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Original Article

MiR-194 promotes hepatocellular carcinoma through negative regulation of CADM1

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Abstract: Aberrant expression of microRNAs may contribute to the initiation and progression of various types of human cancer and they may also constitute biomarkers for cancer diagnosis and treatment. However, the specific function of miR-194 in hepatocellular carcinoma (HCC), and the potential mechanism of its involvement in HCC were unclear. In the present study, we found that miR-194 inhibited CADM1 protein level expression by inhibiting mRNA translation of CADM1; the expression of CADM1 was low in liver cancer cells and tumor tissues, and the high expression of miR-194 was closely related to HCC. MiR-194 promoted proliferation, invasion, migration, and cell cycle progression of HCC cells, and such promotion effect was inhibited by CADM1. In addition, miR-194 may play a tumor-promoting action in a HCC xenograft tumor model. These results suggested that miR-194 may promote the occurrence and development of HCC by inhibiting CADM1. Therefore, miR-194 may be a promising novel therapy for diagnosis of hepatocellular carcinoma.

Keywords: Hepatocellular carcinoma, miR-194, CADM1

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide, and the third leading reason for deaths in the world and the second in China with the increase of its incidence and mortality [1, 2]. Recurrence and metastasis of HCC are responsible for the high mortality [3], but the exact mechanism of HCC is unknown and effective prevention and treatment methods are still lacking at present [4]. Revealing the molecular mechanism of HCC metastasis and taking effective preventive and therapeutic measures can improve efficacy and prognosis of patients with HCC.

The tumor suppressor, Cell adhesion molecule 1 (CADM1) is one of the members of the immunoglobulin superfamily in cell adhesion molecule that is involved in construction of cytoskeleton and maintenance of cell adhesion function. The decrease or inactivate expression of CADM1 is closely related to invasion, metastasis, and prognosis of the malignancy. It has been shown that CADM1 gets underexpressed in many tumors, including HCC [5-8].

Tumor invasion and metastasis is a multi-factor, multi-step dynamic process [9, 10] and involves epigenetic instability, chromosomal structural instability, and protein-coding gene instability. However, the existing metastasis-related molecules are not enough to explain all aspects of HCC metastasis, suggesting that there may be unknown molecules regulating HCC metastasis. Recent studies have demonstrated an association between the expression of microRNAs (miRNAs) and HCC metastasis. miRNAs are small non-coding RNAs of 20-23 nucleotides, recognize and bind with the 3'-UTR of target mRNAs, thus affecting numerous molecular and cellular processes [11-13]. Previous studies have demonstrated that certain miRNAs, including miR-194 [14] and miR-381 [15], are associated with carcinogenesis by regulating cell proliferation, invasion, cell cycle, and angiogenesis. However, to date, the role of miR-194 in HCC tumorigenesis remains poorly

understood and the potential target of miR-194 in HCC has not yet been fully characterized. The present study aimed to investigate the effect of miR-194 on HCC and explore the underlying targets involved.

Materials and methods

Patient information and sample collection

Tissues were obtained from 131 patients (83 males and 48 females; mean age, 65.12±14.56 years) who were undergoing treatment at The Fifth Affiliated Hospital of Zunyi Medical University. The tumor tissues and adjacent tissues (at 2 cm away from the tumor tissue) were collected and stored at -80°C. The present study was approved by The Ethics Committee of The Fifth Affiliated Hospital of Zunyi Medical University, Zhuhai, and informed consent was obtained from all patients.

qRT-PCR

Total miRNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. cDNA was synthesized using the cDNART kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). PCR was performed using a SYBR Green mix kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). Relative expression levels of miRNA and other indicators were calculated using the $2^{-\Delta\Delta Ct}$ method. The primer sequences used were as follows: miR-194-F: 5'-GTGCAGGGTCCGAGGT-3', miR-194-R: 5'-GCCGCTGGCAGTGTCTTAGCTG-3'; U6-F: 5'-CTCGCTTCGGCAGCACA-3', U6-R: 5'-AACGCTTCACGA ATTTGCGT-3'; CADM1-F: 5'-AGGGCAGAATCATCACGAAGT-3', CADM1-R: 5'-AGGGTCTCGATTGGATGGCA-3'; GAPDH-F: 5'-AGAAGGCTGGGGCTCArTTG-3', GAPDH-R: 5'-AGGGGCCATCCACAGTCTTC-3'.

Cell culture

Normal hepatic cell line, THLE-3 and HCC cell lines (SMCC7721, LM3, Hep3B, MHCC97H) were provided from Shanghai Institute of Cellular Biology of Chinese Academy of Sciences. The cells were cultured in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Inc., Waltham, MA, USA), supplemented with 10% fetal calf serum (Gibco; Thermo Fisher Scientific, Inc.) and l00 u/ml penicillin and streptomycin (Gibco; Thermo Fisher Scientific, Inc.) in a humidified water-jacket incubator at 37°C with 5% CO_2.

Cell transfection

HCC cell lines, SMCC7721 or MHCC97H, were seeded into six-well plates (5×10^5/well). When cultured SMCC7721 or MHCC97H had reached >60% confluence, the cells were transfected with miR-194 mimics or miR-NC (both, Thermo Fisher Scientific, Inc., Waltham, MA, USA) respectively, and co-transfected with miR-194 mimics and CADM1 (both, Thermo Fisher Scientific, Inc) using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol.

Dual luciferase assays

The targets and binding sites of miRNA and CADM1 were predicted using TargetScan (http://www.targetscan.org/). MHCC97H cells were co-transfected with WT-CADM1 or Mut-CDMA1 plasmid (Promega Corporation, Madison, WI, USA), along with miRNA mimics or miR-NC using Lipofectamine® 2000 (Gibco; Thermo Fisher Scientific, Inc.), following transient transfection for 24 h. The luciferase activity was determined by comparison with Renilla luciferase activity when the cells had been lysed with a passive lysis buffer, using the Dual-Luciferase Reporter assay system (Promega Corporation).

Western blotting

Proteins were extracted using RIPA Lysis Buffer (C1053, Applygen Technologies Inc. Beijing). Protein concentration was determined using BCA Protein Assays (Thermo Fisher Scientific, Inc.). The proteins (40 μg/well) were separated by 12% SDS-PAGE and transferred to a polyvinylidene difluoride membrane. The membranes were blocked with 5% non-fat dry milk powder for 1 h at 37°C. The primary antibodies (rabbit anti-CADM1, ab3910, 1:1000; GAPDH, ab181602, 1:2000; both Abcam, Cambridge, UK) were incubated at 4°C overnight. Horseradish peroxidase-labeled secondary antibody (ab150077, 1:5000, Abcam, Cambridge, UK) was incubated at room temperature for 1 h. An ECL chemiluminescence detection kit (Kangwei Century Biotechnology Co., Ltd., Beijing, China) was used to visualize the protein bands.

