Oncology Research, Vol. 27, pp. 1025–1034
Printed in the USA.

0965-0407/19 $90.00 + .00
DOI: https://doi.org/10.3727/096504018X15456687424096
E-ISSN 1555-3906
www.cognizantcommunication.com

High Blood miR-802 Is Associated With Poor Prognosis in HCC Patients by Regulating DNA Damage Response 1 (REDD1)-Mediated Function of T Cells

Chao Jiang,* Xueyan Liu,† Meng Wang,* Guoyue Lv,* and Guangyi Wang*

*Department of Hepatobiliary Pancreatic Surgery, First Hospital of Jilin University, Changchun, P.R. China
†Department of Cardiology, China–Japan Union Hospital of Jilin University, Changchun, P.R. China

miR-802 has been reported to be dysregulated in multiple tumors and contribute to tumor progression. However, its role in HCC was still largely unknown. The aim of this study is to investigate the function and mechanism of miR-802 in HCC progression. The results showed that miR-802 was upregulated in the peripheral blood and tumor tissue of HCC patients, and high levels of blood miR-802 predicted poor prognosis. miR-802 had no effect on the proliferation and migration of HCC cell lines. Interestingly, the levels of CD8/CD28 and regulated in development and DNA damage response 1 (REDD1) were declined along with the upregulation of miR-802 in vivo. Hence, it is speculated that miR-802 participated in the regulation of T-cell function in HCC patients. Furthermore, we demonstrated that mir-802 directly targets REDD1 and inhibited its expression. miR-802 increased the expression of programmed cell death protein 1 (PD-1) and decreased the expression of interferon- (IFN-) and $CD8^+CD28^+$ T-cell number. In conclusion, miR-802 was involved in T-cell exhaustion through posttranscriptionally suppressing REDD1, which might offer the suppressive effect of miR-802 on HCC progression.

Key words: Hepatocellular carcinoma (HCC); REDD1; Programmed cell death protein 1 (PD-1); miR-802; T-cell exhaustion

INTRODUCTION

Hepatocellular carcinoma (HCC) is the major form of liver cancer, of which over 700,000 new cases are reported every year[1]. It has been regarded as the third leading cause of cancer-related death globally[2]. Although progressive diagnostics and comprehensive therapies have been applied in clinic treatments, HCC patients still have poor prognosis due to its high rate of metastasis and relapse[3].

Immune T lymphocytes play a critical role in the initiation and progression of HCC[4]. T cells are activated mainly through two independent signaling pathways. The first requires recognition of the antigen-bearing major histocompatibility complex (MHC) by the T-cell receptor (TCR) that is on the surface of antigen-presenting cells[5]. The second is delivered by costimulatory molecules. Positive costimulatory signals are mainly mediated by the CD28 receptor expressed on naive T cells, responsible for T-cell proliferation and cell survival[6]. Negative costimulatory signals, such as programmed cell death protein 1 (PD-1), negatively regulate T-cell receptor TCR signals and contribute to T-cell dysfunction[5]. PD-1 is a cell surface receptor expressed on T cells, which could induce immune inhibition in HCC[4]. In tumors, T cells become progressively "exhausted" due to the persistence of antigen and cancer[7]. At the same time, tumors trigger the immune tolerance leading to the production of tolerant T cells. Hence, tumor cells can escape from T-cell recognition and escape control by the immune system. Therefore, it is expected that restoring T-cell vitality and enhancing immunocompetence of cancer patients will bring breakthrough for future cancer treatment.

MicroRNAs (miRNAs) are a group of short noncoding single-strand RNAs with lengths of 18–24 nt. They regulate translation of genes through binding to the 3 -UTR of the target mRNAs. Numerous reports have revealed that the dysregulated miRNAs (e.g., mir-1246, mir-128-2, and mir-892a) are correlated with the occurrence, development, and prognosis of HCC[1,8–10]. It has been reported that miR-802 is downregulated in breast and prostate cancer tissues, inhibiting cell proliferation and promoting cell apoptosis[11,12]. Indeed, miR-802 is distinctly enriched in the liver, and its expression was important in the regulation of glucose and lipid metabolism and xenobiotic

Address correspondence to Guangyi Wang, Ph.D., Department of Hepatobiliary Pancreatic Surgery, First Hospital of Jilin University, 71 Xinmin Street, Changchun 130021, P.R. China. Tel: 86-431-88782222; E-mail: zhuoruw18@sina.com

response[13–15]. However, little is understood about the biological function of miR-802 in human HCC progression.

