Biochemistry and Biophysics Reports 27 (2021) 101082

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Inverse relationship between the level of miRNA 148a-3p and both TGF-β1 and FIB-4 in hepatocellular carcinoma

Ashraf A. Dawood [a], Amany A. Saleh [a,*], Osama Elbahr [b], Suzy Fawzy Gohar [c], Mona S. Habieb [a]

[a] Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Menoufia University, Egypt
[b] Hepatology and Gastroenterology Department, National Liver Institute, Menoufia University, Egypt
[c] Clinical Oncology Department, Faculty of Medicine, Menoufia University, Egypt

ARTICLE INFO

Keywords:
HCC
miRNA 148a-3p
TGF-β1
RT-PCR
FIB-4

ABSTRACT

Background and aim: Hepatocellular carcinoma (HCC) is a major health burden globally. Dysregulation of miRNA 148a-3p is engaged in carcinogenesis. TGF-β is a profibrogenic cytokine. This study assesses the expression level of miRNA 148a-3p and its relationship with serum TGF-β1 and fibrosis index based on four factors (FIB-4) in Egyptian patients with HCV-associated HCC.

Subjects: and Methods: The study included 72 HCC patients with HCV, 48 HCV cirrhotic patients, and 47 healthy controls. Serum TGF-β1 was assessed by ELISA and the expression of miRNA 148a-3p was measured by RT-PCR.

Results: Patients with HCC had lower plasma miRNA 148a-3p, higher serum TGF-β1, and higher FIB-4 levels than patients with cirrhosis and controls. miRNA 148a-3p discriminated HCC either from control (AUC: 0.997, 95.83% sensitivity, 85.11% specificity) or from cirrhosis (AUC: 0.943, 91.67% sensitivity, 81.25% specificity). Moreover, it distinguished metastatic from nonmetastatic patients (AUC: 0.800, 88.89% sensitivity, 60.0% specificity). The decreased miRNA 148a-3p and the increased TGF-β1 levels were related to distant metastasis, multinodular lesions, advanced TNM stage, and BCLC score (C). A negative correlation between miRNA 148a-3p and each of FIB-4 and TGF-β1 was detected. The decreased miRNA 148a-3p was associated with poor overall survival and poor progression-free survival.

Conclusion: An inverse relationship between miRNA 148a-3p and both TGF-β1 and FIB-4 was observed, which could be involved in HCC pathogenesis. Moreover, this miRNA is a potential diagnostic and prognostic biomarker for HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the 6th frequently identified malignancy and the 4th principle cause of malignancy-related mortality worldwide [1]. HCC is terminal, with most patients being diagnosed at advanced stages and an incidence rate very close to the mortality rate [2]. HCC accounts for approximately 75%–85% of primary hepatic malignancies and is considered a health burden worldwide, with Egypt having the highest estimated age-standardized incidence rates in Africa [3]. Hepatitis C virus (HCV) is the most important risk factor for HCC in Egypt [4].

HCV infection is the prime cause of HCC, which could also develop in patients who report sustained virologic response, and hepatic inflammation and fibrosis markers such as FIB-4 index are used as predictors of HCC development [5]. Moreover, HCV infection enhances the development of HCC principally through various pathways such as sustained inflammation, enhancing fibrosis and cellular proliferation and necrosis; the risk of HCC propagation is associated with the fibrosis stage [6].

MicroRNAs (miRNAs) are the main constituents of the noncoding RNA family. miRNAs are about 23 nucleotides in length; they control gene expression posttranscriptionally. They regulate the expression of specific mRNAs mainly by attaching to the 3′-untranslated region of specific mRNA [7]. Moreover, miRNAs are essential factors for controlling the progression of HCC. They act through various mechanisms such as

* Corresponding author. Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Menoufia University, Shebin el-Kom, Yassin Abdel Ghaffar St., 32511, Egypt.

E-mail addresses: Kingashrafdawood@yahoo.com (A.A. Dawood), amanybio@yahoo.com, amany.saleh@med.menofia.edu.eg (A.A. Saleh), osama.elbahr@liver.menofia.edu.eg, oelbahr@yahoo.com (O. Elbahr), sozy.johar@med.menofia.edu.eg (S.F. Gohar), monahabieb@yahoo.com, mona.habiab@med.menofia.edu.eg (M.S. Habieb).

https://doi.org/10.1016/j.bbrep.2021.101082
Received 26 May 2021; Received in revised form 9 July 2021; Accepted 17 July 2021

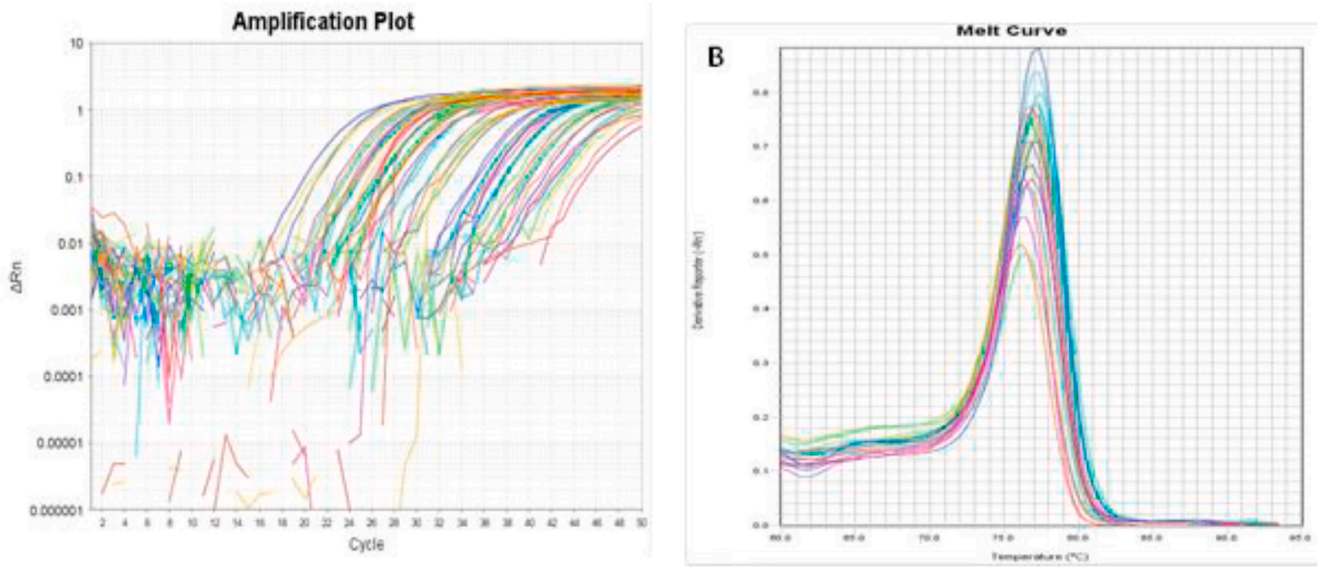

Fig. 1. **(A)** Amplification plot of the miR-148a-3p. **(B)** Melting curve analysis.

cellular proliferation, cell death, metastasis, and epithelial-mesenchymal transition (EMT) [8].

miR-148a, miR-148b, and miR-152 are members of the miR-148/152 family [9]. miR-148a is located on chromosome 7p15.2 and seems to have a stem-loop structure sequence [10]. miRNA 148a-3p is produced from the 3′ terminal of the pre-miRNAs by Dicer cleavage [11]. Recent data have revealed that miRNA-148a-3p is a tumor-suppressive miRNA that is underexpressed in epithelial ovarian cancer [12], breast cancer [13], and HCC [14,15]. Several growth factors, including transforming growth factor beta (TGF-β), are influenced by miRNA-148a as a tumor suppressor; DNA hypermethylation has been linked to miRNA-148a decreased expression in a variety of metastatic cell lines [16]. TGF-β is a biovital factor that promotes cellular growth and proliferation and is a very effective profibrogenic cytokine in hepatocytes. Moreover, TGF-β is involved in cancer progression by initiating EMT [17]. Based on the association of miRNA-148a-3p and TGF-β1 with fibrosis, this study was designed to evaluate the expression level of miRNA 148a-3p and its relationship with both TGF-β1 level and fibrosis index (FIB-4) in patients with HCV-associated HCC.

