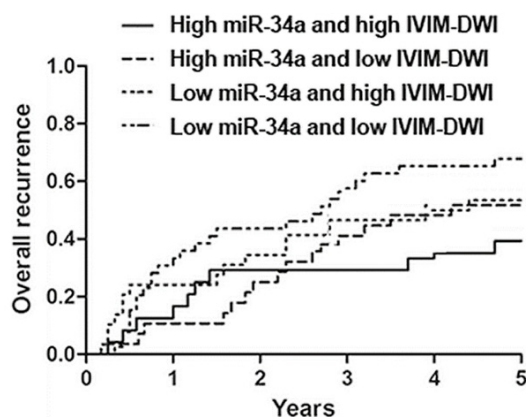


Figure 2. Kaplan-Meier estimates of overall recurrence according to the combined expression of miR-34a by HCC tissues and parameter value of IVIM-DWI.

intrahepatic recurrence. During a median follow-up of 30 months (range 2-52 months), overall recurrence occurred in 69 patients (51.1%) including 45 with early recurrence (33.3%). Finally, 44 patients died (32.6%) and 13 (9.6%) underwent LT.

Cut-off values for each of the clinical and pathological factors including miR-34a expression level were selected, according to the median value for continuous variables, and univariate analysis was carried out to identify those factors significantly associated with recurrence. The results showed that low parameter value of IVIM-DWI and low miR-34a level were associated with overall recurrence with a P value < 0.05 and were entered into the multivariate analysis. In the multivariate analysis, parameter value of IVIM-DWI (HR=1.36; 95% CI=1.14-1.29; $P=0.028$) and low miR-34a level (HR=1.38; 95% CI=1.22-1.56; $P=0.011$) were both independent predictive factors for overall recurrence.

Figure 2 shows overall recurrence according to tumor expression of miR-34a combined with parameter value of IVIM-DWI. The frequency of recurrence differed significantly among the four categories. Among 26 patients with normal parameter value of IVIM-DWI and high miR-34a levels, only 40% had a recurrence during the follow-up period after RFA, while recurrence rate reached 67% in 42 patients with baseline low parameter value of IVIM-DWI and low miR-34a levels ($P=0.006$).

According to the delay of recurrence, independent predictive factors for early recurrence were high parameter value of IVIM-DWI (HR=1.32; 95% CI=1.04-1.55; $P=0.016$) and low miR-34a level by HCC tissues (HR=1.46; 95% CI=1.25-1.89; $P=0.007$) (**Table 2**). The parameters obtained from IVIM-DWI were significantly positive correlated with the microvessel density ($r=0.573$ and 0.526 , $P=0.000$ and 0.0000 , respectively). When analyzed with a Cox regression model, the independent predictive factors for overall recurrence were low parameter value of IVIM-DWI (hazard ratio [HR]=1.26; 95% confidence interval [CI]=1.13-1.46; $P=0.028$), low miR-34a level (HR=1.38; 95% CI=1.22-1.56; $P=0.011$), high microvessel density (HR=1.42; 95% CI=1.03-1.62; $P=0.010$). In the evaluation of early recurrence after RFA, the sensitivity and specificity of miR-34a and IVIM-DWI combined is better than miR-34a or IVIM-DWI alone.

Discussion

Predictive factors of recurrence after treatment of early-stage HCC are needed to improve therapeutic strategies. Given the increasing use of non-surgical treatment, such as RFA, which has similar results as compared to surgical resection [5], there is a need to identify tissue biomarkers predictive of early recurrence using the unique pre-treatment small biopsy samples. miRNAs are small non-protein-coding RNAs that control gene expression post-transcriptionally by binding to various mRNA targets, and act as molecular switches in the extensive regulatory web that contains thousands of transcripts. Numerous aberrantly expressed miRNAs have been reported to be involved in hepatocarcinogenesis. Previously, Pineau *et al.* [13] found that miR-34a was over-expressed in HCC and was linked to disease progression from normal liver through cirrhosis to full-blown HCC. However, Li *et al.* [16] reported that downregulation of miR-34a expression was highly significant in 19 of 25 (76%) human HCC tissues compared with adjacent normal tissues. The results by Dang *et al.* [14] further confirmed the report from Li *et al.* in a larger size of patients of 83 cases, which also showed that miR-34a expression in HCC tissues decreased significantly compared to that in adjacent liver tissues. In addition, a study by

Table 2. Univariate and multivariate Cox analyses of clinical variables and miR-34a expression for overall and early recurrence

