Pathology - Research and Practice 215 (2019) 152406

Contents lists available at ScienceDirect

Pathology - Research and Practice

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

miR-152-3p Modulates hepatic carcinogenesis by targeting cyclin-dependent kinase 8

Tao Yin[a,b], Ming-Ming Liu[b], Ruo-Tian Jin[b], Jian Kong[a], Shao-Hong Wang[a], Wen-Bing Sun[a,*]

[a] Department of Hepatobiliary Surgery, Beijing Chao-Yang Hospital, Capital Medical University, Beijing 100043, China
[b] Department of General Surgery, Affiliated Hospital of Chifeng University, Chifeng 024005, China

ARTICLE INFO

Keywords:
Hepatocellular carcinoma
Cyclin-dependent kinase 8
miR-152-3p
Proliferation
Apoptosis

ABSTRACT

Background: Cyclin-dependent kinase 8 (*CDK8*) as a Mediator complex-associated transcriptional regulator has been shown to play important role in the initiation and progression of various cancers. The present study aimed to explore miR-152-3p-modulated post-transcriptional repression of *CDK8* in hepatic carcinogenesis.
Methods: Eighty-nine pairs of hepatocellular carcinoma (HCC) and adjacent non-tumor tissues were collected for molecular biological analysis. Cell viability and apoptosis assays were detected using CCK8 and Annexin V-fluorescein isothiocyanate/propidium iodide (Annexinv-FITC) double staining, respectively. Bioinformatics algorithms and luciferase reporter assay were performed to validate CDK8 as a direct target of miR-152-3p. Gene and protein expression levels were monitored using RT-qPCR, western blotting or immunohistochemical (IHC) staining.
Results: *CDK8* expression levels were up-regulated and miR-152-3p was down-regulated in HCC tissues. The correlation analysis had documented a significant negative correlation between miR-152-3p and *CDK8* in the HCC tissues. Both *CDK8* and miR-152-3p could serve as the independent prognostic factors for predicting the OS and DFS in HCC patients. Bioinformatics and experimental measurement revealed that *CDK8* was a direct target of miR-152-3p. After co-transfection with the miR-152-3p mimics and the *CDK8* overexpressed plasmids, the anti-proliferative and pro-apoptotic roles of miR-152-3p were restricted by *CDK8*.
Conclusion: The present results obtained forcefully proved that miR-152-3p exhibited an antineoplastic activity via targeting *CDK8* and might be served as a potential therapeutic target for the treatment of HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignant tumors and the third cause of cancer-related death worldwide [1]. An estimated report of cancer statistics in China deduces that about 466,100 Chinese are newly diagnosed with HCC, and approximately 422,100 Chinese die from HCC in 2015 year [2]. Although the diagnostic and therapeutic strategies make significant progress in the clinical practice of HCC, a 1-year survival rate is less than 50% [3], which may be attributed to the complicated pathogenic mechanism of HCC. Therefore, it is imperative to further explore the molecular mechanism underlying the hepatic tumorigenesis, which may reveal the new therapeutic targets for improving the survival prognosis of HCC patients.

Cyclin-dependent kinase 8 (*CDK8*) is a ubiquitously expressed transcription-regulating serine/threonine kinases and is belong to CDK family [4]. Recent study reveals that *CDK8* plays a positive role in gene-specific transcription by the phosphorylation of transcription factors or the association with the Mediator complex [4]. *CDK8*-modulated signaling pathways, such as Wnt/β-catenin, estrogen receptor-responsive genes and drosophila mothers against decapentaplegic transcriptional signaling, have been implicated in regulating oncogenesis in breast cancer, pancreatic cancer and colorectal cancer [4–8]. Overexpression of *CDK8* is presented in melanoma, breast cancer and prostate cancer [6,9,10]. The suppression of *CDK8* expression by small interfering RNA or short hairpin leads to inhibit proliferation in colon cancer, prostate cancer and breast cancer cells [6,8,10]. In addition, *CDK8*-mediated phosphorylation restrains natural killer (NK) cells cytotoxicity and tumor surveillance, while specific *CDK8* deletion in NK cells enhances antitumor responses [11,12]. These promising preclinical data suggest that specific inhibition of *CDK8* had the ability to neutralize its oncogenic activity.

* Corresponding author at: Department of Hepatobiliary Surgery, Beijing Chao-Yang Hospital, Capital Medical University, No. 5 Jingyuan Street, Beijing 100043, China.
E-mail address: sun_wenbing@aliyun.com (W.-B. Sun).

https://doi.org/10.1016/j.prp.2019.03.034
Received 21 January 2019; Received in revised form 18 March 2019; Accepted 31 March 2019