2. Subjects and Methods

Ethical approval

This study was performed in accordance with the Declaration of Helsinki. Written informed consent was signed by all participants and approved by the Ethics Committee of Medical Research, Faculty of Medicine and National Liver Institute, Menoufia University, Egypt (no. BIO-12-2018).

2.1. Selection of patients

This study was conducted in the Medical Biochemistry and Molecular Biology Department in collaboration with the Clinical Oncology Department, Faculty of Medicine, and the National Liver Institute, Menoufia, Egypt, from December 2018 to December 2020. The study included 120 individuals divided into three groups: group I, 72 patients with HCC on top of chronic HCV infection; group II, 48 patients with cirrhosis due to chronic HCV infection; group III, 47 age- and gender-matched healthy volunteers as the controls who were negative for HCV antibody, HB surface antigen (HBsAg), and HB core antibody (HBcAb). The selection of patients was based on specific inclusion and exclusion criteria. The inclusion criteria were patients older than 18 years with HCV infection confirmed by anti-HCV detection and positive HCV RNA. The diagnosis of cirrhosis was made based on history, clinical examination, laboratory findings, and imaging criteria such as ultrasonography and computed tomography (CT) [18]. On the other hand, HCC diagnosis was reached by triphasic spiral CT and/or magnetic resonance imaging, showing characteristic HCC features, elevated alpha-fetoprotein (AFP), and/or liver biopsy [19]. HCC staging was defined according to the Barcelona Clinic Liver Cancer staging system (BCLC) [20]. The Child–Pugh score was used to assess the severity of the liver disease [21]. FIB-4 is a convenient and noninvasive index used for assessing and predicting liver fibrosis. It was calculated according to the following formula: aspartate aminotransferase (AST) (U/L) × age (years)/[platelet count (× 10^9/L) × alanine aminotransferase (ALT) (U/L) $^{1/2}$] [22].

The exclusion criteria included patients with positive HBsAg and/or HBcAb, secondary liver cancer, other malignancies, chronic hepatitis, or cirrhosis due to any cause other than HCV infection, those who had previous HCC treatment and history of antiviral treatment, and candidates for localized treatment.

Patients with good liver functions, Child–Pugh A classification, received sorafenib treatment (400 mg twice daily). Patients' responses to treatment were evaluated every month by clinical examination, CBC test, liver and kidney functions tests and every 3 months by CTs and AFP test. Complete response (CR) is the disappearance of the disease, while partial response (PR) is characterized by a decrease of at least 50% in the sum of the products of the two greatest perpendicular diameters of measurable lesions, for at least four weeks. Stable disease (SD) is defined as a decrease of less than 50% or an increase of less than 25% in the tumor size, while progressive disease (PD) is characterized by an increase of more than 25% in the sum of the products of two perpendicular diameters of at least one tumor or the appearance of a new lesion. Treatment-related toxicities in the group were evaluated in all participants using the NCI-CTC systemV5.0. All patients were followed up for 24 months at least. Furthermore, patients with Child–Pugh B or C classifications were treated with supportive treatment.

2.2. Specimen requirements

Seven milliliters of venous blood was drawn using sterile venipuncture and distributed in three different tubes. Four milliliters of blood was placed in a plain tube, allowed to clot, and then centrifuged for 10 min at 4000 rpm. The serum obtained was utilized to measure liver function

Table (1)
Comparison between the three studied groups according to different parameters.

	HCC (n = 72)	Cirrhosis (n = 48)	Control (n = 47)	Test of sig.	P
Age					
Min. – Max.	47–74	46.0–66.0	45.0–68.0	F = 4.705**	0.010**
Mean ± SD.	59.1 ± 5.6	56.2 ± 5.5	56.8 ± 5.1		
Sig. bet. grps	$p_1 = 0.015$*,$p_2 = 0.069$,$p_3 = 0.863$				
Sex					
Male	57 (79.2%)	30 (62.5%)	29 (61.7%)	$\chi^2 = 5.628$	0.060
Female	15 (20.8%)	18 (37.5%)	18 (38.3%)		
BMI					
Min. – Max.	20–33	18.5–35	18–32.5	F = 1.967	0.143
Mean ± SD.	25.7 ± 3.1	26.9 ± 4.1	25.5 ± 4		
History of bilhaziasis	44 (61.1%)	19 (39.6%)	0 (0%)	$\chi^2 = 45.304$***	<0.001***
Smoking					
Non smoker	33 (45.8%)	24 (50%)	39 (83%)	$\chi^2 = 17.599$***	<0.001***
Smoker	39 (54.2%)	24 (50%)	8 (17%)		
ALT(IU/L)					
Min. – Max.	12–77	10–88	6–43	H = 33.792***	<0.001***
Median (IQR)	37 (28–52.5)	31 (22–43)	22 (19–32.5)		
Sig. bet. grps	$p_1 = 0.017$*,$p_2 < 0.001$*,$p_3 = 0.002$*				
AST(IU/L)					
Min. – Max.	34–137	30–114	7–43	H = 93.523***	<0.001***
Median (IQR)	57 (47.5–70.5)	56 (42–69.5)	22 (19–33)		
Sig. bet. grps	$p_1 = 0.359$,$p_2 < 0.001$*,$p_3 < 0.001$*				
Platelets (103/mm3)					
Min. – Max.	46–200	79–200	195–452	F = 191.643***	<0.001***
Mean ± SD.	121.8 ± 39.6	145.4 ± 27.4	277.1 ± 60.4		
Sig. bet. grps	$p_1 = 0.012$*,$p_2 < 0.001$*,$p_3 < 0.001$*				
Serum albumin (g/dL)					
Min. – Max.	1.8–35	2–4.6	3–5.4	H = 28.951***	<0.001***
Median (IQR)	3.4 (3–4.1)	3.7 (3.2–4.2)	4 (3.8–4.8)		
Sig. bet. Grps	$p_1 = 0.165$,$p_2 < 0.001$*,$p_3 < 0.001$*				
AFP (ng/ml)					
Min. – Max.	3–8910	2.1–614	1–8	H = 92.527***	<0.001***
Median (IQR)	152 (31.8–982.5)	17 (8.1–69.8)	6 (5–6)		
Sig. bet. Grps	$p_1 < 0.001$*,$p_2 < 0.001$*,$p_3 < 0.001$*				
TGF-β1 (ng/ml)					
Min. – Max.	1.9–6.1	0.6–3	0.2–2.5	H = 138.508***	<0.001***
Median (IQR)	3.7 (3.5–4)	1.6 (1.2–1.8)	0.3 (0.3–1.7)		
Sig. bet. grps	$p_1 < 0.001$*,$p_2 < 0.001$*,$p_3 = 0.026$*				
miRNA 148a-3p					
Min. – Max.	0.1–4.9	0.4–22.4	1.8–81.3	H = 108.667***	<0.001***
Median (IQR)	0.9 (0.3–1.7)	9.4 (4–17.3)	22 (7.5–36.2)		
Sig. bet. grps	$p_1 < 0.001$*,$p_2 < 0.001$*,$p_3 = 0.028$*				

χ^2: Chi square test.
F: F for ANOVA test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Tukey).
H: H for Kruskal Wallis test, Pairwise comparison bet. each 2 groups was done using Post Hoc Test (Dunn's for multiple comparisons test).
p: p value for comparing between the studied groups.
p_1: p value for comparing between group I and group II.
p_2: p value for comparing between group I and group III.
p_3: p value for comparing between group II and group III.
*: Statistically significant at $p \leq 0.05$.
**: Statistically significant at $p \leq 0.01$.
***: Statistically significant at $p \leq 0.001$ IQR: Inter quartile range.