Immunohistochemical analysis

The HCC tissue and normal adjacent tissue were fixed in 4% paraformaldehyde Fix Solution for 48 h, and routinely dehydrated permeabilized and embedded in paraffin. The paraffin blocks were sliced into 5 μm serial paraffin sections, which were placed in an oven (65°C) overnight. Hydrated tissue sections were treated with 3% H_2O_2 to block endogenous peroxidase; the sections were subsequently acid-fixed using a pre-configured citrate buffer. The antigen was repaired a microwave heating method. Following repair, blocking was performed with 5% normal goat serum (Thermo Fisher Scientific, Inc.) at room temperature for 20 min. The primary antibodies (rabbit anti-CADM1, ab3910, 1:100; Anti-Ki67, ab197234, 1:100; both Abcam, Cambridge, UK) were incubated at 4°C overnight. Horseradish peroxidase-labeled secondary antibody (ab150077, 1:200, Abcam, Cambridge, UK) was incubated at room temperature for 30 min. The sections were performed by using DAB Horseradish Peroxidase Color Development Kit. Hematoxylin staining was performed at room temperature for 2 min, followed by dehydration and neutral resin packing. Positive images were observed with a vertical microscope.

Transwell assay

Migration assay: the cell density was controlled at around 1×10^6/mL, and transwells with the polycarbonate membranes were seeded in 24-well plates. 100 μl cell suspension was added into the upper chamber. After 24 h, the cells in the upper compartment of the membrane were swabbed with a cotton swab. The cells in the lower chamber were stained bycrystal violet for 20 min, which were observed and counted under the microscope. Invasion assay: the matrigel was placed overnight under 4°C; the liquefied matrigel and medium was diluted with proportion of 1:6. 50 μl matrigel diluent was added into the upper chamber to coat the filter membrane, and we let the coating liquid dry. The consequent steps followed that in the transwell migration assay.

CCK8 assay

Transfected cells were seeded into 96-well plates and assayed for proliferation with the CCK-8 kit (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h in accordance with the manufacturer's protocol. Cell proliferation was analyzed by measurement of absorbance at 450 nm using an automatic microplate reader.

Wound healing assay

The cells were seeded in a 6-well plate (2×10^5/ml, 2 ml/well). The culture solution was removed after the cells were grown to a monolayer. The middle of every hole was scratched and photographed under a microscope. The cell movement changes in the scratched area in 24 h were observed.

Flow cytometry

The cells were suspended, collected, and washed twice with saline before they were fixed with 70% ethyl alcohol, and fixed overnight at 4°C. 400 μl Propidium Iodide was added to each group for staining at 37°C in the dark for 30 min. Finally, cell cycle was detected by flow cytometry.

Mouse xenograft model

BALB/c nude mice (n=12; male; age, 4-5 week; weight, 13-17 g) were given adaptive feeding under specific-pathogen-free for 1 week. MHCC97H cells were transfected with miR-194 mimics or miR-NC, and the cell density was controlled at around 2×10^7/mL. Nude mice were divided into two groups (miR-194 mimics group, n=6; miR-NC group, n=6) and subcutaneously injected with 0.2 ml cell suspension into the right forearm, subcutaneous to the axilla. Tumor growth was measured every 3 days. Tumor volume (V) was monitored by measuring the maximum diameter (a) and the minimum diameter (W) with calipers and calculated using the following formula: $V=1/2\times a\times b^2$, After 3 weeks, the nude mice were euthanized by cervical dislocation. The tumors were excised and weighed, before being preserved in 4% paraformaldehyde at 4°C. All procedures were approved by the Animal Experimental Ethics Committee of The Fifth Affiliated Hospital of Zunyi Medical University, Zhuhai.

Statistical analysis

The data are presented as means ± SD. Student's t-test was used to compare the different expression of miR-194 and CADM1 bet-