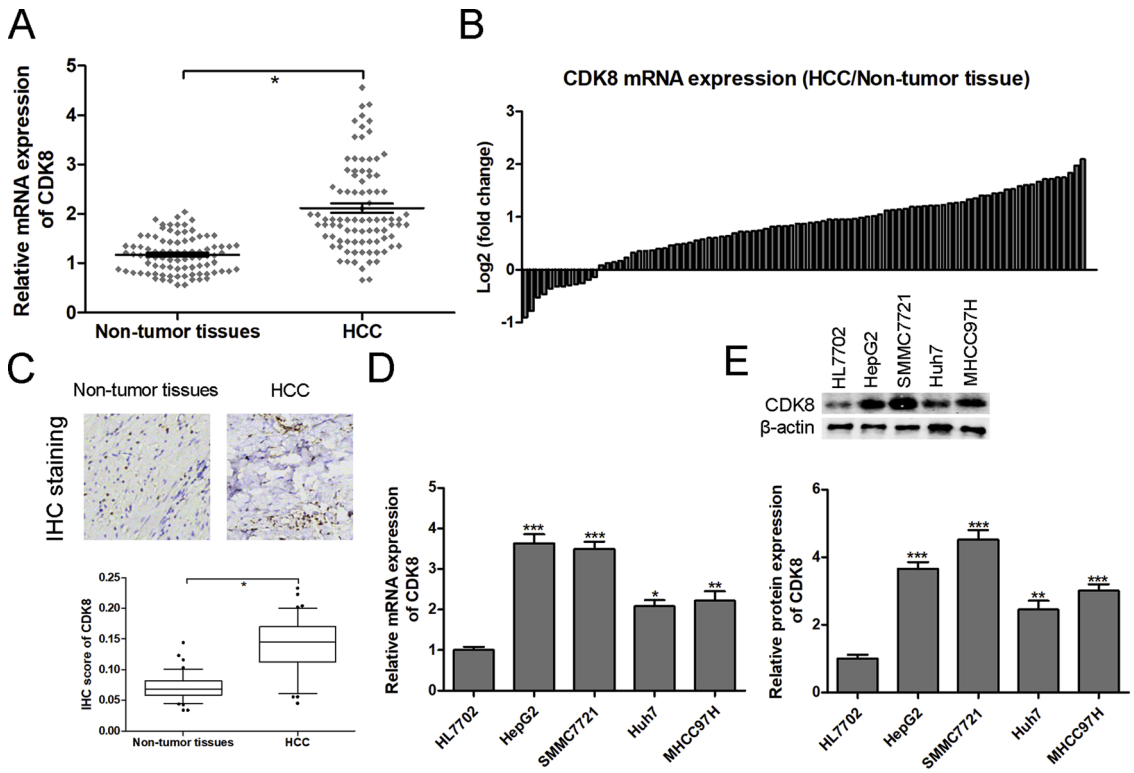

Fig. 1. Up-regulation of *CDK8* is observed in HCC tissues and cell lines. The expression levels of *CDK8* are detected using RT-qPCR (A and B) and IHC staining (C) in eighty-nine pairs of hepatocellular carcinoma (HCC) and adjacent non-tumor tissues. mRNA and protein expression levels of *CDK8* are measured in the four HCC cell lines (HepG2, SMMC7721, Huh7 and MHCC97 H) and a human normal hepatic cell line HL7702 using RT-qPCR (D) and western blotting (E), respectively. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with corresponding control group.

Table 1
Correlation between CDK8 mRNA level and clinicopathological variables.

Variables	n	Low expression (n = 54)	High expression (n = 35)	*P* value
Age (years)				0.083
≤ 50	58	39	19	
> 50	31	15	16	
Gender				0.126
M	64	42	22	
F	25	12	13	
Tumor size (cm)				< 0.001
≤ 5	47	39	8	
> 5	42	15	27	
TNM stages				0.001
I-II	35	29	6	
III-IV	54	25	29	
HBV				0.244
–	39	21	18	
+	50	33	17	
Serum AFP (U/mL)				0.636
< 400	46	29	17	
≥ 400	43	25	18	

CDK8, cyclin-dependent kinase 8; M, male; F, female; HBV, hepatitis B virus; -, negative; +, positive; AFP, alpha fetoprotein.

MicroRNAs (miRNAs) have emerged as a novel class of noncoding RNAs and are characterized by short, noncoding and single-stranded RNAs of ˜22 nucleotides [13,14]. In mammal, more than one-third of genes can be silenced by miRNAs by post-transcriptionally regulatory mechanism via binding with the 3′-untranslated region (3′-UTR) of target genes to repress transcription or translation [13,14]. Numerous studies indicate that miRNAs participate in a variety of biological processes, including carcinogenesis [15]. So far, numerous miRNAs are determined to regulate the progression of HCC, including miR-148-3p/152-3p family members [16]. For example, a decrease in miR-152 expression levels is observed in human HCC tissues [17]. Down-regulation of miR-152 is frequently reported in HBV-related HCC and induces aberrant DNA methylation facilitating the process of HCC [18]. Low level of circulating miR-152 is detected in serum of HCC patients with hepatitis C virus infection as compared to the patients with hepatitis C virus infection alone [19]. These results support a tumor-suppressive role of miR-152 in the progression of HCC. However, the roles of miR-152-3p in hepatic carcinogenesis via targeting *CDK8* are still unclear.

The present study found a significant negative correlation between miR-152-3p and *CDK8* expression in human HCC tissues. We also validated that *CDK8* was a direct target of miR-152-3p, and overexpression of miR-152-3p inhibited proliferation and induced apoptosis in HCC cells by suppressing *CDK8* expression.

2. Material and methods

2.1. Sample collection

Eighty-nine pairs of HCC and adjacent non-tumor tissues were collected from HCC patients in the Beijing Chao-Yang Hospital, Capital Medical University (Beijing China) and the Affiliated Hospital of Chifeng University (Chifeng, China) between Jan 2010 and Jan 2013. All of the patients were recruited according to the histopathological evaluation without radiotherapy or chemotherapy before surgical operation. The specimens were rapidly stored in liquid nitrogen for molecular biological analysis. Informed consent forms were obtained from the HCC patients. This study was permitted by the Ethics Committee of the Beijing Chao-Yang Hospital, Capital Medical University (Beijing China) and the Affiliated Hospital of Chifeng University (Chifeng, China).

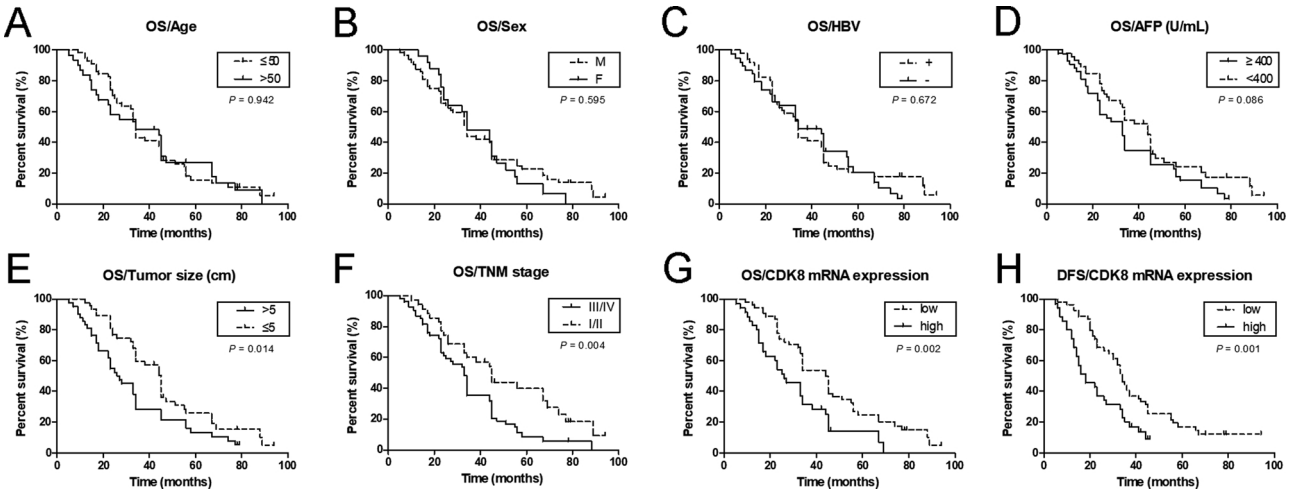

Fig. 2. The correlation between survival prognosis and clinicopathological variables. Kaplan–Meier analysis and logrank test reveal that age (A), gender (B), HBV infection (C) and serum AFP (D) have no relevance with survival prognosis in HCC patients. Tumor size (E) and TNM stages (F) are correlated with OS in HCC patients. High *CDK8* mRNA expression is correlated with poor OS (G) and DFS (H) in HCC patients.