(ALT, AST, and albumin), hepatitis viral markers, AFP, and TGF-β1 levels. The remaining 3 mL was put in an EDTA tube for complete blood count (CBC), HCV real-time polymerase chain reaction (RT-PCR), and expression analysis of miRNA-148a-3p.

2.3. Laboratory techniques

CBC was measured by a Sysmex XN-1000 (Japan (19723); BM Egypt Company). Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were evaluated using the kinetic UV optimized method IFCC (LTEC Kit, England) [23,24]. Serum albumin was quantified utilizing the technique with enhanced specificity of bromocresol green colorimetric assay (Diamond Diagnostics Kit, Germany) [25]. Electrochemiluminescence immunoassay (ECLA) on COBAS immunoassay analyzer was employed for assessing HCV antibody (anti-HCV) [26], while HBsAg was detected using Sorin Biomedica Co. kit (Italy) [27]. AFP was measured by enzyme-linked immunosorbent assay (ELISA) (IMMULITE 1000 system by a kit provided by Siemens Medical Solutions Diagnostics, USA) [28]. Moreover, the ELISA technique was used to estimate the serum level of TGF-β1 (Human TGF-β ELISA Kit, SunRed, China) [29]. HCV RNA was detected by RT-PCR using COBAS TaqMan HCV quantitative test, version 2.0 (Roche Molecular Systems, Inc., Branchburg, NJ, USA) [30].

2.4. Expression assessment of the circulating miRNA 148a-3p

To extract miRNA from plasma, we used the miRNeasy® Mini kit (QIAGEN, Germany). The quantity and the purity of extracted miRNA were estimated by a NanoDrop instrument (Thermo Scientific, USA). Purified miRNA was stored at −80 °C. The extracted miRNA was reverse-transcribed to yield single-stranded cDNA using a miScript II RT kit (QIAGEN, Germany). Reverse transcription reaction was performed on ice in a total volume of 20 μl for every reaction. The mixture consisted of 4 μl of 5x miScript HiSpec RT buffer, 2 μl of 10x miScript Nucleics

Table (2)
Comparison between patients with HCC and cirrhosis according to different parameters.

	HCC (n = 72)	Cirrhosis (n = 48)	Test of sig.	p
Splenomegaly	54 (75%)	31 (64.6%)	$\chi^2 = 1.513$	0.219
Ascites				
No	53 (73.6%)	41 (85.4%)	$\chi^2 = 2.566$	$^{MC}p=$
Mild	18 (25%)	7 (14.6%)		0.214
Moderate	1 (1.4%)	0 (0%)		
Comorbidities				
DM	27 (37.5%)	18 (37.5%)	$\chi^2 = 0.0$	1.000
HTN	15 (22.1%)	17 (35.4%)	$\chi^2 = 2.513$	0.113
Heart disease	6 (8.3%)	0 (0%)	$\chi^2 = 4.211$	$^{FE}p = 0.080$
Child Pugh class				
A	41 (56.9%)	33 (68.8%)	$\chi^2 = 3.944$	$^{MC}p=$
B	26 (36.1%)	15 (31.3%)		0.124
C	5 (6.9%)	0 (0%)		
FIB4				
Min. – Max.	3–15.3	3.1–6	U =	<0.001***
Median (IQR)	4.5 (3.4–6.3)	3.5 (3.2–3.8)	1022.0***	

χ^2: Chi square test MC: Monte Carlo FE: Fisher Exact.
t: Student t-test U: Mann Whitney test.
p: p value for comparing between the studied groups.
*: Statistically significant at $p \leq 0.05$.
**: Statistically significant at $p \leq 0.01$.
***: Statistically significant at $p \leq 0.001$ IQR: Inter quartile range.

Table (3)
Clinical parameters and characteristics of patients with HCC (n = 72).

	No. (%)
Vascular invasion	27 (37.5%)
Distant Metastasis	
No	45 (62.5%)
Yes	27 (37.5%)
Tumor	
Number	
Single	26 (36.1%)
Multiple	46 (63.9%)
Size	
≤5 cm	37 (51.4%)
>5 cm	35 (48.6%)
Site	
Right Lobe	38 (52.8%)
Left Lobe	13 (18.1%)
Caudate lobe	2 (2.8%)
Both	19 (26.4%)
TNM staging	
I	16 (22.2%)
II	4 (5.6%)
III	23 (31.9%)
IV	29 (40.3%)
LN metastasis	18 (25%)
BCLC	
A	9 (12.5%)
B	31 (43.1%)
C	32 (44.4%)
Survival	
Alive	38 (52.8%)
Died	34 (47.2%)
Progression	
No	23 (31.9%)
Yes	49 (68.1%)

Mix, and 2 μl of miScript™ reverse transcriptase; 2 μl of nuclease-free water was pipetted into each well; then, 10 μl of extracted miRNA was added. Measurement was completed in a 2720 Applied Biosystems thermal cycler (Bioline, Singapore, USA) for one cycle under the following conditions: 37 °C for 60 min and 95 °C for 5 min. The cDNA was stored at −20 °C. The real-time polymerase chain reaction was achieved using a miScript® SYBR® Green PCR kit (QIAGEN, Germany). Before amplification, cDNA samples were diluted with nuclease-free water at a ratio of 1:5. A total volume of 25 μl was used:12.5 μl of SYBR Green Master Mix, 3.5 μl of nuclease-free water, 4 μl of diluted cDNA, 2.5 μl of miScript universal primer, and 2.5 μl of miScript primer assay. The miRNA RNU6 was used as a reference miRNA. The miScript primer assay (Cat# MS00003556) containing miRNA-specific forward primers was used to detect mature miRNA-148a-3p (UCAGUGCACUACAGAACUUUGU). The amplification was run in an ABI 7500 real-time PCR instrument (software version 2.0.1), guided by the following steps: preliminary activation at 95 °C for 15 min and 40 cycles at 94 °C for 15 s, 55 °C for 30 s, and 70 °C for 30 s. The expression level of miRNA-148a-3p was normalized to that of RNU6, and the relative quantification of the miRNA was estimated using the comparative $2^{-\Delta\Delta Ct}$ method. Furthermore, melting curve analysis confirmed the specificity of the amplification and absence of primer dimers (Fig. 1) [31].

2.5. Statistical analysis

Analysis of data was carried out using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The comparisons between groups for categorical variables were assessed using the chi-square test (Fisher or Monte Carlo). For comparing two groups, Student's *t*-test was used for normally distributed quantitative variables, whereas Mann–Whitney test was used for abnormally distributed quantitative variables. For comparing the three groups under study, ANOVA was used for normally distributed variables followed by Tukey's post hoc test. In contrast, Kruskal–Wallis test was utilized for abnormally distributed quantitative variables followed by post hoc test (Dunn's for multiple comparisons test). Spearman's coefficient was used to investigate the relationship between quantitative variables. Moreover, the receiver operating characteristic curve (ROC) curve was used to assess the diagnostic performance of the markers. Kaplan–Meier survival and Cox regression analyses were conducted. The significance of the obtained results was judged at the 5% level.

3. Results

Our data analysis revealed that age was not significantly different when comparing HCC and cirrhosis groups to controls ($p_2 = 0.069$ and $p_3 = 0.863$), whereas patients with HCC were significantly older than patients with cirrhosis ($p_1 = 0.015$). Gender and BMI ($p = 0.060$ and $p = 0.143$, respectively) did not show significant variations among the groups under study. However, statistically significant differences in the history of bilharziasis and smoking status were observed in our groups. Differences in serum levels of ALT, AST, albumin, AFP, TGF-β1, and platelets count were statistically significant among groups ($p < 0.001$) with elevated ALT, AST, AFP, and TGF-β1 and decreased serum albumin and platelets count in HCC and cirrhosis versus controls and in HCC versus cirrhosis. The assessment of the miRNA 148a-3p relative expression level revealed significantly decreased levels in HCC patients compared to both cirrhosis and control groups ($p_1 < 0.001$). Moreover, cirrhosis group showed decreased expression levels compared to controls ($p_3 = 0.028$) (Table 1).