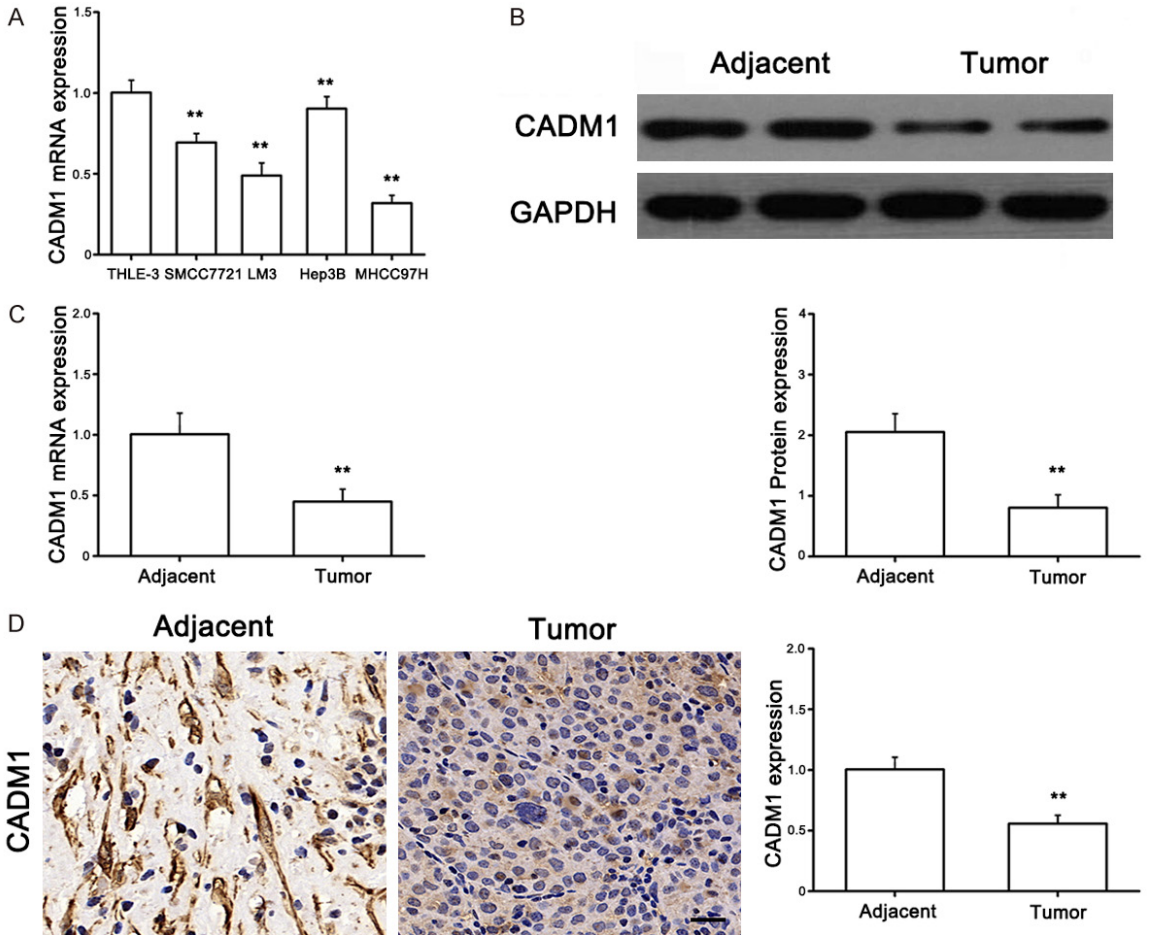

Figure 1. Expression of CADM1 in HCC tissues and HCC cell lines. A. CADM1 mRNA expression in HCC cell lines (SMCC7721, MHCC97H, LM3, Hep3B) and normal human hepatocytes (THLE-3) was measured by qRT-PCR. B. CADM1 mRNA expression in HCC tissues and adjacent normal tissues was measured by qRT-PCR. C. HMGA2 protein expression in HCC tissues and adjacent normal tissues was measured by western blot. D. CADM1 expression in HCC tissues and adjacent normal tissues was measured by IHC (scale bar =50 um). **P<0.01.

ween the HCC tumor tissues and adjacent normal tissues. One-way ANOVA was adopted to analyze the relationship of miR-194 and CADM1 in different cell lines. The statistical analysis was performed using SPSS 22.0 software. P<0.05 was considered a significant difference.

Results

Expression of CADM1 in HCC cell lines and tissue samples

qRT-PCR was employed to detect CADM1 mRNA expression in HCC cell lines (SMCC7721, LM3, Hep3B, MHCC97H) and human liver epithelial cells THLE-3. As shown in **Figure 1A**, the mRNA expression level of CADM1 was reduced in each HCC cell line when compared with human liver epithelial cells; moreover, the detection showed that mRNA expression level of CADM1 in HCC tumor tissues was significantly downregulated when compared with adjacent normal tissue (**Figure 1B**). Western blot was used to detect the expression of CADM1 protein expression and similar results were obtained (**Figure 1C**). IHC results also showed that the expression of CADM1 in HCC was significantly decreased (**Figure 1D**). These results indicate that CADM1 expression is correlated with HCC.

miR-194 suppresses CADM1 expression via targeting the 3'-untranslated region (UTR) of CADM1

Using TargetScan, it was found that 3'UTR base site of CADM1 formed complementary binding with miR-185, miR-142, miR-182, miR-194,

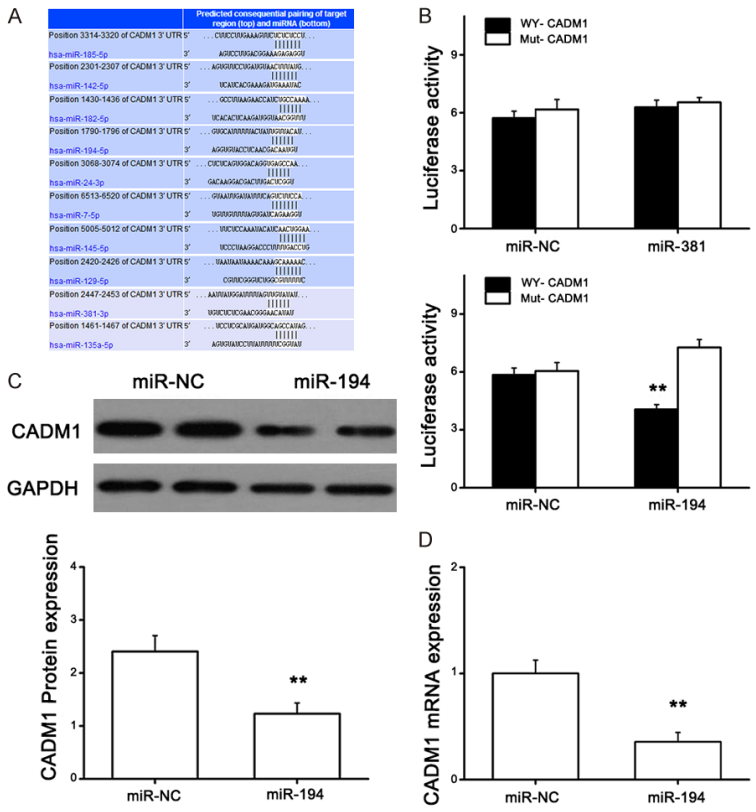

Figure 2. MiR-194 regulates CADM1 expression by binding to the 3'-UTR of CADM1. A. TargetScan was used to predict the interaction between 10 miRNAs and target gene CADM1. B. Luciferase activity was detected by dual luciferase assays; C. CADM1 proteins were determined by western blotting. D. The expression of CADM1 mRNA was detected by qRT-PCR. **P<0.01.

miR-24, miR-7, miR-145, miR-129, miR-381 and miR-135a (**Figure 2A**). Until now, miR-381 and miR-194 have not been reported. We mutated the miR-194 binding sites in the WT-CADM1 (wild-type) reporter to generate a mutant Mut-CADM1 reporter. The WT-CADM1 or Mut-CADM1 reporter and miR-194 mimics or miR-NC were co-transfected into MHCC97H cells. The results of dual luciferase assays showed that miR-194 significantly inhibited the luciferase activity of pMIR-CADM1-WT, but had no effect on the luciferase activity of pMIR-CADM1-Mut. However, using the above method, we found that miR-381 could not bind to the 3'-UTR of CADM1 (**Figure 2B**). To further investigate the regulation of miR-194 on CADM1 expression, we detected the expression levels of CADM1 protein by western blot. The results showed that miR-194 significantly reduced CADM1 protein expression (**Figure 2C**). Meanwhile, the qRT-PCR results showed the high expression of miR-194 down regulated the CADM1 mRNA expression (**Figure 2D**). These results suggest that miR-194 may inhibit the CADM1 protein expression by inhibiting the mRNA translation of CADM1.