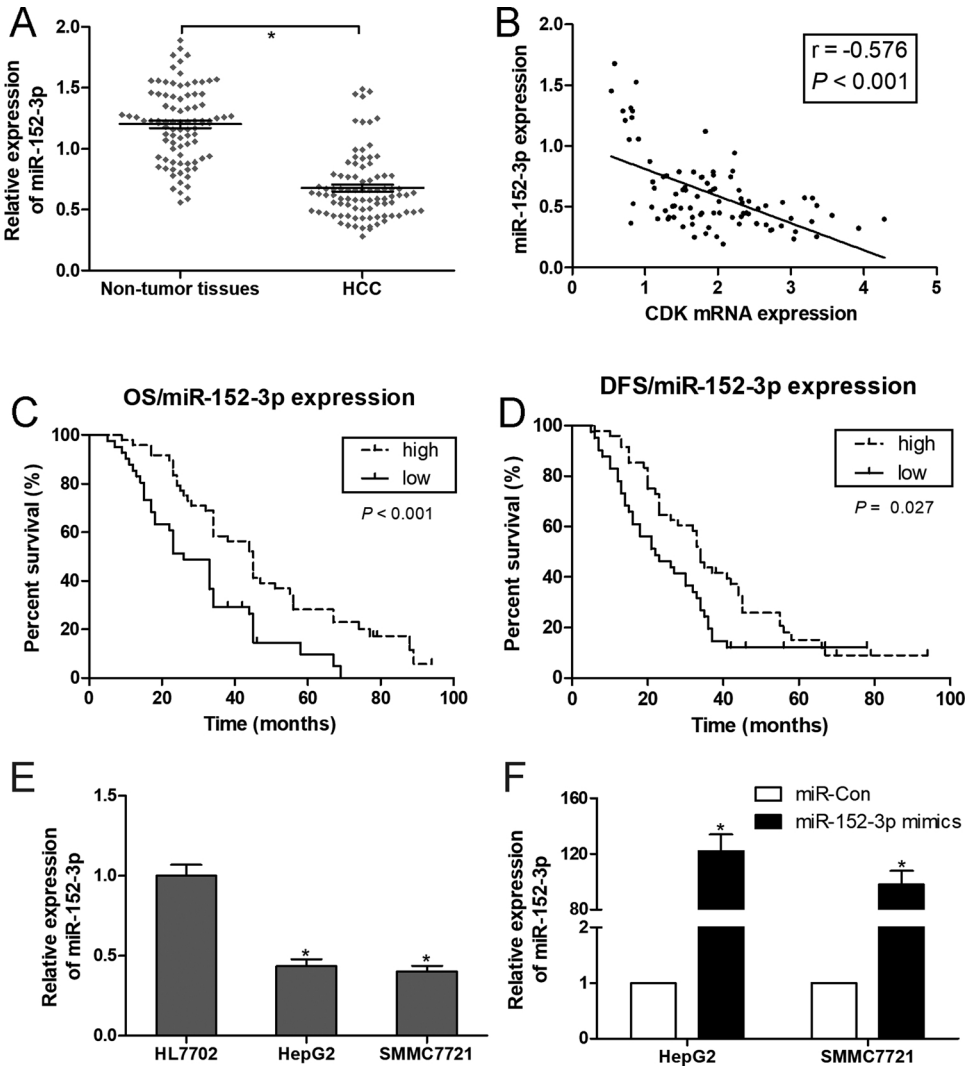

Fig. 3. miR-152-3p is down-regulated and associated with survival prognosis in HCC patients. RT-qPCR assay shows that miR-152-3p is decreased (A) and negatively correlated with *CDK8* expression (B) in HCC tissues. Kaplan–Meier survival analyses uncover that low miR-152-3p expression is significantly correlated with poor OS and DFS in HCC patients (C and D). miR-152-3p expression levels are measured in a human normal hepatic cell line HL7702 and HCC cell lines (HepG2 and SMMC7721) using RT-qPCR (E). After transfection with miR-152-3p mimics into HepG2 and SMMC7721 cells, miR-152-3p expression levels are measured using RT-qPCR (F). $^{*}P < 0.05$.

Table 2
Correlation between miR-152-3p level and clinicopathological variables.

Variables	n	Low expression (n = 41)	High expression (n = 48)	*P* value
Age (years)				0.309
≤ 50	58	29	29	
> 50	31	12	19	
Gender				0.819
M	64	29	35	
F	25	12	13	
Tumor size (cm)				< 0.001
≤ 5	47	7	40	
> 5	42	34	8	
TNM stages				0.002
I-II	35	9	26	
III-IV	54	32	22	
HBV				0.383
–	39	20	19	
+	50	21	29	
Serum AFP (U/mL)				0.136
< 400	46	20	26	
≥ 400	43	31	22	

CDK8, cyclin-dependent kinase 8; M, male; F, female; HBV, hepatitis B virus; -, negative; +, positive; AFP, alpha fetoprotein.

2.2. Cell culture

Human normal liver cell line (HL-7702) and four HCC cell lines (HepG2, SMMC7721, Huh7 and MHCC97 H) were obtained from the Institute of Biochemistry and Cell Biology of the Chinese Academy of Sciences (Shanghai, China). Cells were cultured according to previously described [20].

2.3. Immunohistochemical (IHC) staining

IHC staining was performed in HCC and adjacent non-tumor tissues according to previously described [21]. The primary antibody of *CDK8* was purchased from Santa Cruz Biotechnology Inc., (cat.no. sc-13155; dilution: 1:50; Dallas, TX, USA). Mouse IgGκ binding protein-HRP second antibody was purchased from Santa Cruz Biotechnology Inc., (cat.no. sc-516102; dilution: 1:200; Dallas, TX, USA). The pictures of IHC staining were visual under a microscope (Leica DM 2500; Leica Microsystems GmbH, Wetzlar, Germany). IHC score of *CDK8* positive staining was using image Pro-Plus 6 software (Media Cybernetics, Inc., Rockville, MD, USA).

2.4. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) was used to extract total RNA, according to the manufacturer's protocol. TaqMan® RT kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) and TaqMan® MicroRNA assay (Applied Biosystems; Thermo Fisher Scientific, Inc.) were used to perform RT-qPCR of miR-152-3p, according to the manufacturer's protocol. U6 small nuclear RNA was used as an endogenous control. The primers were used as follows: miR-152-3p: **stem-loop primer, 5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCAGTCACGT-3′**; forward, 5′-ACACTCCAGCTGGGTCAGTGCATGACAG-3′, and reverse, 5′-CTCAACTGGTGTCGTGGAGTCGGCAATTCAGTTGAGCCAAGTT-3′; U6 forward, 5′-CAAATTCGTGAAGCGTTCCATA-3′, and reverse, 5′-AGTGCAGGGTCCGAGGTA TTC-3′.