Variables	Overall recurrence				Early recurrence			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Female sex	0.83 (0.55-1.34)	0.843			0.78 (0.59-1.28)	0.753		
Age	0.98 (0.67-1.56)	0.781			0.89 (0.61-1.38)	0.827		
Serum bilirubin	0.87 (0.44-1.61)	0.882			0.79 (0.53-1.22)	0.584		
Serum albumin	0.96 (0.55-1.46)	0.913			0.95 (0.78-1.35)	0.773		
Platelets	1.11 (0.66-1.25)	0.856			0.93 (0.68-1.31)	0.485		
HCC size	1.08 (0.84-1.35)	0.426			1.15 (0.99-1.22)	0.092		
IVIM-DWI	1.26 (1.13-1.46)	0.007	1.36 (1.14-1.29)	0.028	1.44 (1.24-1.78)	0.002	1.33 (1.06-1.57)	0.016
Edmondson grade 3 or 4	1.27 (0.94-1.54)	0.165			1.09 (0.96-1.25)	0.084		
miR-34a level	1.56 (1.09-1.86)	<0.001	1.38 (1.22-1.56)	0.011	1.66 (1.25-2.14)	<0.001	1.46 (1.25-1.89)	0.007

CI confidence interval, HCC hepatocellular carcinoma, HR hazard ratio.

Tryndyak *et al.* [15] demonstrated in in vitro experiments that the development of HCC is characterized by prominent inhibition of expression of several miRNAs including miR-34a. In the current study, we have shown that low miR-34a expression level was associated with larger tumor size and low parameter value of IVIM-DWI, which is consistent with the notion that dysregulation of miR-34a expression is an important contributing factor in the development of HCC. Furthermore, low expression of miR-34a in HCC tissues was related to both high overall and early recurrence of HCC with a relative risk of 1.38 [1.22; 1.56] and 1.46 [1.25; 1.89], respectively, which may help select a group of patients that will benefit from adjuvant therapy after RFA.

In the present study, we showed that miR-34a expression and parameter value of IVIM-DWI could have an additional prognostic value. Indeed, the classification of patients according to the tumor expression of miR-34a combined with IVIM-DWI led to the identification of four groups with significantly different overall recurrence rates varying from 40% in patients with normal parameter value of IVIM-DWI and high miR-34a levels ($n=26$) to 67% in patients with baseline normal parameter value of IVIM-DWI and low miR-34a levels ($n=42$) ($P=0.006$). Our results could improve decision in the HCC therapeutic area. Stratification according to normal parameter value of IVIM-DWI and tumor miR-34a expression would allow a better selection of adequate candidates for liver transplantation following RFA, restricted to patients with normal parameter value of IVIM-DWI and high miR-34a levels, and would be helpful for design-

ing clinical trials for HCC, aimed at assessing the benefit of adjuvant therapy in patients with unfavorable prognostic factors (i.e., baseline IVIM-DWI value and/or low miR-34a expression in HCC tissues). However, to be included into guidelines of clinical management of early HCC, external validation studies are needed.

Targeting miR-34a may represent an innovative therapeutic approach for HCC. Preliminary experimental data showed that oncolytic adenoviral vector co-expressing miR-34a and IL-24 (AdCN205-IL-24-miR-34a) significantly induced dramatic antitumor activity and resulted in complete tumor regression without recurrence in a mouse xenograft tumor model of HCC [17]. Another study assessing the therapeutic activity of erlotinib combined with miR-34a in HCC showed strong synergy between erlotinib and miR-34a, which suggests that miR-34a can sensitize cancer cells to erlotinib [18]. As a single therapeutic agent, a liposomal formulation, MRX34, loaded with miR-34a mimics has been developed and has recently entered clinical testing for patients with HCC and other cancers with metastatic lesions in the liver [19]. Moreover, as transferring of miRNA-34a into HCC cells can inhibit the expression of its target genes (e.g., Bcl-2), miR-34a may also induce sensitivity to the antitumor effect of sorafenib in human HCC cells by inhibiting Bcl-2 expression [20].

In conclusion, miR-34a expression and IVIM-DWI in HCC biopsy specimens are independent predictors of overall and early recurrence after RFA, with additional prognostic values. This simple and reproducible detection of tissue

miR-34a, which is applicable to paraffin HCC small biopsy specimens, can help the decision-making of the therapeutic process in HCC patients by providing a more accurate estimation of prognosis for each patient.

Acknowledgements

This research was supported by grants from Shandong province Primary Research and development projection (2008GG30002004 and 2012YD18064), Project of Medicine and Health Development Plan of Shandong Province (2011HW067), and Shandong Provincial Natural Science Foundation (2013ZRB14309) and Shandong Provincial Science and Technology Development Plan (2013GGB14098) of China.

Disclosure of conflict of interest

None.