In assessing markers of fibrosis, patients with HCC had elevated FIB-4 compared to patients with cirrhosis ($p_1 < 0.001$), whereas both groups did not show significant variations regarding presence of splenomegaly, ascites, different comorbidities, or Child–Pugh class (Table 2).

The detailed clinical characteristics of the patients with HCC are listed in Table 3. From patients with HCC, 27 (37.5%) patients had vascular invasion and a similar percentage represented those with distant metastasis. Regarding imaging findings, 35 (48.6%) patients had large sized tumors (>5 cm), while 37 (51.4%) patients had tumors ≤5 cm. The main site of tumor in the liver was the right lobe (52.8%);

Table (4)
Agreement (sensitivity, specificity) for miRNA 148a-3p and AFP to differentiate between different groups.

	AUC	P	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
HCC patients (n = 72) from control (n = 47)								
miRNA 148a-3p	0.997	<0.001***	0.960–0.998	≤3.62	95.83	85.11	90.8	93.0
AFP (ng/ml)	0.987	<0.001***	0.962–1.012	≥6	98.61	91.49	94.67	97.73
HCC patients (n = 72) from cirrhosis (n = 48)								
miRNA 148a-3p	0.943	<0.001***	0.903–0.984	≤3.1	91.67	81.25	88.0	86.7
AFP (ng/ml)	0.777	<0.001***	0.695–0.859	≥18	90.28	54.17	74.7	78.8
metastatic patients (n = 27) from non-metastatic (n = 45) in HCC group								
miRNA 148a-3p	0.800	<0.001***	0.697–0.903	≤0.9	88.89	60.0	57.1	90.0

AUC: Area Under a Curve p value: Probability value.
CI: Confidence Intervals.
NPV: Negative predictive value PPV: Positive predictive value.
#Cut off was choose according to Youden index.
*: Statistically significant at $p \leq 0.05$.
**: Statistically significant at $p \leq 0.01$.
***: Statistically significant at $p \leq 0.001$.

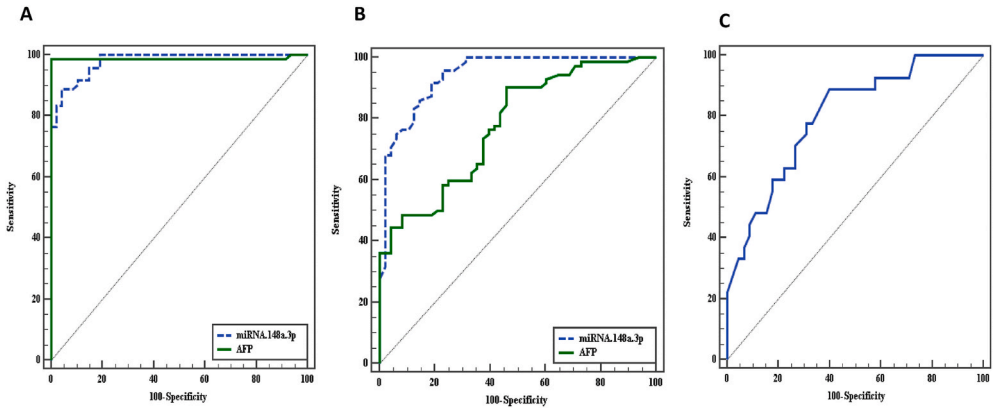

Fig. 2. **(A)** ROC curves of miRNA-148a-3p and AFP to differentiate patients with HCC from controls. **(B)** ROC curves of miRNA-148a-3p and AFP to differentiate patients with HCC from cirrhosis. **(C)** ROC curves for miRNA-148a-3p to differentiate metastatic patients from nonmetastatic ones.

tumors in both lobes were found in 26.4% of patients and tumors of the left lobe in 18.1% of patients. The caudate lobe tumor was reported in only 2% of HCC patients. In terms of tumor staging, 22.2%, 5.6%, 31.9%, and 40.3% of the patients were in TNM stage I, II, III, and IV, respectively. Lymph node metastasis was detected in 25% of patients. Regarding BCLC scoring, A, B, and C scores were reported in 12.5%, 43.1%, and 44.4% of the patients, respectively. At the end of follow-up, disease progression was reported in 49 (68.1%) patients and 34 (47.2%) patients died.

ROC curve was performed to assess the potential value of our markers in the diagnosis. ROC results showed that AFP at a cutoff >6 had sensitivity of 98.61% and specificity of 91.49% and miRNA-148a-3p had a sensitivity of 95.83% and specificity of 85.11% at a cutoff ≤3.62 (Table 4; Fig. 2A) for discriminating patients with HCC from controls. However, miRNA 148a-3p had a better performance at a cutoff ≤3.1 with a sensitivity of 91.67% and a specificity of 81.25% and AFP revealed a sensitivity of 90.28% and a specificity of only 54.17% at a cutoff >18 (Table 4; Fig. 2B) for distinguishing patients with HCC from patients with cirrhosis. Additionally, miRNA 148a-3p showed a good performance in predicting metastatic patients from nonmetastatic ones with a sensitivity of 88.89% and specificity of 60% at a cutoff of ≤0.9 (Table 4; Fig. 2C). Combining the values of miRNA-148a-3p and AFP did not improve the results compared to the values of miRNA 148a-3p alone.

The miRNA-148a-3p expression level was significantly decreased in metastatic tumors compared to localized tumors ($p < 0.001$), multiple tumors compared to single tumors ($p = 0.007$), and advanced tumor stages (stages III and VI) compared to stage I and II tumors ($p < 0.001$), indicating aggressive tumor behavior. Moreover, patients with progressive BCLC score (C) had lower expression level than patients with A and B scores ($p = 0.011$). The Child–Pugh class, tumor site, and size were not associated with the miRNA 148a-3p expression level (Table 5). Regarding the relationship between serum TGF-β1 and different parameters, a significant prevalence of elevated TGF-β1 levels was observed in metastatic tumors compared to that in localized tumors ($p < 0.001$), tumor represented in both lobes of the liver compared to single lobe tumors ($p < 0.033$), advanced tumor stage (VI) compared to stage I, II, and III tumors ($p < 0.001$), and progressive BCLC score (C) compared to patients with A and B score ($p = 0.008$). Futhermore, no significant variations were observed in terms of the Child–Pugh class of the patients, tumor number, and size (Table 5).

In patients with HCC, the miRNA 148a-3p expression level was significantly negatively correlated with fibrosis markers, TGF-β1 ($r = -0.696$, $p < 0.001$) and FIB-4 ($r = -0.615$, $p < 0.001$) (Fig. 3A and B).

In terms of identifying the predictors of survival in HCC patients at the end of the 24-month follow-up period, the univariate analysis revealed that the miRNA 148a-3p expression levels (low expression versus high expression), TGF-β1 serum levels (high levels versus low levels), tumor number (multiple versus single tumors), tumor stage (III

Table (5)
Relation between miRNA 148a-3p expression level and serum TGF-β1 and different parameters in patients with HCC (n = 72).