The cDNA was synthesized by reverse transcription reactions with 2 μg of total RNA using moloney murine leukemia virus reverse transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. RT-qPCR for *CDK8* was performed by Applied Biosystems 7300 Real-Time PCR System (Thermo Fisher Scientific, Inc.) with the TaqMan Universal PCR Master Mix (Thermo Fisher Scientific, Inc.). The Cq (quantification cycle fluorescence value) was calculated using SDS software, version 2.1 (Applied Biosystems; Thermo Fisher Scientific, Inc.), and the relative expression levels of mRNA were calculated using the $2^{-\Delta\Delta Cq}$ method [22] and normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers were used as follows: *CDK8*: forward, 5′-GCCGGTTGTCAAATCCCTTAC-3′, and reverse, 5′-TGTGACTGCTGTCTTGATTCCCT-3′; GAPDH forward, 5′-CAAATTCGTGAAGCGTTCCATA-3′, and reverse, 5′-AGTGCAGGGTCCGAGGTATTC-3′.

2.5. Western blotting

Radio immunoprecipitation assay (RIPA) buffer (Cat.No: P0013B; Beyotime Institute of Biotechnology) was used to extract the protein in HCC tissues and cell lines. Western blotting was performed according to previously described [21]. The primary antibody of *CDK8* was purchased from Santa Cruz Biotechnology Inc., (cat.no. sc-13155; dilution: 1:500; Dallas, TX, USA). Mouse IgGκ binding protein-HRP second antibody was purchased from Santa Cruz Biotechnology Inc., (cat.no. sc-516102; dilution: 1:5,000; Dallas, TX, USA). β-actin (1:2,000; cat. no. sc-130065; Santa Cruz Biotechnology, Inc.) was used as the control antibody. Signals were analyzed with Quantity One® software version 4.5 (Bio Rad Laboratories, Inc., Hercules, CA, USA).

2.6. Cell transfection and plasmid constructs

miR-control (miR-Con; 5′-UAGCCACGGUUGUGUAAAGUCUG-3′) and miR-152-3p mimics (5′-UCAGUGCAACUGACAGAACUUGG-3′) were synthesized by Guangzhou RiboBio Co., Ltd. (Guangzhou, China). Cells were seeded in 6-well plates and transfected with miR-Con and miR-152-3p mimics using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h at 37 °C, according to the manufacturer's protocols.

A mammalian expression plasmid designed to specially express the full-length open reading frame of human *CDK8* without 3′-UTR, which did not contain the conserved complementary sequence binding with

Table 3
Univariate and multivariate regression analysis of HCC patients for overall survival.

	Univariate		Multivariate	
Variables	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Age (> 50 vs ≤ 50)	1.12 (0.60-2.77)	0.456		
Gender (M vs F)	1.41 (0.87-3.03)	0.341		
Tumor size (> 5 vs ≤ 5)	1.27 (0.70-2.88)	0.347		
HBV (+ vs -)	1.62 (0.933-3.54)	0.103		
Serum AFP (≥ 400 vs < 400)	1.56 (0.91-2.96)	0.116		
TNM stages (III-IV vs I-II)	2.88 (1.39-6.71)	0.011	2.59 (1.15-5.39)	0.030
CDK8 (high vs low)	3.35 (1.84-7.51)	0.003	2.92 (1.36-7.03)	0.014

HCC, hepatocellular carcinoma; CDK8, cyclin-dependent kinase 8; M, male; F, female; HBV, hepatitis B virus; -, negative; +, positive; AFP, alpha fetoprotein.

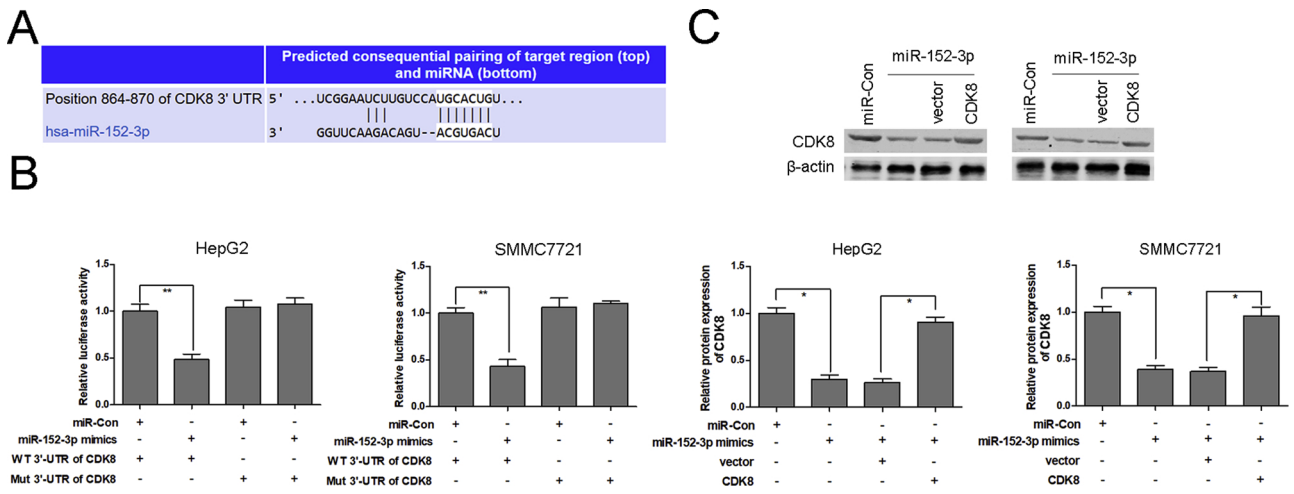

Fig. 4. *CDK8* is a direct target of miR-152-3p. Bioinformatics algorithm is executed by Targetscan (www.targetscan.org) to predict the binding sites of miR-152-3p in the 3′-UTR of *CDK8* (A). Luciferase reporter assay is performed in HepG2 and SMMC7721 cells co-transfection with miR-152-3p mimics or the plasmid containing WT or Mut 3′-UTR of *CDK8* (B). After co-transfection with *CDK8* overexpressed plasmids and miR-152-3p mimics into HepG2 and SMMC7721 cells, the protein expression of *CDK8* is measured using western blotting (C). $^{*}P < 0.05$, $^{**}P < 0.01$.