Expression of miR-194 in HCC cell lines and tissue

qRT-PCR was employed to detect miR-194 expression in HCC cell lines (SMCC7721, MHCC97H, LM3, Hep3B) and the human liver epithelial cells, THLE-3, and the results showed that the expression level of miR-194 in each HCC cell line was significantly higher than that in THLE-3 (**Figure 3A**). In addition, miR-194 expression was significantly up-regulated in HCC tumor tissues when compared with adjacent normal tissues (**Figure 3B**). Transwell invasion assay was used to detect the invasion ability of SMCC7721 and MHCC97H cells. The results showed that the invasion ability of MHCC97H cells was significantly stronger than that of SMCC7721 cells (**Figure 3C**). Therefore, MHCC97H cells were selected as the HCC cell model for high invasion ability in this study, while SMCC7721 cells were selected for low invasion ability. The miR-194 mimics were transfected into MHCC97H cells and SMCC7721 cells, respectively, to enhance the expression of endogenous miR-194 (**Figure 3D**), and the invasion abilities of both of the two HCC cells lines were improved by the high expression of miR-194 (**Figure 3E**). These results indicate that the abnormal expression of miR-194 may be relate to HCC, and its overexpression may promote the invasion ability of HCC cells.

Effects of miR-194 and CADM1 on the proliferation, migration, and cell cycle progression of HCC cells

To assess the impact of miR-194 and CADM1 on HCC cells, miR-NC, miR-194 mimics, or CADM1 were transfected into SMCC7721 with low invasion ability. Cell proliferation ability of each group was detected by CCK8 assay, and

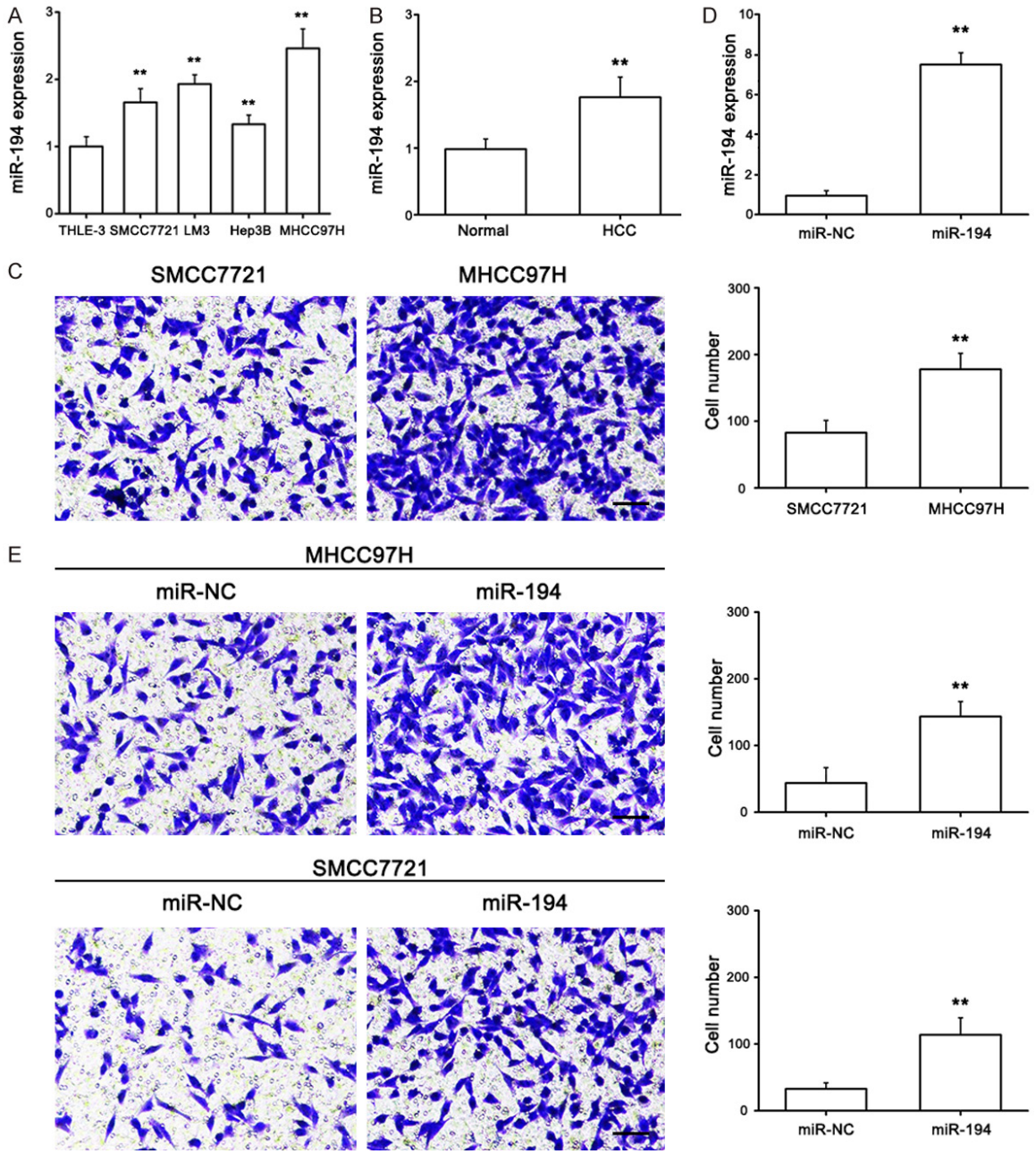

Figure 3. MiR-194 is differentially expressed in HCC cells and in tumor tissues. A. qRT-PCR assay was used to detect the expression of miR-194 in HCC cell lines (SMCC7721, MHCC97H, LM3, Hep3B) and normal human hepatocytes (THLE-3). B. qRT-PCR was used to measure the expression level of miR-194 in HCC tumor tissues and adjacent normal tissues. C. Transwell invasion assay (scale bar =25 um); D. The expression of miR-194 in cells transfected with miR-194 mimics or miR-NC was determined by qRT-PCR. E. Transwell invasion assay was used to detect the effect of changing the expression of endogenous miR-194 on the invasion ability of two HCC cell lines with different invasion abilities (scale bar =25 um). **P<0.01.