	N	miRNA 148a-3p	TGF-β1 (ng/ml)
		Median (IQR)	Median (IQR)
Child Pugh class			
A	**41**	0.8 (0.4–1.3)	3.7 (3.5–4)
B	**26**	0.9 (0.7–2.4)	3.6 (3.4–4)
C	**5**	0.1 (0.1–0.9)	4 (4–4.1)
H(p)		4.015 (0.134)	2.550 (0.279)
Distant Metastasis			
No	**45**	1.1 (0.8–2.3)	3.6 (3.4–3.9)
Yes	**27**	0.4 (0.2–0.8)	4 (3.8–4.6)
U(p)		242.50*** (<0.001***)	262.0*** (<0.001***)
Tumor Number			
Single	**26**	1.3 (0.8–2.1)	3.6 (3–4)
Multiple	**46**	0.7 (0.3–0.9)	3.9 (3.5–4.2)
U(p)		368.0** (0.007**)	432.0 (0.052)
Size			
≤5 cm	**37**	0.9 (0.4–1.4)	3.6 (3.5–4)
>5 cm	**35**	0.8 (0.3–1.9)	3.9 (3.5–4.1)
H(p)		554.0 (0.319)	4.32 (0.376)
Site			
Rt Lobe	**38**	0.9 (0.4–2)	3.6 (3.5–3.9)
Lt Lobe	**13**	0.9 (0.8–1.6)	3.6 (3–4)
Caudate lobe	**2**	0.6 (0.4–0.8)	3.9 (3.7–4.1)
Both	**19**	0.4 (0.3–0.9)	4 (3.6–4.4)
H(p)		4.716 (0.194)	8.735* (0.033*)
TNM staging			
I	**16**	1.5 (1.2–3)	3.5 (2.9–3.6)
II	**4**	2.1 (1.5–2.6)	3.2 (2.8–3.6)
III	**23**	0.8 (0.4–0.9)	3.7 (3.5–4.1)
IV	**29**	0.4 (0.2–0.9)	4 (3.8–4.6)
H(p)		20.196*** (<0.001***)	20.282*** (<0.001***)
BCLC			
A	**9**	1.4 (1.3–2)	3 (2.8–3.6)
B	**31**	0.9 (0.4–1.7)	3.6 (3.5–4.1)
C	**32**	(0.2–1.1)	3.9 (3.6–4.1)
H(p)		8.954** (0.011*)	9.594** (0.008**)

U: Mann Whitney test H: H for Kruskal Wallis test.
p: p value for comparing between the studied categories.
*: Statistically significant at $p \leq 0.05$.
**: Statistically significant at $p \leq 0.01$.
***: Statistically significant at $p \leq 0.001$.

+ IV versus I + II), and BCLC score (C versus A + B) were significantly associated with decreased mean survival. However, AFP levels, Child–Pugh class, presence of metastasis, and tumor size did not influence patients' survival significantly (Table 6). Moreover, a multivariate Cox regression analysis was performed to identify the independent markers affecting patients' survival. The miRNA-148a-3p expression level (low expression versus high expression) was the only independent predictor of the decreased overall survival ($p = 0.007$) (Table 6).

By applying the log-rank (Mantel–Cox test) of the Kaplan–Meier survival curve analysis to patients with HCC, a low miRNA-148a-3p expression was observed to be significantly linked to the decreased overall survival ($p < 0.001$) (mean: 13.25; 95% CI: 10.99–15.51) (Fig. 3C). Additionally, the low miRNA 148a-3p expression was significantly associated with the decreased progression-free survival ($p < 0.001$) (mean: 9.53; 95% CI: 7.47–11.58) (Fig. 3D).

4. Discussion

Circulating miRNA can mirror cancer cell biology, in addition to being a feasible analytic test for the HCC patients, which gives it an advantage over tissue miRNA [32]. The miRNA-148a-3p and TGF-β1 biomarkers were the focus of this study because of their close association with fibrosis, which is one of the dynamic processes involved in liver cancer, as mentioned by Giannelli et al. (2011) [33].

First, we have observed lower levels of plasma miRNA-148a-3p and higher serum levels of TGF-β1 in HCC patients compared to their levels in cirrhotic patients and controls. Consistently, previous researchers have found decreased miRNA-148a-3p as the patients progressed from cirrhosis to HCC [34–36]. This stepwise decrease of the circulatory miRNA-148a-3p is a warning sign of liver cancer.

Altered homeostasis of some miRNA can direct a normal cell toward forming cancer [37]. As a tumor suppressor, miRNA-148a was found to be decreased in many tumors, including HCC [38]. Sengupta et al. (2018) and Long et al. (2014) have attributed this deficiency to miRNA-148a promoter hypermethylation [16,39]. miRNA-148a could hinder growth, metastasis, EMT, and angiogenesis of hepatoma cells through different regulatory targets such as ubiquitin-specific protease 4 (USP4), Met/Snail, Wnt, and TGFβ-SMAD2 signaling pathways [40].

Using ROC curve analysis, we demonstrated the discriminatory ability of this miRNA in predicting HCC with remarkable sensitivity and specificity. Herein, miRNA-148a-3p was superior to AFP in distinguishing HCC patients from cirrhotic patients. Han et al. (2019) [36] have concluded that plasma miRNA-148a could be used for HCC screening, especially in patients with reduced levels of AFP.

In this study, patients with HCC had higher serum values of TGF-β1 and higher fibrosis scores (FIB-4) than patients with cirrhosis. Consistent with our results, Lee et al. (2012) [41], Shehata et al. (2013) [42], and Kohla et al. (2017) [43] have found higher serum levels of TGF-β1 in HCC patients than those in cirrhotic patients and controls. Giannelli et al. (2011) have stated that TGF-β induces a signaling pathway that initiates hepatic fibrosis and cirrhotic changes ending with HCC [33], meaning that the profibrogenic TGF-β1 has a promoting role in cancer growth and development [17]. Depending on the stage of the tumor, TGF-β can be either beneficial or harmful. In the early stages, it offers a tumor inhibitory role, whereas in the late stages, it promotes genomic instability, EMT, angiogenesis, immune evasion, cell migration, and metastasis [44].

In this study, a higher fibrosis score (FIB-4) was found in patients HCC compared to patients with cirrhosis. Upon HCV infection, hepatocytes secrete TGF-β and then stimulate hepatic stellate cells. Both secrete fibrogenic effectors leading to HCV-advanced fibrosis. Moreover, TGF-β continues to trigger an immune reaction maintaining the inflammation that ultimately leads to progressive fibrosis and liver cancer [33]. Zhu and his colleagues have discovered that the regulatory circuit consisting of H19/miR-148a/USP4 can induce hepatic fibrosis by promoting TGF-β signaling cascade in hepatocytes and hepatic stellate cells [45].

Unrestrained metastasis is a major contributing factor to the bad prognosis of HCC [46]. Therefore, identifying and understanding markers of HCC metastasis could ameliorate tumor outcomes, thus improving the patient's quality of life. In this analysis, we noticed that lower median levels of miRNA-148a-3p and higher levels of TGF-β1 were related to some aggressive tumor phenotypes, namely, distant metastasis, multinodular lesions, advanced TNM stage, and BCLC score (C). Moreover, we reported for the first time a negative correlation between miRNA-148a-3p and FIB-4 score and TGF-β1, indicating their potential in the progression of the carcinogenic process and their possible role in fibrosis. Heo et al. (2014) [47] have found that the underexpression of miRNA-148a concomitant with overexpression of its target USP4 could contribute to the deterioration of HCC by stimulating the TGF-β1 signaling cascades.

The journey of a tumor cell from the site of origin to settle in a distant destination is initiated by EMT [48], which is a fundamental step in tumor invasion and metastasis [49]. TGF-β can induce EMT in cancer by inhibiting genes encoding the epithelial protein, E-cadherin, and stimulation of genes encoding the mesenchymal proteins, *N*-cadherin, and