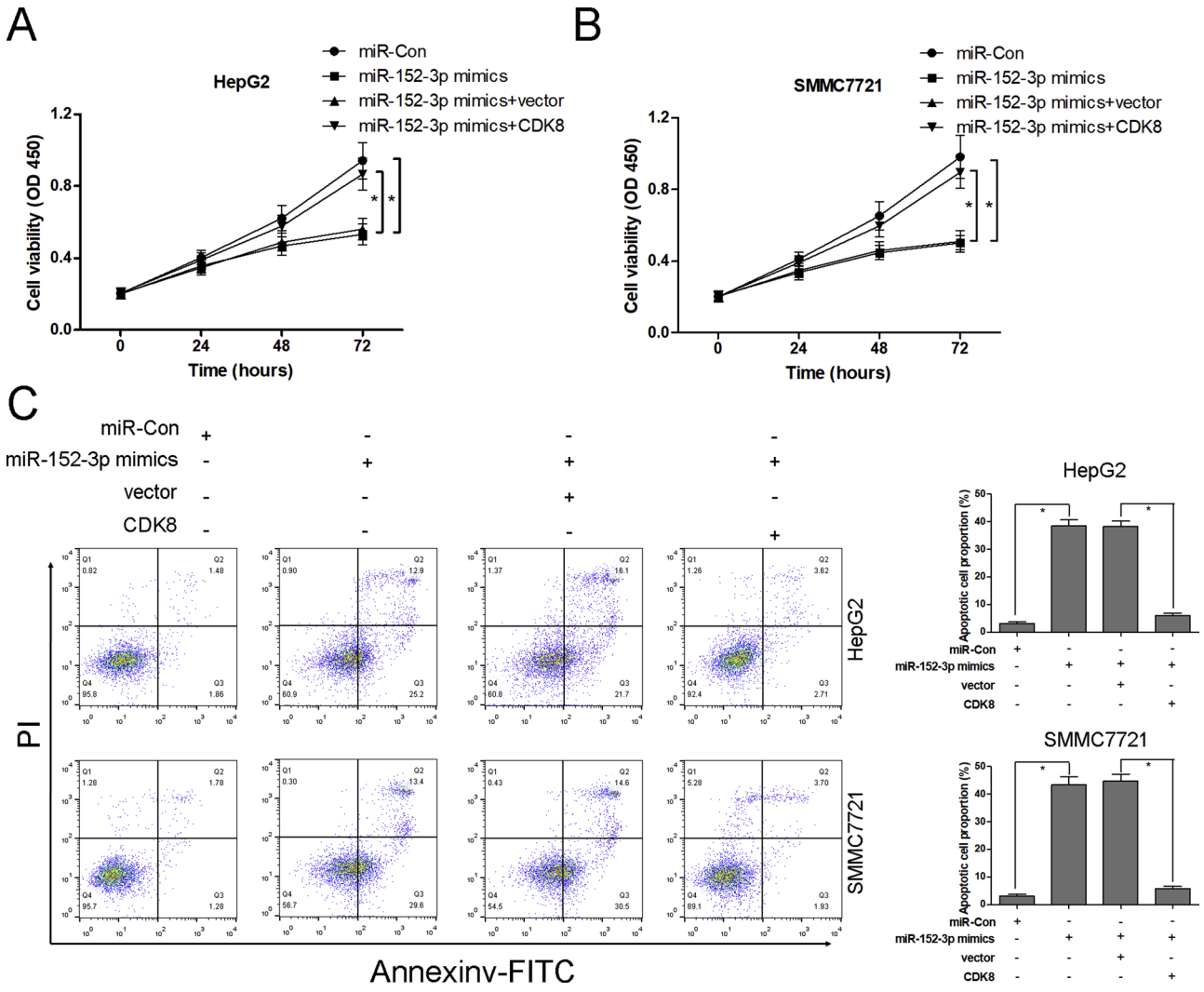

Fig. 5. Overexpression of miR-152-3p inhibits proliferation and induces apoptosis in HCC cells. After co-transfection with *CDK8* overexpressed plasmids and miR-152-3p mimics into HepG2 and SMMC7721 cells, Cell viability (A and B) and apoptosis assays (C) are detected using CCK8 and Annexin V-fluorescein isothiocyanate/propidium iodide (Annexinv-FITC) double staining, respectively. $^{*}P < 0.05$.

miR-152-3p, was purchased from GeneCopoeia, Inc. (Rockville, MD, USA). An empty plasmid served as the negative control. Vector-Con and vector-*CDK8* were transfected into HCC cells using Lipofectamine 2000 for 48 h at 37 °C, according to the manufacturer's protocols.

2.7. Luciferase reporter assay

The 3′-UTR of representative transcript (ENST00000536792.1) for *CDK8* (ENSG00000132964.7) was used to predict the binding sites with miR-152-3p by Targetscan (www.targetscan.org). The wild-type (WT) and mutant-type (Mut) 3′-UTR of *CDK8* were constructed with synthetic oligonucleotides (Beijing AuGCT DNA-SYN Biotechnology, China) and inserted into the multiple cloning sites of the luciferase expressing pMIR-REPORT vector (Ambion; Thermo Fisher Scientific, Inc.). For the luciferase assay, chondrocytes containing the WT or Mut 3′-UTR of *CDK8* (0.5 μg) were co-transfected with miR-Con or miR-152-3p mimics using Lipofectamine 2000, according to the manufacturer's protocols. The luciferase activity was measured using a luciferase reporter assay kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's protocols.

2.8. Cell counting kit 8 (CCK8) assay

Cell viability was detected using CCK-8 kit (Beyotime Institute of Biotechnology, Haimen, China). Absorbance was recorded at 450 nm with a SpectraMax M5 ELISA plate reader (Molecular Devices, LLC, Sunnyvale, CA, USA), according to the manufacturer's instructions.

2.9. Annexinv-FITC/PI double staining for apoptosis analysis

Annexinv-FITC kit (BD Biosciences, Franklin Lakes, NJ, USA) was used to stain cells for 15 min, and then apoptotic cell was analyzed by flow cytometry (FACScan, BD Biosciences, San Jose, CA, USA) using CELL Quest 3.0 software (FACScan, BD Biosciences, San Jose, CA, USA).

2.10. Statistical analysis

Data were presented as the mean ± standard error of the mean. Statistical analysis was performed using SPSS software version 19.0 (IBM Corp., Armonk, NY, USA) and GraphPad Prism Version 7.0 (GraphPad Software, Inc., La Jolla, CA, USA). A Student's *t*-test or Kruskal-Wallis test was used to analyze two-group differences. Differences between multiple groups were analyzed by one-way analysis of variance, followed by a post-hoc Tukey test. Pearson χ^2 tests were used to evaluate differences between the clinical characteristics and *CDK8* or miR-152-3p expression levels in HCC patients. Spearman's rank analysis was used to identify the correlation between *CDK8* and miR-152-3p expression levels in HCC patients. Overall survival (OA) and disease free survival (DFS) was calculated using the Kaplan-Meier method with the log-rank test applied for comparison. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. Up-regulation of CDK8 is observed in HCC tissues and cell lines

The mRNA and protein expression levels of *CDK8* in HCC and adjacent non-tumor tissues were detected using RT-qPCR and IHC staining, respectively. mRNA expression level of *CDK8* was significantly up-regulated in the HCC tissues compared with the adjacent non-tumor tissues (Fig. 1A). The up-regulation of *CDK8* was observed in the majority of HCC tissues (77/89, 86.5%; Fig. 1B). As expected, the protein levels of *CDK8* showed a significant increase in HCC tissues compared with that of in the adjacent non-tumor tissues (Fig. 1C). In addition, RT-qPCR and western blotting assays demonstrated that both mRNA and protein expression levels were markedly higher in the four HCC cell lines (HepG2, SMMC7721, Huh7 and MHCC97 H) than that of in the human normal hepatic cell line HL7702 (Fig. 1**D and E**).