the results showed that the cells transfected with miR-194 mimics exhibited increased cell proliferation when compared with the control, while transfection with miR-194 mimics and CADM1 revealed significantly attenuated cell proliferation (**Figure 4A**). Flow cytometry assay showed that S-phase fraction was significantly higher in cells transfected with miR-194 mimics when compared with the control, Conversely, treatment with miR-194 mimics and CADM1 showed dramatically decreased the S-phase fraction (**Figure 4B**). Transwell assay showed

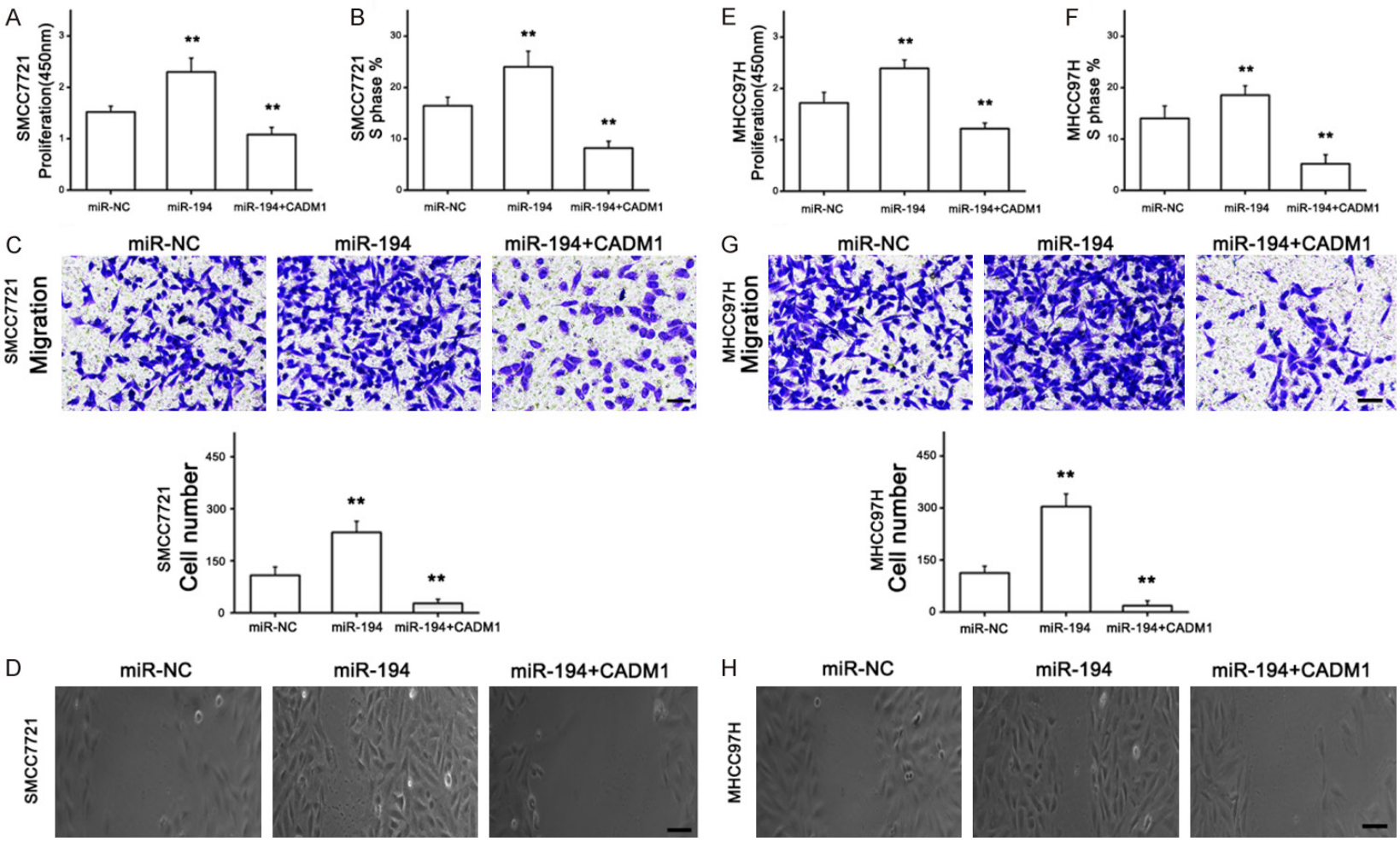

Figure 4. Effects of miR-194 and CADM1 on the proliferation, migration, and cell cycle progression of HCC cells. A. CCK8 assay was used to measure SMCC7721 cell proliferation. B. The S-phase cells of SMCC7721 were determined by flow cytometry. C. Transwell migration assay was used to measure the migration ability of MCC7721 cells (scale bar =25 um). D. The migration ability of SMCC7721 cells was detected by wound healing assay (scale bar =50 um). E. CCK8 assay was used to measure MHCC97H cell proliferation. F. The S-phase cells of MHCC97H were determined by flow cytometry. G. Transwell migration assay was used to measure the migration ability of MHCC97H cells (scale bar =25 um). H. The migration ability of MHCC97H cells was detected by wound healing assay (scale bar =50 um). **P<0.01.