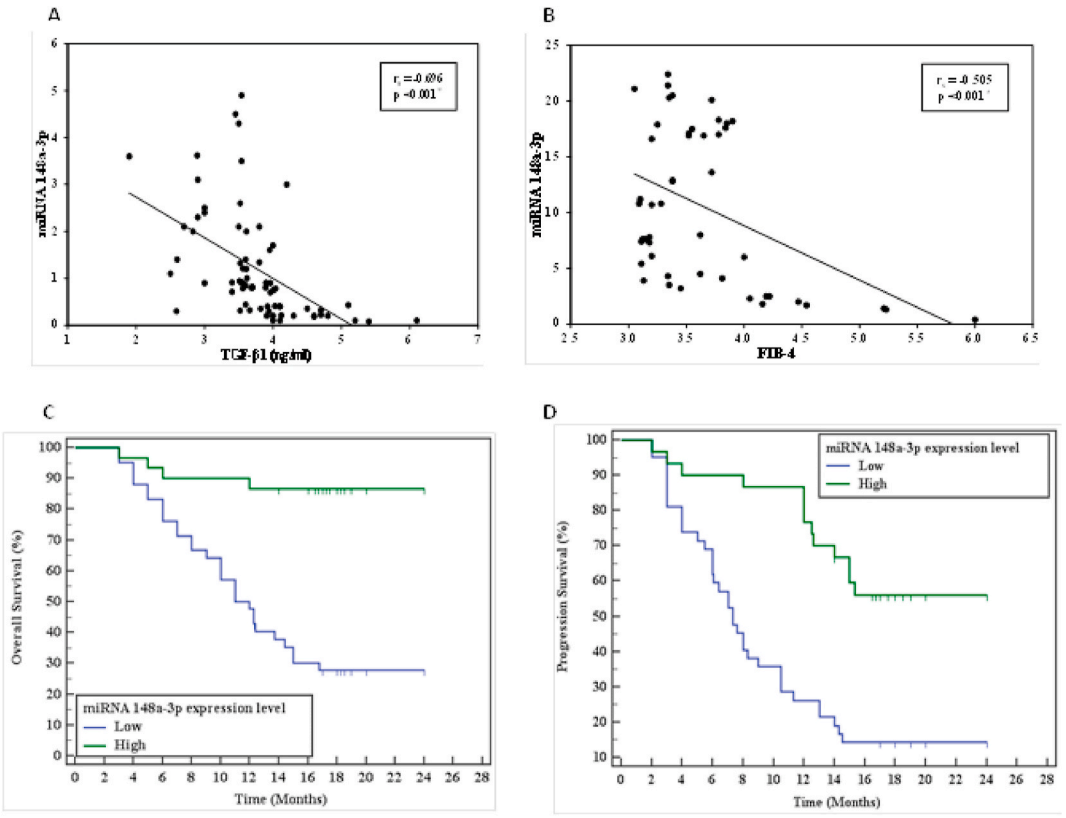

Fig. 3. **(A)** Correlation between the miRNA-148a-3p and TGF-β1 in patients with HCC. **(B)** Correlation between the miRNA-148a-3p and FIB4 in patients with HCC. **(C)** Kaplan–Meier survival curve for the overall survival with miRNA-148a-3p in patients with HCC. **(D)** Kaplan–Meier survival curve for progression-free survival with miRNA-148a-3p in patients with HCC.

vimentin [50].

In this study, we discovered that miRNA-148a-3p can be used not only to effectively distinguish patients with HCC from patients with cirrhosis and controls but also to distinguish HCC metastatic patients from nonmetastatic patients.

The results regarding the association of these markers with signs of tumor aggressiveness were supported by several previous studies. Yan et al. (2014) [51] have reported low expression of miRNA-148a in metastatic and poorly differentiated HCC tissues. They have concluded that miRNA-148a suppresses metastasis by preventing EMT and cancer stem cell-like properties by targeting the Wnt regulatory pathway. Heo et al. (2014) [47] have stated that the deficient miRNA-148a was related to the high levels of AFP, advanced TNM stage, poor overall survival, and the recurrence-free survival rate in HCC patients. Similarly, Pan et al. (2014) [34] have confirmed the correlation with TNM stage, metastasis, capsular infiltration, and numbers of tumor nodes.

Survival analysis is a major tool used for the estimation of a patient's prognosis [52]. By applying the Kaplan–Meier survival analysis, this study showed a significant relationship between the reduced median values of miRNA-148a-3p and poor overall survival and progression-free survival among HCC patients. Moreover, the univariate analysis revealed that reduced expression of miRNA-148a-3p, high TGF-β1 level, multiple tumors, advanced TNM stage (III + IV), and BLCC score (C) were correlated with worse survival outcomes. Using Cox regression analysis, we identified that the low level of plasma miRNA-148a-3p was an independent prognostic marker predicting poor overall survival. These results agreed with previous reports [32,35,53]. In terms of the relationship between advanced stage and poorly differentiated HCC tumors, Cheng et al. (2017) [35] have also demonstrated that the low expression of tissue miR-148a3p/miR-148a5p had a significantly worse survival than the high expression, implying its involvement in the pathogenesis of HCC. Futhermore, Song et al. (2019) [53] have concluded that the downregulated miRNA-148a-3p was correlated with gross vascular invasion and poor survival of patients after hepatic resection for HCC.

5. Conclusion

This study showed a significant inverse relationship between miRNA-148a-3p and both TGF-β1 and FIB4. Moreover, the miRNA-148a-3p exhibited good diagnostic and prognostic power. Taken together, the plasma miRNA-148a-3p and TGF-β1 are valuable biomarkers of potential clinical significance in HCC.

Funding

There was no funding for this study.

Data availability

The data are available from the authors on reasonable request.

Declaration of competing interest

All authors declare no conflicts of interest.

Table (6)
Univariate and multivariate COX regression analysis for predict overall survival in HCC groups.

	Univariate				#Multivariate		
	Mean (95%C.I)	SE	Log rank	P	Wald	HR (95%C.I)	P
Child Pugh class							
A	16.10 (13.6–18.61)	1.28	0.636	0.728			
B	17.27 (14.13–20.41)	1.60			–	–	–
C	15.89 (13.30–18.48)	1.32					
Metastatic site							
No	17.32 (14.84–19.80)	1.26	1.316	0.251			
Yes	14.22 (12.14–16.29)	1.06			–	–	–
Tumor Number							
Single	19.89 (17.25–22.54)	1.35	6.106**	0.013**	0.046	0.902 (0.35–2.32)	0.992
Multiple	14.97 (12.61–17.33)	1.20					
Tumor Size							
≤5 cm	17.33 (14.74–19.92)	1.32	0.467	0.494	–	–	–
>5 cm	14.20 (12.13–16.26)	1.05					
TNM staging							
I + II	22.30 (20.06–24.54)	1.14	12.656***	<0.001***			
III + IV	14.62 (12.45–16.79)	1.11			1.692	3.170 (0.56–18.03)	0.193
BCLC							
A + B	18.84 (16.53–21.15)	1.18	6.401**	0.011**			
C	12.63 (10.46–14.80)	1.11			2.104	1.713 (0.83–3.54)	0.147
AFP (ng/ml)							
Low median	17.36 (14.80–19.93)	1.31	0.319	0.572	–	–	–
High median	16.09 (13.39–18.79)	1.38					
TGF-β1 (ng/ml)							
Low median	18.737 (16.29–21.18)	1.25	5.530**	0.019**			
High median	12.992 (10.93–15.06)	1.06			0.355	0.791 (0.37–1.71)	0.551
miRNA 148a-3p							
Low median	13.25 (10.99–15.51)	1.15	20.924***	<0.001***	7.308	5.089 (1.56–16.56)	0.007**
High median	21.67 (19.49–23.84)	1.11					

HR: Hazard ratio.
C.I: Confidence interval LL: Lower limit UL: Upper Limit.
#: All variables with $p < 0.05$ was included in the multivariate.
*: Statistically significant at $p \leq 0.05$.
**: Statistically significant at $p \leq 0.01$.
***: Statistically significant at $p \leq 0.001$.

References

[1] Gbd 2015 Mortality and Causes of Death Collaborators, Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015, A systematic analysis for the global burden of disease study 2015, Lancet 388 (2016) 1459–1544, https://doi.org/10.1016/S0140-6736(16)31012-1.

[2] G.B. of D.L.C. Collaboration, The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: results from the global burden of disease study 2015, JAMA Oncol 3 (2017) 1683–1691, https://doi.org/10.1001/jamaoncol.2017.3055.

[3] A.G. Singal, P. Lampertico, P. Nahon, Epidemiology and surveillance for hepatocellular carcinoma: new trends, J. Hepatol. 72 (2020) 250–261, https://doi.org/10.1016/j.jhep.2019.08.025.

[4] W.M. Rashed, M.A.M. Kandeil, M.O. Mahmoud, S. Ezzat, Hepatocellular Carcinoma (HCC) in Egypt: a comprehensive overview, J. Egypt. Natl. Canc. Inst. 32 (2020) 1–11, https://doi.org/10.1186/s43046-020-0016-x.