3.2. High CDK8 mRNA expression is correlated with poor OS and DFS in HCC patients

First, the correlation between *CDK8* mRNA level and clinicopathological variables was performed to evaluate whether *CDK8* could serve as an independent prognostic factor in the progression of HCC. We found that high *CDK8* mRNA expression was closely related with tumor size and TNM stages, but not associated with age, gender, HBV infection and serum AFP in HCC patients (Table 1). Kaplan–Meier analysis and log-rank test revealed that age, gender, HBV infection and serum AFP had no relevance with survival prognosis of HCC patients (Fig. 2**A-D**). However, bigger tumor size and higher TNM stages were significantly correlated with poorer OS in HCC patients (Fig. 2**E and F**). We also found that high *CDK8* mRNA expression was correlated with poor OS and DFS in HCC patients (Fig. 2**G and H**). These findings indicated that high mRNA expression of *CDK8* might be an independent factor for predicting a poorer OS and DFS in HCC patients.

3.3. miR-152-3p was down-regulated and associated with survival prognosis in HCC patients

To determine the role of miR-152-3p in hepatic carcinogenesis, we first detected the expression levels of miR-152-3p in HCC tissues. The RT-qPCR assays showed that miR-152-3p was significantly decreased (Fig. 3A) and negatively correlated with *CDK8* mRNA expression (Fig. 3B) in HCC tissues. Moreover, a lower miR-152-3p level was correlated with bigger tumor size and higher TNM stages, but not associated with age, gender, HBV infection and serum AFP in HCC patients (Table 2). Kaplan–Meier survival analyses uncovered that low miR-152-3p expression was significantly correlated with poor OS and DFS in HCC patients (Fig. 3**C and D**). COX univariate and multivariate analyses uncovered that miR-152-3p was an independent risk factor for predicting the prognosis of HCC patients (Table 3). We also found a decrease in miR-152-3p expression levels in HepG2 and SMMC7721 cells compared with human normal HL7702 cells (Fig. 3E). As shown in Fig. 3F, transfection with miR-152-3p mimics into HepG2 and SMMC7721 cells led to approximately 100-folds higher than that of in the control group.

3.4. CDK8 is a direct target of miR-152-3p

To investigate the association between *CDK8* and miR-152-3p, bioinformatics algorithm was executed by Targetscan (www.targetscan.org) to predict the binding sites of miR-152-3p in the 3′-UTR of *CDK8*. As shown in Fig. 4A, the conserved complementary sequences between the 3′-UTR of *CDK8* and miR-152-3p were predicted. Luciferase reporter assays showed that miR-152-3p mimics significantly reduced the luciferase activity in HepG2 and SMMC7721 cells transfection with the plasmid containing WT 3′-UTR of *CDK8*, while the luciferase activity had no obvious change in HepG2 and SMMC7721 cells with Mut 3′-UTR of *CDK8* (Fig. 4B). Moreover, overexpression of miR-152-3p led to the down-regulation of *CDK8* protein expression in HepG2 and SMMC7721 cells (Fig. 4C). Meanwhile, we performed a rescue experiment in HepG2 and SMMC7721 cells co-transfection with *CDK8* overexpressed plasmids, which did not contain the 3′-UTR of *CDK8*. Therefore, it was not targeted by miR-152-3p. We found that miR-152-3p-induced the down-regulation of *CDK8* protein expression was rescued by transfection with the *CDK8* overexpressed plasmids (Fig. 4C).

3.5. Overexpression of miR-152-3p inhibits proliferation and induces apoptosis in HCC cells

The roles of miR-152-3p on proliferation and apoptosis were

evaluated using CCK8 and Annexinv-FITC/PI double staining. The results indicated that overexpression of miR-152-3p had the ability to inhibit proliferation (Fig. 5**A and B**) and induces apoptosis in HepG2 and SMMC7721 cells (Fig. 5C), while the anti-proliferative and pro-apoptotic roles of miR-152-3p were neutralized by *CDK8* overexpressed plasmids in HepG2 and SMMC7721 cells (Fig. 5**A-C**).

4. Discussion

CDK8 is a functional transcription factor in various cancers and may be a potential anticancer target [4]. In fact, *CDK8* seems not to be a necessary factor for transcription, knockdown of *CDK8* has not achieved histopathological abnormalities in the young adult mouse [23]. In contrast to that, *CDK8* deficiency results in the developmental arrest of embryogenesis in mice [24]. These findings imply that *CDK8* plays multiple roles during different physiological and pathological phases. Recently, *CDK8* as an oncogene promotes the growth and metastasis of colon cancer [25]. In addition, *CDK8* is amplified in several cancer types, including colorectal cancer [8], breast cancer [26] and laryngeal squamous cell carcinoma [27]. Intriguingly, Zhang et al reveals that up-regulation of *CDK8* is observed in HCC tissues and associated with lung metastasis [28]. However, the association between *CDK8* and survival prognosis in HCC patients has not been clarified in this paper [28]. In our study, we also found that *CDK8* expression levels were significantly elevated in HCC tissues, and higher *CDK8* expression level was dramatically correlated with poorer OS and DFS in HCC patients. These findings showed *CDK8* as an independent factor for predicting the survival prognosis of HCC patients.

On the other hand, *CDK8* as a direct target of miR-152-3p could be post-transcriptionally repressed by miR-152-3p. The expression of *CDK8* was significantly inversely correlated with miR-152-3p expression in HCC tissues. Overexpression of miR-152-3p had the ability to inhibit proliferation and induce apoptosis in HCC cells by inhibiting *CDK8* signaling. Similarly, miR-26a inhibits the progression and metastasis of HCC by *CDK8*-modulated c-Myc/EZH2 signaling [28]. However, the downstream signaling pathways of *CDK8* had not been dabbled in our study. These may be the limitations in the present study.