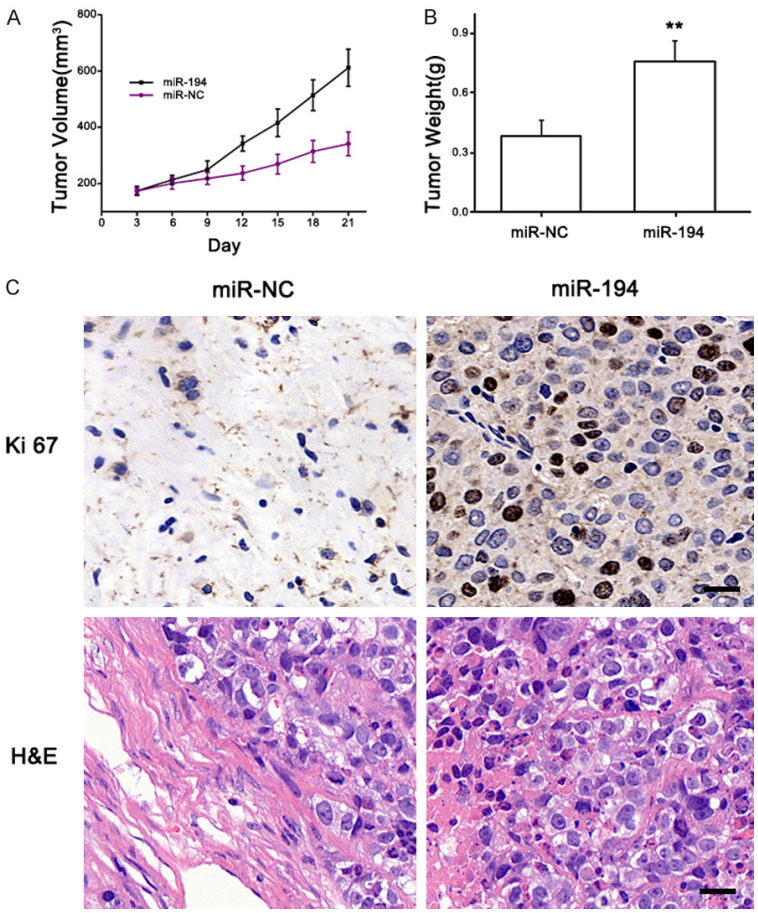

Figure 5. Effect of miR-194 in xenograft tumor model of HCC in nude mice. A. Tumor volume. B. Tumor weight. C. Ki67 protein expression and H&E staining (scale bar =50 um). **P<0.01.

that in the miR-194 mimics group the numbers of migrated cells were greater when compared with the controls, whereas miR-194 mimics plus CADM1 transfected group showed an inverse effect (**Figure 4C**). The wound healing assays also demonstrated that overexpression of miR-194 significantly promoted the cell migration, while overexpression of CADM1 could reverse this effect (**Figure 4D**). The above experiment was repeated with MHCC97H cells with high invasion ability. Results showed that mir-194 promoted the proliferation, migration, and S-phase arrest of MHCC97H cells, but this promotion effect was inhibited by CADM1 (**Figure 4E-H**). These results suggest that miR-194 may promote malignant biologicbehavior of HCC cells by inhibiting CADM1.

Effect of miR-194 in HCC xenograft tumor model in nude mice

MHCC97H cells were used to construct the HCC xenograft tumor model in nude mice, The cells transfected with miR-194 mimics or miR-NC were injected subcutaneously into the right forearm subcutaneous to the axilla of nude mice and tumor growth was monitored. After 21 days, the results showed that the tumor volume and weight of miR-194 mimics transfected group were significantly higher than that of NC control group (**Figure 5A**, **5B**). In addition, the results of Ki67 protein expression and H&E staining showed that, compared with NC control group, overexpression of miR-194 mimics significantly promoted cell proliferation *in vivo* (**Figure 5C**). These results suggest that miR-194 may play a tumor-promoting action in HCC xenograft tumor model.

Discussion

The initial steps of tumorigenesis are dysfunction of cell adhesion molecules, loss of intracellular adhesion molecules, and abscission of basal membrane cells [16]. These factors change the extracellular matrix and ultimately lead to tumor metastasis and invasion. Cell adhesion molecule 1 (CADM1), is a member of the immunoglobulin superfamily and is identified as a tumor suppressor gene in human non-small-cell carcinoma through functional supplementary assay. At present, a series of studies on CADM1 and various tumors including non-small-cell carcinoma [17], colorectal cancer [18], HCC [19], neuroblastoma [20], gastric cancer [21], pancreatic cancer [22], and breast cancer [23], have been conducted. In this study, we found that mRNA expression levels of CADM1 in HCC cells (SMCC7721, MHCC97H, LM3, Hep3B) were significantly lower than that in human liver epithelial cells, THLE-3, and mRNA and protein expression levels of CADM1 in HCC tumor tissues were significantly lower than that in adjacent normal tissues. Moreover, IHC analysis results also demonstrated under-expression of CADM1 in tumor tissue of HCC patients. It was demonstrated that the abnormal expression of

CADM1 may be closely related to the occurrence and development of HCC.

The regulatory effect of miRNA on genes depends on complementary base pairing. Mature miRNAs generally bind to the 3'UTR of target genes [24-26], reducing the expression of the target gene [27]. TargetScan was applied to screening in this study, and results show CADM1 was the potential target gene of miR-185, miR-142, miR-182, miR-194, miR-24, miR-7, miR-145, miR-129, miR-381 and miR-135a. However, to date, miR-381 and miR-194 were not reported. We found that miR-194 indeed bind to the 3'-UTR region of CADM1 in a complementary manner using dual luciferase assays. Further studies conclusively showed that high expression level of miR-194 inhibited mRNA and protein expression of CADM1. These results suggest that miR-194 has a targeted regulatory effect on CADM1 expression.

A series of miRNA are highly conserved among species and participate in various biological processes, such as proliferation, migration, apoptosis, differentiation and cell cycle progression [28, 29]. Mounting evidence indicated that several miRNAs are implicated in the development of certain cancers. For instance, miR-21 has been shown to promote the invasion and metastasis of various tumors, such as breast cancer [30], colorectal cancer [31], glioma [32] and pancreatic cancer [33]. Yang, et al. reported that miR-34 inhibited invasion and migration of breast cancer by targeting Fra-1 [34]. Lin, et al. recently found that miR-135b promoted lung cancer metastasis by regulating Hippo and LZTS1 [35]. In this study, we detected the expression level of miR-194 in HCC cell lines and human liver epithelial cells, THLE-3, and found that the expression level of miR-194 in each HCC cell was significantly higher than in human liver epithelial cellsl. In addition, the expression level of miRNA-194 in HCC tissues were increased compared with the adjacent tissues. These results all indicate that the abnormal expression of miR-194 may closely relate to HCC.

Transwell invasion assay is a cell invasion ability detection assay which uses polycarbonate membranes to separate upper and lower growth mediums in transwell chamber, and lays a layer of matrigel on the upper chamber of the membrane [36]. Transwell invasion assay results in this study demonstrated that the invasion ability of MHCC97H was obviously better than that of SMCC7721. Therefore, MHCC97H was selected as the hepatoma model for high invasion and migration ability, while SMCC-7721 was selected as hepatoma model for low invasion and migration ability; then we found that after miR-194 mimics were transfected into MHCC97H and SMCC7721, the invasion ability of the two HCC cell lines was significantly enhanced, suggesting that overexpression of miR-194 may improve the invasive ability of HCC cells.