Address correspondence to: Chengkun Qin, Department of Hepatobiliary Surgery, Shandong Provincial Hospital Affiliated to Shandong University, 324 Jingwu Road, Jinan, China. Tel: +86 531 6877 6363; E-mail: prof_ck_qin@126.com; Zhenguo Yuan, Shandong Medical Imaging Research Institute, Shandong University, 324 Jingwu Road, Jinan, China. Tel: +86 531 6877 6363; E-mail: gy1673@163.com

References

- [1] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; 61: 69-90.
- [2] Huang J, Yan L, Cheng Z, Wu H, Du L, Wang J, Xu Y, Zeng Y. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann Surg* 2010; 252: 903-12.
- [3] Llovet JM, Ducreux M, Lencioni R, Di Bisceglie AM, Galle PR, Dufour JF; European Association for The Study of The Liver; European Organisation for Research and Treatment of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; 56: 908-43.
- [4] Shiina S, Tateishi R, Arano T, Uchino K, Enooku K, Nakagawa H, Asaoka Y, Sato T, Masuzaki R, Kondo Y, Goto T, Yoshida H, Omata M, Koike K. Radiofrequency ablation for hepatocellular carcinoma: 10-year outcome and prognostic factors. *Am J Gastroenterol* 2012; 107: 569-77.
- [5] Cho YK, Rhim H, Noh S. Radiofrequency ablation versus surgical resection as primary treatment of hepatocellular carcinoma meeting the Milan criteria: a systematic review. *J Gastroenterol Hepatol* 2011; 26: 1354-60.
- [6] Imamura H, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003; 38: 200-7.
- [7] Kumada T, Nakano S, Takeda I, Sugiyama K, Osada T, Kiriya S, Sone Y, Toyoda H, Shimada S, Takahashi M, Sassa T. Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. *Hepatology* 1997; 25: 87-92.
- [8] Llovet JM, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999; 30: 1434-40.
- [9] Roayaie S, Blume IN, Thung SN, Guido M, Fiel MI, Hiotis S, Labow DM, Llovet JM, Schwartz ME. A system of classifying microvascular invasion to predict outcome after resection in patients with hepatocellular carcinoma. *Gastroenterology* 2009; 137: 850-5.
- [10] Ishizawa T, Hasegawa K, Aoki T, Takahashi M, Inoue Y, Sano K, Imamura H, Sugawara Y, Kokudo N, Makuuchi M. Neither multiple tumors nor portal hypertension are surgical contraindications for hepatocellular carcinoma. *Gastroenterology* 2008; 134: 1908-16.
- [11] Hummel R, Hussey DJ, Haier J. MicroRNAs: predictors and modifiers of chemo- and radiotherapy in different tumour types. *Eur J Cancer* 2010; 46: 298-311.
- [12] Bader AG. miR-34-a microRNA replacement therapy is headed to the clinic. *Front Genet* 2012; 3: 120.
- [13] Pineau P, Volinia S, McJunkin K, Marchio A, Battiston C, Terris B, Mazzaferro V, Lowe SW, Croce CM, Dejean A. miR-221 overexpression contributes to liver tumorigenesis. *Proc Natl Acad Sci U S A* 2010; 107: 264-9.
- [14] Dang Y, Luo D, Rong M, Chen G. Underexpression of miR-34a in hepatocellular carcinoma and its contribution towards enhancement of proliferating inhibitory effects of agents targeting c-MET. *PLoS One* 2013; 8: e61054.
- [15] Tryndyak VP, Ross SA, Beland FA, Pogribny IP. Down-regulation of the microRNAs miR-34a, miR-127, and miR-200b in rat liver during hepatocarcinogenesis induced by a methyl-deficient diet. *Mol Carcinog* 2009; 48: 479-87.
- [16] Li N, Fu H, Tie Y, Hu Z, Kong W, Wu Y, Zheng X. miR-34a inhibits migration and invasion by

- down-regulation of c-Met expression in human hepatocellular carcinoma cells. *Cancer Lett* 2009; 275: 44-53.
- [17] Lou W, Chen Q, Ma L, Liu J, Yang Z, Shen J, Cui Y, Bian XW, Qian C. Oncolytic adenovirus co-expressing miRNA-34a and IL-24 induces superior antitumor activity in experimental tumor model. *J Mol Med (Berl)* 2013; 91: 715-25.
- [18] Zhao J, Kelnar K, Bader AG. In-depth analysis shows synergy between erlotinib and miR-34a. *PLoS One* 2014; 9: e89105.
- [19] Kelnar K, Peltier HJ, Leatherbury N, Stoudemire J, Bader AG. Quantification of therapeutic miRNA mimics in whole blood from nonhuman primates. *Anal Chem* 2014; 86: 1534-42.
- [20] Yang F, Li QJ, Gong ZB, Zhou L, You N, Wang S, Li XL, Li JJ, An JZ, Wang DS, He Y, Dou KF. MicroRNA-34a targets Bcl-2 and sensitizes human hepatocellular carcinoma cells to sorafenib treatment. *Technol Cancer Res Treat* 2014; 13: 77-86.