Previous studies substantiate that inhibition of *CDK8* can impede tumor growth in several types of cancers, which have expedited the development of *CDK8* inhibitors as potential anticancer agents [4]. Confusedly, some researchers have suggested that *CDK8* knockout is distinct from pharmacological inhibition of *CDK8*. For example, *CDK8* inhibitor has not enough activity to resist proliferation in HCT116 cells, while *CDK8* knockout exerts the anti-proliferative activity in these cells [29]. As an endogenous inhibitor of *CDK8*, miR-152-3p has been reported as an onco-suppressor in prostate cancer [30], glioma [31] and breast cancer [32]. In our study, we also found that miR-152-3p had an antineoplastic activity *in vitro*, reflecting that overexpression of miR-152-3p restrained cell proliferation and increased the apoptotic cell proportion in HepG2 and SMMC7721 cells. However, the anti-proliferative and pro-apoptotic roles of miR-152-3p were restricted by *CDK8* after co-transfection with the miR-152-3p mimics and the *CDK8* overexpressed plasmids. These findings suggested that *CDK8* and miR-152-3p might play the reciprocal roles in the progression of HCC. Additionally, high miR-152-3p expression was associated with longer OS and DFS in HCC patients. The correlation analysis had documented a significant negative correlation between miR-152-3p and *CDK8* in the HCC tissues. The present results obtained forcefully proved that miR-152-3p exhibited a similar function of *CDK8* inhibitor and might be vital for the therapeutic application in the treatment of HCC.

The correlation between miR-152-3p and *CDK8* mRNA expression levels in HCC tissues was significantly negatively correlated (Fig. 3B). *in vitro* experiments, we found that only the protein expression of *CDK8* was decreased in HepG2 and SMMC7721 cells after transfection with miR-152-3p mimics (Fig. 4C). Therefore, there is no direct evidence that miR-152-3p shows the ability to degrade *CDK8* mRNA. One possible reason is that accumulation of multiple gene mutations and their interactions contribute to the initiation and progression of HCC. The present study revealed that miR-152-3p as a translational repressor decreased the protein expression of *CDK8*, and miR-152-3p/*CDK8* signaling pathway, at least partially, participated in hepatic tumorigenesis.

5. Conclusions

Taken together, the roles of *CDK8* and miR-152-3p in the process of HCC likely performed opposite effect completely. Both *CDK8* and miR-152-3p could serve as the independent prognostic factors for predicting the OS and DFS in HCC patients. We identified that miR-152-3p as a post-transcriptional regulator of *CDK8* might possess favorable pharmacologic properties to prevent the progression of HCC.

Funding

Not applicable

Competing interests

The authors declare that they have no competing interests.

References

[1] Y. Xie, J. Du, Z. Liu, D. Zhang, X. Yao, Y. Yang, MiR-6875-3p promotes the proliferation, invasion and metastasis of hepatocellular carcinoma via BTG2/FAK/Akt pathway, J. Exp. Clin. Cancer Res. 38 (2019) 7.
[2] W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, F. Bray, A. Jemal, X.Q. Yu, J. He, Cancer statistics in China, 2015, CA Cancer J. Clin. 66 (2016) 115–132.
[3] L.A. Torre, F. Bray, R.L. Siegel, J. Ferlay, J. Lortet-Tieulent, A. Jemal, Global cancer statistics, 2012, CA Cancer J. Clin. 65 (2015) 87–108.
[4] S. Philip, M. Kumarasiri, T. Teo, M. Yu, S. Wang, Cyclin-dependent kinase 8: a new hope in targeted cancer therapy? J. Med. Chem. 61 (2018) 5073–5092.
[5] C. Alarcon, A.I. Zaromytidou, Q. Xi, S. Gao, J. Yu, S. Fujisawa, A. Barlas, A.N. Miller, K. Manova-Todorova, M.J. Macias, G. Sapkota, D. Pan, J. Massague, Nuclear CDKs drive Smad transcriptional activation and turnover in BMP and TGF-beta pathways, Cell 139 (2009) 757–769.
[6] M.S. McDermott, A.A. Chumanevich, C.U. Lim, J. Liang, M. Chen, S. Altilia, D. Oliver, J.M. Rae, M. Shtutman, H. Kiaris, B. Gyorffy, I.B. Roninson, E.V. Broude, Inhibition of CDK8 mediator kinase suppresses estrogen dependent transcription and the growth of estrogen receptor positive breast cancer, Oncotarget 8 (2017) 12558–12575.
[7] W. Xu, Z. Wang, W. Zhang, K. Qian, H. Li, D. Kong, Y. Li, Y. Tang, Mutated K-ras activates CDK8 to stimulate the epithelial-to-mesenchymal transition in pancreatic cancer in part via the Wnt/beta-catenin signaling pathway, Cancer Lett. 356 (2015) 613–627.
[8] R. Firestein, A.J. Bass, S.Y. Kim, I.F. Dunn, S.J. Silver, I. Guney, E. Freed, A.H. Ligon, N. Vena, S. Ogino, M.G. Chheda, P. Tamayo, S. Finn, Y. Shrestha, J.S. Boehm, S. Jain, E. Bojarski, C. Mermel, J. Barretina, J.A. Chan, J. Baselga, J. Tabernero, D.E. Root, C.S. Fuchs, M. Loda, R.A. Shivdasani, M. Meyerson, W.C. Hahn, CDK8 is a colorectal cancer oncogene that regulates beta-catenin activity, Nature 455 (2008) 547–551.
[9] A. Kapoor, M.S. Goldberg, L.K. Cumberland, K. Ratnakumar, M.F. Segura, P.O. Emanuel, S. Menendez, C. Vardabasso, G. Leroy, C.I. Vidal, D. Polsky, I. Osman, B.A. Garcia, E. Hernando, E. Bernstein, The histone variant macroH2A suppresses melanoma progression through regulation of CDK8, Nature 468 (2010) 1105–1109.
[10] J. Bragelmann, N. Klumper, A. Offermann, A. von Massenhausen, D. Bohm, M. Deng, A. Queisser, C. Sanders, I. Syring, A.S. Merseburger, W. Vogel, E. Sievers, I. Vlasic, J. Carlsson, O. Andren, P. Brossart, S. Duensing, M.A. Svensson, Z. Shaikhibrahim, J. Kirfel, S. Perner, Pan-cancer analysis of the mediator complex transcriptome identifies CDK19 and CDK8 as therapeutic targets in advanced prostate cancer, Clin. Cancer Res. 23 (2017) 1829–1840.
[11] E.M. Putz, D. Gotthardt, G. Hoermann, A. Csiszar, S. Wirth, A. Berger, E. Straka, D. Rigler, B. Wallner, A.M. Jamieson, W.F. Pickl, E.M. Zebedin-Brandl, M. Muller, T. Decker, V. Sexl, CDK8-mediated STAT1-S727 phosphorylation restrains NK cell cytotoxicity and tumor surveillance, Cell Rep. 4 (2013) 437–444.
[12] A. Witalisz-Siepracka, D. Gotthardt, M. Prchal-Murphy, Z. Didara, I. Menzl, D. Prinz, L. Edlinger, E.M. Putz, V. Sexl, NK cell-specific CDK8 deletion enhances antitumor responses, Cancer Immunol. Res. 6 (2018) 458–466.
[13] A. Soroosh, M. Koutsioumpa, C. Pothoulakis, D. Iliopoulos, Functional role and therapeutic targeting of microRNAs in inflammatory bowel disease, Am. J. Physiol. Gastrointest. Liver Physiol. 314 (2018) g256–g262.
[14] B.P. Lewis, C.B. Burge, D.P. Bartel, Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets, Cell 120 (2005) 15–20.