The relation between tumor invasion and metastasis and miRNAhas been the focus of studies in recent years [37, 38]. Invasion and migration are important tumor characteristics, and they affect the final prognosis of tumors [39, 40]. To investigate the effect of miR-194 on the biologic behavior of HCC cells. MiR-NC, miR-194 mimics, and CADM1 were transfected into SMCC7721/MHCC97H cells with low/high invasion ability. A series of assays showed that up-regulated miR-194 expression promoted the proliferation, invasion, migration and S-phase arrest of SMMC772/MHCC97H cells, but overexpression of CADM1 reversed this promoting effect. These results suggest that miR-194 may promote the malignant biologic behavior of HCC cells by targeting CADM1. Furthermore, to investigate the effect of miR-194 on HCC *in vivo*, a HCC xenograft tumor model was established in nude mice. The results showed that the up-regulated expression of miR-194 promoted the proliferation of HCC *in vivo*, in addition, the tumor volume and weight were significantly increased. These findings confirm that miR-194 promoted the growth of HCC tumors *in vivo.*

Conclusions

In conclusion, miR-194 has an important regulatory role with respect to CADM1. miR-194 promoted the proliferation, invasion, migration, and S-phase arrest of HCC cells by inhibiting CADM1. Therefore, miR-194 in this study may become a new target for HCC diagnosis and therapy.

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Disclosure of conflict of interest

None.

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References

[1] Giordano S and Columbano A. Met as a therapeutic target in HCC: facts and hopes. J Hepatol 2014; 60: 442-452.

[2] Qin S, Kruger E, Tan SC, Cheng S, Wang N and Liang J. Cost-effectiveness analysis of FOLFOX4 and sorafenib for the treatment of advanced hepatocellular carcinoma in China. Cost Eff Resour Alloc 2018; 16: 29.

[3] Reig M, Mariño Z, Perelló C, Iñarrairaegui M, Ribeiro A, Lens S, Díaz A, Vilana R, Darnell A, Varela M, Sangro B, Calleja JL, Forns X and Bruix J. Unexpected high rate of early tumor recurrence in patients with HCV-related HCC undergoing interferon-free therapy. J Hepatol 2016; 65: 719-726.

[4] Kew MC. Hepatocellular carcinoma in developing countries: prevention, diagnosis and treatment. World J Hepatol 2012; 4: 99-104.

[5] Wikman H, Westphal L, Schmid F, Pollari S, Kropidlowski J, Sielaff-Frimpong B, Glatzel M, Matschke J, Westphal M, Iljin K, Huhtala H, Terracciano L, Kallioniemi A, Sauter G, Müller V, Witzel I, Lamszus K, Kemming D and Pantel K. Loss of CADM1 expression is associated with poor prognosis and brain metastasis in breast cancer patients. Oncotarget 2014; 5: 3076-87.

[6] Yao J, Chen Y, Wang Y, Liu S, Yuan X, Pan F and Geng P. Decreased expression of a novel lncRNA CADM1-AS1 is associated with poor prognosis in patients with clear cell renal cell carcinomas. Int J Clin Exp Pathol 2014; 7: 2758-67.

[7] Hartsough EJ, Weiss MB, Heilman SA, Purwin TJ, Kugel CH 3rd, Rosenbaum SR, Erkes DA, Tiago M, HooKim K, Chervoneva I and Aplin AE. CADM1 is a TWIST1-regulated suppressor of invasion and survival. Cell Death Dis 2019; 10: 281.

[8] Saito M, Goto A, Abe N, Saito K, Maeda D, Ohtake T, Murakami Y and Takenoshita S. Decreased expression of CADM1 and CADM4 are associated with advanced stage breast cancer. Oncol Lett 2018; 15: 2401-2406.

[9] Ji Q, Zheng GY, Xia W, Chen JY, Meng XY, Zhang H, Rahman K and Xin HL. Inhibition of invasion and metastasis of human liver cancer HCCLM3 cells by portulacerebroside A. Pharm Biol 2015; 53: 773-80.

[10] Sun M, Ding J, Li D, Yang G, Cheng Z and Zhu Q. NUDT21 regulates 3'-UTR length and microRNA-mediated gene silencing in hepatocellular carcinoma. Cancer Lett 2017; 410: 158-168.

[11] Li T, Huang H, Liao D, Ling H, Su B and Cai M. Genetic polymorphism in HLA-G 3'UTR 14-bp ins/del and risk of cancer: a meta-analysis of case-control study. Mol Genet Genomics 2015; 290: 1235-1245.

[12] Macedo GS, Araujo Vieira I, Brandalize AP, Giacomazzi J, Inez Palmero E, Volc S, Rodrigues Paixão-Côrtes V, Caleffi M, Silva Alves M, Achatz MI, Hainaut P and Ashton-Prolla P. Rare germline variant (rs78378222) in the TP53 3'UTR: evidence for a new mechanism of cancer predisposition in Li-Fraumeni syndrome. Cancer Genet 2016; 209: 97-106.

[13] Zhang S, Xue J, Zheng J, Wang S, Zhou J, Jiao Y, Geng Y, Wu J, Hannafon BN and Ding WQ. The superoxide dismutase 1 3'UTR maintains high expression of the SOD1 gene in cancer cells: the involvement of the RNA-binding protein AUF-1. Free Radic Biol Med 2015; 85: 33-44.

[14] Peng Y, Zhang X, Lin H, Deng S, Huang Y, Qin Y, Feng X, Yan R, Zhao Y, Cheng Y, Wei Y, Wang J, Chen W, Fan X, Ashktorab H, Smoot D, Meltzer SJ, Li S, Zhang Z and Jin Z. Inhibition of miR194 suppresses the Wnt/βcatenin signalling pathway in gastric cancer. Oncol Rep 2018; 40: 3323-3334.

[15] Tu C, Wang F and Wan J. MicroRNA-381 inhibits cell proliferation and invasion in endometrial carcinoma by targeting the IGF-1R. Mol Med Rep 2018; 17: 4090-4098.

[16] Zahnd C, Kawe M, Stumpp MT, de Pasquale C, Tamaskovic R, Nagy-Davidescu G, Dreier B, Schibli R, Binz HK, Waibel R and Plückthun A. Efficient tumor targeting with high-affinity designed ankyrin repeat proteins: effects of affinity and molecular size. Cancer Res 2010; 70: 1595-605.

[17] Kikuchi S, Iwai M, Sakurai-Yageta M, Tsuboi Y, Ito T, Maruyama T, Tsuda H, Kanai Y, Onizuka M, Sato Y and Murakami Y. Expression of a splicing variant of the CADM1 specific to small cell lung cancer. Cancer Sci 2012; 103: 1051-1057.