[5] F.M. Sou, C.K. Wu, K.C. Chang, S.N. Lu, J.H. Wang, C.H. Hung, C.H. Chen, K. M. Kee, Y.H. Yen, M.T. Lin, M.C. Tsai, T.H. Hu, Clinical characteristics and prognosis of HCC occurrence after antiviral therapy for HCV patients between sustained and non-sustained responders, J. Formos. Med. Assoc. 118 (2019) 504–513, https://doi.org/10.1016/j.jfma.2018.10.017.

[6] A. Ozakyol, Global epidemiology of hepatocellular carcinoma (HCC epidemiology), J. Gastrointest. Canc. 48 (2017) 238–240, https://doi.org/10.1007/s12029-017-9959-0.

[7] R. Rupaimoole, G.A. Calin, G. Lopez-Berestein, A.K. Sood, MiRNA deregulation in cancer cells and the tumor microenvironment, Canc. Discov. 6 (2016) 235–246, https://doi.org/10.1158/2159-8290.CD-15-0893.

[8] X. Xu, Y. Tao, L. Shan, R. Chen, H. Jiang, Z. Qian, F. Cai, L. Ma, Y. Yu, The role of MicroRNAs in hepatocellular carcinoma, J. Canc. 9 (2018) 3557–3569, https://doi.org/10.7150/jca.26350.

[9] F. Wang, H. Ying, B. He, Y. Pan, H. Sun, S. Wang, Circulating miR-148/152 family as potential biomarkers in hepatocellular carcinoma, Tumor Biol. 37 (2016) 4945–4953, https://doi.org/10.1007/s13277-015-4340-z.

[10] G.A. Calin, C. Sevignani, C.D. Dumitru, T. Hyslop, E. Noch, S. Yendamuri, M. Shimizu, S. Rattan, F. Bullrich, M. Negrini, C.M. Croce, Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers, Proc. Natl. Acad. Sci. U. S. A 101 (2004) 2999–3004, https://doi.org/10.1073/pnas.0307323101.

[11] S. Lin, R.I. Gregory, MicroRNA biogenesis pathways in cancer, Nat. Rev. Canc. 15 (2015) 321–333, https://doi.org/10.1038/nrc3932.

[12] W. Wang, J. Dong, M. Wang, S. Yao, X. Tian, X. Cui, S. Fu, S. Zhang, miR-148a-3p suppresses epithelial ovarian cancer progression primarily by targeting c-Met, Oncol. Lett. 15 (2018) 6131–6136, https://doi.org/10.3892/ol.2018.8110.

[13] X. Chen, Y.-W. Wang, P. Gao, SPIN1, negatively regulated by miR-148/152, enhances Adriamycin resistance via upregulating drug metabolizing enzymes and transporter in breast cancer, J. Exp. Clin. Canc. Res. 37 (2018) 100, https://doi.org/10.1186/s13046-018-0748-9.

[14] L. Li, Y. Liu, Y. Guo, B. Liu, Y. Zhao, P. Li, F. Song, H. Zheng, J. Yu, T. Song, R. Niu, Q. Li, X.W. Wang, W. Zhang, K. Chen, Regulatory MiR-148a-ACVR1/BMP circuit defines a cancer stem cell-like aggressive subtype of hepatocellular carcinoma, Hepatology 61 (2015) 574–584, https://doi.org/10.1002/hep.27543.

[15] K.H. Jung, J. Zhang, C. Zhou, H. Shen, M. Gagea, C. Rodriguez-Aguayo, G. Lopez-Berestein, A.K. Sood, L. Beretta, Differentiation therapy for hepatocellular carcinoma: multifaceted effects of miR-148a on tumor growth and phenotype and liver fibrosis, Hepatology 63 (2016) 864–879, https://doi.org/10.1002/hep.28367.

[16] D. Sengupta, M. Deb, S.K. Patra, Antagonistic activities of miR-148a and DNMT1: ectopic expression of miR-148a impairs DNMT1 mRNA and dwindle cell proliferation and survival, Gene 660 (2018) 68–79, https://doi.org/10.1016/j.gene.2018.03.075.

[17] F. Jiang, J. Mu, X. Wang, X. Ye, L. Si, S. Ning, Z. Li, Y. Li, The repressive effect of miR-148a on TGF beta-SMADs signal pathway is involved in the glabridin-induced inhibition of the cancer stem cells-like properties in hepatocellular carcinoma cells, PloS One 9 (2014), https://doi.org/10.1371/journal.pone.0096698.

[18] Z.H. Lin, Y.N. Xin, Q.J. Dong, Q. Wang, X.J. Jiang, S.H. Zhan, Y. Sun, S.Y. Xuan, Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis, Hepatology 53 (2011) 726–736, https://doi.org/10.1002/hep.24105.

[19] J. Bruix, M. Sherman, Management of hepatocellular carcinoma: an update, Hepatology 53 (2011) 1020–1022, https://doi.org/10.1002/hep.24199.

[20] J.M. Llovet, C. Brú, J. Bruix, Prognosis of hepatocellular carcinoma: the BCLC staging classification, Semin. Liver Dis. 19 (1999) 329–337, https://doi.org/10.1055/s-2007-1007122.

[21] R.N.H. Pugh, I.M. Murray-Lyon, J.L. Dawson, M.C. Pietroni, R. Williams, Transection of the oesophagus for bleeding oesophageal varices, Br. J. Surg. 60 (1973) 646–649, https://doi.org/10.1002/bjs.1800600817.

[22] R.K. Sterling, E. Lissen, N. Clumeck, R. Sola, M.C. Correa, J. Montaner, M. S. Sulkowski, F.J. Torriani, D.T. Dieterich, D.L. Thomas, D. Messinger, M. Nelson,

Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection, Hepatology 43 (2006) 1317–1325, https://doi.org/10.1002/hep.21178.
[23] H.U. Bergmeyer, P. Scheibe, A.W. Wahlefeld, Optimization of methods for aspartate aminotransferase and alanine aminotransferase, Clin. Chem. 24 (1978) 58–73, https://doi.org/10.1093/clinchem/24.1.58.
[24] K.S. Henley, IFCC method for alanine aminotransferase, Clin. Chim. Acta 105 (1980) 155–166, https://doi.org/10.1016/0009-8981(80)90106-0.
[25] A. Affonso, F.D. Lasky, Bromocresol purple dye-binding method for the estimation of serum albumin adapted to the SMA 12/60, Clin. Biochem. 18 (1985) 285–289, https://doi.org/10.1016/S0009-9120(85)80033-3.
[26] F.B. Wu, H.Q. Ouyan, X.Y. Tang, Z.X. Zhou, Double-antigen sandwich time-resolved immunofluorometric assay for the detection of anti-hepatitis C virus total antibodies with improved specificity and sensitivity, J. Med. Microbiol. 57 (2008) 947–953, https://doi.org/10.1099/jmm.0.47835-0.
[27] H. Adachi, T. Fukuda, S. Funahashi, T. Kurahori, E. Ishikawa, Sandwich enzymoimmunoassay of hepatitis B surface antigen (HBsAg), Vox Sang. 35 (1978) 219–223, https://doi.org/10.1111/j.1423-0410.1978.tb02925.x.
[28] E. Engall, H. Van Vunakis, J. J Langone, Methods in Enzymology, vol. 70, Academic Press, New York, 1980, pp. 419–492.
[29] E. Fathi, R. Farahzadi, I. Vietor, S. Javanmardi, Cardiac differentiation of bone-marrow-resident c-kit+ stem cells by L-carnitine increases through secretion of VEGF, IL6, IGF-1, and TGF-β as clinical agents in cardiac regeneration, J. Biosci. 45 (2020) 1–11, https://doi.org/10.1007/s12038-020-00063-0.
[30] M.G. Ghany, D.B. Strader, D.L. Thomas, L.B. Seeff, Diagnosis, management, and treatment of hepatitis C: an update, Hepatology 49 (2009) 1335–1374, https://doi.org/10.1002/hep.22759.
[31] E. Fathi, R. Farahzadi, S. Javanmardi, I. Vietor, L-carnitine extends the telomere length of the cardiac differentiated CD117+- expressing stem cells, Tissue Cell 67 (2020), https://doi.org/10.1016/j.tice.2020.101429.
[32] Y. Zhang, C. Wei, C.C. Guo, R.X. Bi, J. Xie, D.H. Guan, C.H. Yang, Y.H. Jiang, Prognostic value of microRNAs in hepatocellular carcinoma: a meta-analysis, Oncotarget 8 (2017) 107237–107257, https://doi.org/10.18632/oncotarget.20883.
[33] G. Giannelli, A. Mazzocca, E. Fransvea, M. Lahn, S. Antonaci, Inhibiting TGF-β signaling in hepatocellular carcinoma, Biochim. Biophys. Acta Rev. Canc 1815 (2011) 214–223, https://doi.org/10.1016/j.bbcan.2010.11.004.
[34] L. Pan, S. Huang, R. He, M. Rong, Y. Dang, G. Chen, Decreased expression and clinical significance of miR-148a in hepatocellular carcinoma tissues, Eur. J. Med. Res. 19 (2014) 68, https://doi.org/10.1186/s40001-014-0068-2.
[35] L. Cheng, Y. Zhu, H. Han, Q. Zhang, K. Cui, H. Shen, J. Zhang, J. Yan, E. Prochownik, Y. Li, MicroRNA-148a deficiency promotes hepatic lipid metabolism and hepatocarcinogenesis in mice, Cell Death Dis. 8 (2017), https://doi.org/10.1038/cddis.2017.309 e2916.
[36] J. Han, J. Li, Y. Qian, W. Liu, J. Liang, Z. Huang, S. Wang, C. Zhao, Identification of plasma miR-148a as a noninvasive biomarker for hepatocellular carcinoma, Clin. Res. Hepatol. Gastroenterol. 43 (2019) 585–593, https://doi.org/10.1016/j.clinre.2018.12.008.
[37] M. Tuna, A.S. Machado, G.A. Calin, Genetic and epigenetic alterations of microRNAs and implications for human cancers and other diseases, Gene Chromosome Canc. 55 (2016) 193–214, https://doi.org/10.1002/gcc.22332.
[38] X. Li, L. Wang, X. Cao, L. Zhou, C. Xu, Y. Cui, Y. Qiu, J. Cao, Casticin inhibits stemness of hepatocellular carcinoma cells via disrupting the reciprocal negative regulation between DNMT1 and miR-148a-3p, Toxicol. Appl. Pharmacol. 396 (2020) 114998, https://doi.org/10.1016/j.taap.2020.114998.
[39] X.R. Long, Y. He, C. Huang, J. Li, MicroRNA-148a is silenced by hypermethylation and interacts with DNA methyltransferase 1 in hepatocellular carcinogenesis, Int. J. Oncol. 45 (2014) 1915–1922, https://doi.org/10.3892/ijo.2014.2373.
[40] Z. Huang, J. Wen, J. Yu, J. Liao, S. Liu, N. Cai, H. Liang, X. Chen, Z. Ding, B. Zhang, MicroRNA-148a-3p inhibits progression of hepatocelluar carcimoma by repressing SMAD2 expression in an Ago2 dependent manner, J. Exp. Clin. Canc. Res. 39 (2020) 150, https://doi.org/10.1186/s13046-020-01649-0.
[41] D. Lee, Y.H. Chung, J.A. Kim, Y.S. Lee, D. Lee, M.K. Jang, K.M. Kim, Y.S. Lim, H. C. Lee, Y.S. Lee, Transforming growth factor beta 1 overexpression is closely related to invasiveness of hepatocellular carcinoma, Oncology 82 (2012) 11–18, https://doi.org/10.1159/000335605.
[42] F. Shehata, N.A. Monem, M. Sakr, S. Kasem, M. Balbaa, Epidermal growth factor, its receptor and transforming growth factor-β1 in the diagnosis of HCV-induced hepatocellular carcinoma, Med. Oncol. 30 (2013) 1–7, https://doi.org/10.1007/s12032-013-0673-x.
[43] M.A.S. Kohla, A. Attia, N. Darwesh, M. Obada, H. Taha, M.F. Youssef, Association of serum levels of transforming growth factor b1 with disease severity in patients with hepatocellular carcinoma, Hepatoma Res 3 (2017) 294, https://doi.org/10.20517/2394-5079.2017.40.
[44] H. Zhao, J. Wei, J. Sun, Roles of TGF-β signaling pathway in tumor microenvirionment and cancer therapy, Int. Immunopharm. 89 (2020), https://doi.org/10.1016/j.intimp.2020.107101.
[45] J. Zhu, Z. Luo, Y. Pan, W. Zheng, W. Li, Z. Zhang, P. Xiong, D. Xu, M. Du, B. Wang, J. Yu, J. Zhang, J. Liu, H19/miR-148a/USP4 axis facilitates liver fibrosis by enhancing TGF-β signaling in both hepatic stellate cells and hepatocytes, J. Cell. Physiol. 234 (2019) 9698–9710, https://doi.org/10.1002/jcp.27656.
[46] Y. Luo, K. Huang, J. Zheng, J. Zhang, L. Zhang, TGF-β1 promotes cell migration in hepatocellular carcinoma by suppressing reelin expression, Gene 688 (2019) 19–25, https://doi.org/10.1016/j.gene.2018.11.033.
[47] M.J. Heo, Y.M. Kim, J.H. Koo, Y.M. Yang, J. An, S.K. Lee, S.J. Lee, K.M. Kim, J. W. Park, S.G. Kim, microRNA-148a dysregulation discriminates poor prognosis of hepatocellular carcinoma in association with USP4 overexpression, Oncotarget 5 (2014) 2792–2806, https://doi.org/10.18632/oncotarget.1920.
[48] J.P. Thiery, H. Acloque, R.Y.J. Huang, M.A. Nieto, Epithelial-mesenchymal transitions in development and disease, Cell 139 (2009) 871–890, https://doi.org/10.1016/j.cell.2009.11.007.
[49] Y. Li, X. Deng, X. Zeng, X. Peng, The role of Mir-148a in cancer, J. Canc. 7 (2016) 1233–1241, https://doi.org/10.7150/jca.14616.
[50] J. Xu, S. Lamouille, R. Derynck, TGF-B-induced epithelial to mesenchymal transition, Cell Res. 19 (2009) 156–172, https://doi.org/10.1038/cr.2009.5.
[51] H. Yan, X. Dong, X. Zhong, J. Ye, Y. Zhou, X. Yang, J. Shen, J. Zhang, Inhibitions of epithelial to mesenchymal transition and cancer stem cells-like properties are involved in miR-148a-mediated anti-metastasis of hepatocellular carcinoma, Mol. Carcinog. 53 (2014) 960–969, https://doi.org/10.1002/mc.22064.
[52] Y. Jin, Y.S. Wong, B.K.P. Goh, C.Y. Chan, P.C. Cheow, P.K.H. Chow, T.K.H. Lim, G. B.B. Goh, T.L. Krishnamoorthy, R. Kumar, T.P. Ng, S.S. Chong, H.H. Tan, A.Y. F. Chung, L.L.P.J. Ooi, J.P.E. Chang, C.K. Tan, C.G.L. Lee, Circulating microRNAs as potential diagnostic and prognostic biomarkers in hepatocellular carcinoma, Sci. Rep. 9 (2019), https://doi.org/10.1038/s41598-019-46872-8, 10464–10464.
[53] S.K. Song, W.Y. Jung, S.K. Park, C.W. Chung, Y. Park, Significantly different expression levels of microRNAs associated with vascular invasion in hepatocellular carcinoma and their prognostic significance after surgical resection, PloS One 14 (2019), https://doi.org/10.1371/journal.pone.0216847.