[15] C.M. Croce, Causes and consequences of microRNA dysregulation in cancer, Nat. Rev. Genet. 10 (2009) 704–714.
[16] F. Duan, W. Liu, X. Fu, Y. Feng, L. Dai, S. Cui, Z. Yang, Evaluating the prognostic value of miR-148/152 family in cancers: based on a systemic review of observational studies, Oncotarget 8 (2017) 77999–78010.
[17] I. Kindrat, V. Tryndyak, A. de Conti, S. Shpyleva, T.K. Mudalige, T. Kobets, A.M. Erstenyuk, F.A. Beland, I.P. Pogribny, MicroRNA-152-mediated dysregulation of hepatic transferrin receptor 1 in liver carcinogenesis, Oncotarget 7 (2016) 1276–1287.
[18] J. Huang, Y. Wang, Y. Guo, S. Sun, Down-regulated microRNA-152 induces aberrant DNA methylation in hepatitis B virus-related hepatocellular carcinoma by targeting DNA methyltransferase 1, Hepatology 52 (2010) 60–70.
[19] E. Miquelestorena-Standley, A. Tallet, C. Collin, E. Piver, A. De Muret, E. Salame, P. Bourlier, T. Kervarrec, S. Guyetant, J.C. Pages, Interest of variations in microRNA-152 and -122 in a series of hepatocellular carcinomas related to hepatitis C virus infection, Hepatol. Res. 48 (2018) 566–573.
[20] H. Cai, B. Hu, L. Ji, X. Ruan, Z. Zheng, Hsa_circ_0103809 promotes cell proliferation and inhibits apoptosis in hepatocellular carcinoma by targeting miR-490-5p/SOX2 signaling pathway, Am. J. Transl. Res. 10 (2018) 1690–1702.
[21] J. Gong, Z.X. Wang, Z.Y. Liu, miRNA1271 inhibits cell proliferation in neuroglioma by targeting fibronectin 1, Mol. Med. Rep. 16 (2017) 143–150.
[22] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(-delta delta C(T)) Method, Methods 25 (2001) 402–408.
[23] M.L. McCleland, T.M. Soukup, S.D. Liu, J.H. Esensten, F. de Sousa e Melo, M. Yaylaoglu, S. Warming, M. Roose-Girma, R. Firestein, Cdk8 deletion in the Apc (Min) murine tumour model represses EZH2 activity and accelerates tumourigenesis, J. Pathol. 237 (2015) 508–519.
[24] T. Westerling, E. Kuuluvainen, T.P. Makela, Cdk8 is essential for preimplantation mouse development, Mol. Cell. Biol. 27 (2007) 6177–6182.
[25] J. Liang, M. Chen, D. Hughes, A.A. Chumanevich, S. Altilia, V. Kaza, C.U. Lim, H. Kiaris, K. Mythreye, CDK8 selectively promotes the growth of colon cancer metastases in the liver by regulating gene expression of TIMP3 and matrix metalloproteinases, Cancer Res. 78 (2018) 6594–6606.
[26] E.V. Broude, B. Gyorffy, A.A. Chumanevich, M. Chen, M.S. McDermott, M. Shtutman, J.F. Catroppo, I.B. Roninson, Expression of CDK8 and CDK8-interacting genes as potential biomarkers in breast cancer, Curr. Cancer Drug Targets 15 (2015) 739–749.
[27] M. Li, L. Tian, H. Ren, X. Chen, Y. Wang, J. Ge, S. Wu, Y. Sun, M. Liu, H. Xiao, MicroRNA-101 is a potential prognostic indicator of laryngeal squamous cell carcinoma and modulates CDK8, J. Transl. Med. 13 (2015) 271.
[28] X. Zhang, X. Zhang, T. Wang, L. Wang, Z. Tan, W. Wei, B. Yan, J. Zhao, K. Wu, A. Yang, R. Zhang, L. Jia, MicroRNA-26a is a key regulon that inhibits progression and metastasis of c-Myc/EZH2 double high advanced hepatocellular carcinoma, Cancer Lett. 426 (2018) 98–108.
[29] H.E. Pelish, B.B. Liau, I.I. Nitulescu, A. Tangpeerachaikul, Z.C. Poss, D.H. Da Silva, B.T. Caruso, A. Arefolov, O. Fadeyi, A.L. Christie, K. Du, D. Banka, E.V. Schneider, A. Jestel, G. Zou, C. Si, C.C. Ebmeier, R.T. Bronson, A.V. Krivtsov, A.G. Myers, N.E. Kohl, A.L. Kung, S.A. Armstrong, M.E. Lemieux, D.J. Taatjes, M.D. Shair, Mediator kinase inhibition further activates super-enhancer-associated genes in AML, Nature 526 (2015) 273–276.
[30] J. Ramalho-Carvalho, C.S. Goncalves, I. Graca, D. Bidarra, E. Pereira-Silva, S. Salta, M.I. Godinho, A. Gomez, M. Esteller, B.M. Costa, R. Henrique, C. Jeronimo, A multiplatform approach identifies miR-152-3p as a common epigenetically regulated onco-suppressor in prostate cancer targeting TMEM97, Clin. Epigenet. 10 (2018) 40.
[31] J. Sun, X. Tian, J. Zhang, Y. Huang, X. Lin, L. Chen, S. Zhang, Regulation of human glioma cell apoptosis and invasion by miR-152-3p through targeting DNMT1 and regulating NF2 : MiR-152-3p regulate glioma cell apoptosis and invasion, J. Exp. Clin. Cancer. Res 36 (2017) 100.
[32] J.H.M. Marques, A.L. Mota, J.G. Oliveira, J.Z. Lacerda, J.P. Stefani, L.C. Ferreira, T.B. Castro, A.F. Aristizabal-Pachon, D. Zuccari, Melatonin restrains angiogenic factors in triple-negative breast cancer by targeting miR-152-3p: in vivo and in vitro studies, Life Sci. 208 (2018) 131–138.