[18] Chen K, Wang G, Peng L, Liu S, Fu X, Zhou Y, Yu H, Li A, Li J, Zhang S, Bai Y and Zhang Y. CADM1/TSLC1 inactivation by promoter hypermethylation is a frequent event in colorectal carcinogenesis and correlates with late stages of the disease. Int J Cancer 2015; 128: 266-273.

[19] Zhang W, Xie HY, Ding SM, Xing CY, Chen A, Lai MC, Zhou L and Zheng SS. CADM1 regulates the G1/S transition and represses tumorigenicity through the Rb-E2F pathway in hepatocellular carcinoma. Hepatobiliary Pancreat Dis Int 2016; 15: 289-296.
[20] Michels E, Hoebeeck J, De Preter K, Schramm A, Brichard B, De Paepe A, Eggert A, Laureys G, Vandesompele J and Speleman F. CADM1 is a strong neuroblastoma candidate gene that maps within a 3.72 Mb critical region of loss on 11q23. BMC Cancer 2008; 8: 173.
[21] Yang Z, Wang R, Zhang T and Dong X. MicroRNA-126 regulates migration and invasion of gastric cancer by targeting CADM1. Int J Clin Exp Pathol 2015; 8: 8869-80.
[22] Wang HL, Zhou R, Liu J, Chang Y, Liu S, Wang XB, Huang MF and Zhao Q. MicroRNA-196b inhibits late apoptosis of pancreatic cancer cells by targeting CADM1. Sci Rep 2017; 7: 11467.
[23] Takahashi Y, Iwai M, Kawai T, Arakawa A, Ito T, Sakurai-Yageta M, Ito A, Goto A, Saito M, Kasumi F and Murakami Y. Aberrant expression of tumor suppressors CADM1 and 4.1B in invasive lesions of primary breast cancer. Breast Cancer 2012; 19: 242-252.
[24] Lu Y, Yue X, Cui Y, Zhang J and Wang KW. MicroRNA-124 suppresses growth of human hepatocellular carcinoma by targeting STAT3. Biochem Biophys Res Commun 2013; 441: 873-879.
[25] Landi D, Gemignani F and Landi S. Role of variations within microRNA-binding sites in cancer. Mutagenesis 2012; 27: 205-10.
[26] Ouellet DL, Perron MP, Gobeil LA, Plante P and Provost P. MicroRNAs in gene regulation: when the smallest governs it all. J Biomed Biotechnol 2006; 2006: 69616.
[27] Xiao Y, Guan J, Ping Y, Xu C, Huang T, Zhao H, Fan H, Li Y, Lv Y, Zhao T, Dong Y, Ren H and Li X. Prioritizing cancer-related key miRNA-target interactions by integrative genomics. Nucleic Acids Res 2012; 40: 7653-65.
[28] Lujambio A, Calin GA, Villanueva A, Ropero S, Sánchez-Céspedes M, Blanco D, Montuenga LM, Rossi S, Nicoloso MS, Faller WJ, Gallagher WM, Eccles SA, Croce CM and Esteller M. A microRNA DNA methylation signature for human cancer metastasis. Proc Natl Acad Sci U S A 2008; 105: 13556-13561.
[29] Huang XH, Chen JS, Wang Q, Chen XL, Wen L, Chen LZ, Bi J, Zhang LJ, Su Q and Zeng WT. miR-338-3p suppresses invasion of liver cancer cell by targeting smoothened. J Pathol 2011; 225: 463-472.
[30] Savad S, Mehdipour P, Miryounesi M, Shirkoohi R, Fereidooni F, Mansouri F and Modarressi MH. Expression analysis of MiR-21, MiR-205, and MiR-342 in breast cancer in Iran. Asian Pac J Cancer Prev 2012; 13: 873-7.
[31] Schee K, Boye K, Abrahamsen TW, Fodstad Ø and Flatmark K. Clinical relevance of microRNA miR-21, miR-31, miR-92a, miR-101, miR-106a and miR-145 in colorectal cancer. BMC Cancer 2012; 12: 505.
[32] Ren Y, Kang CS, Yuan XB, Zhou X, Xu P, Han L, Wang GX, Jia Z, Zhong Y, Yu S, Sheng J and Pu PY. Co-delivery of as-miR-21 and 5-FU by poly (amidoamine) dendrimer attenuates human glioma cell growth in vitro. J Biomater Sci Polym Ed 2010; 21: 303-314.
[33] Ali S, Ahmad A, Banerjee S, Padhye S, Dominiak K, Schaffert JM, Wang Z, Philip PA and Sarkar FH. Gemcitabine sensitivity can be induced in pancreatic cancer cells through modulation of miR200 and miR21 expression by curcumin or its analogue CDF. Cancer Res 2010; 70: 3606-3617.
[34] Yang S, Li Y, Gao J, Zhang T, Li S, Luo A, Chen H, Ding F, Wang X and Liu Z. MicroRNA-34 suppresses breast cancer invasion and metastasis by directly targeting Fra-1. Oncogene 2013; 32: 4294-4303.
[35] Lin CW, Chang YL, Chang YC, Lin JC, Chen CC, Pan SH, Wu CT, Chen HY, Yang SC, Hong TM and Yang PC. MicroRNA-135b promotes lung cancer metastasis by regulating multiple targets in the Hippo pathway and LZTS1. Nat Commun 2013; 4: 1877.
[36] Deng Q, Zhang Z, Feng X, Li T, Liu N, Lai J, Shuai L, Xiong Q, Fu C, Zou H, Wang Y, Li X, Ma K and Bie P. TRAIL-secreting mesenchymal stem cells promote apoptosis in heat-shock-treated liver cancer cells and inhibit tumor growth in nude mice. J Hepatol 2014; 60: S92.
[37] Nelson PT, Kiriakidou M, Mourelatos Z, Tan GS, Jennings MH, Xie K and Wang WX. High-throughput experimental studies to identify miRNA targets directly, with special focus on the mammalian brain. Brain Res 2010; 1338: 122-130.
[38] Sempere LF. Tissue slide-based microRNA characterization of tumors: how detailed could diagnosis become for cancer medicine. Expert Rev Mol Diagn 2014; 14: 853-869.
[39] Coulouarn C, Factor VM, Andersen JB, Durkin ME and Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene 2009; 28: 3526-3536.
[40] Meng Z, Fu X, Chen X, Zeng S, Tian Y, Jove R, Xu R and Huang W. miR-194 is a marker of hepatic epithelial cells and suppresses metastasis of liver cancer cells in mice. Hepatology 2010; 52: 2148-2157.