In the present study, we found that miR-802 was upregulated in peripheral blood and tumor of HCC patients and participated in the progression of HCC tumor. In addition, high levels of blood miR-802 predicted poor outcomes of HCC patients. It is confusing to us that miR-802 has no effect on the proliferation and migration of HCC cancer cells. Interestingly, the expression of CD8, CD28, and regulated in development and DNA damage response 1 (REDD1) was declined along with the upregulation of miR-802. Hence, based on the speculation that miR-802 participates in the regulation of T lymphocytes in HCC patients, the role of miR-802 in regulating T-cell function was explored in HCC patients.

MATERIALS AND METHODS

Patient and Sample Collection

One hundred and seventy-two patients with HCC who underwent resection of their tumors without preoperative chemotherapy, hormone therapy, or radiotherapy at the Department of Hepatobiliary Pancreatic Surgery, First Hospital of Jilin University, between 2012 and 2015, were recruited for the study after giving informed consent. A complete medical history was obtained, and tumor assessment was performed at baseline. The protocol of the study was approved by the Institutional Ethics Committee of First Hospital of Jilin University, P.R. China. In addition, 60 healthy persons were included in this study. After surgery, all the patients were reviewed every 3 months during the first year, every 2 months during the subsequent 2–5 years, and once per year thereafter until death or data censored.

Peripheral blood samples were acquired from 172 HCC patients and 60 healthy donors after written informed consent. Tumor tissues and adjacent tissues were gathered from 48 HCC patients.

Cell Lines

Human hepatocellular carcinoma cell lines (SMMC-7721, MHCC97, Bel-7402, Huh7, and Hep3B cells), normal hepatocytes (HL-7702 and THLE-3 cells) and HEK 293T cell lines were obtained from Shanghai Cell Collection, Chinese Academy of Sciences. HL-7702, THLE-3 cells, SMMC-7721, MHCC97, and Bel-7402 cells were cultured in RPMI-1640 medium (Gibco, Grand Island, NY, USA). Huh7, Hep3B, and HEK 293T cells were cultured in DMEM medium with high glucose (Gibco). All culture medium was supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were cultured in a humidified incubator at 37°C with 5% CO_2.

RNA Extraction

Total RNA was isolated from the fresh whole blood of donors using Stabilized Blood-To-CT™ Nucleic Acid Preparation Kit according to the manufacturer's instructions (Thermo Fisher Scientific, Waltham, MA, USA). Tumor tissue and adjacent tissue samples were cut into small fragments and digested with pancreatic enzymes. Tissue total RNA was isolated using a TRIzol™ plus RNA purification kit according to the manufacturer's instructions (Thermo Fisher Scientific).

Real-Time Quantitative PCR

Expression level of miR-802 was assayed using a Taqman MicroRNA assay (Thermo Fisher Scientific) according to the manufacturer's instructions. RT-qPCR was performed using a 7900 Real-Time PCR system. Transcript levels for miR-802 were normalized to GADPH cDNA level.

Cell Sorting

Peripheral blood mononuclear cells were isolated from fresh whole blood of healthy donors using lymphocyte separation medium by density gradient centrifugation. $CD8^+$ T cells were selected using a MagCellect Human $CD8^+$ T Cell Isolation Kit according to the manufacturer's instructions (R&D Systems, Minneapolis, MN, USA). Then $CD8^+CD28^+$ T cells were selected using human CD28 MicroBead Kit (Miltenyi Biotech, Auburn, CA, USA). The isolated $CD8^+$ and $CD8^+CD28^+$ T cells were cultured in RPMI-1640 medium containing 10% FBS and 2 mM L-glutamine.

Construction and Transduction of the miR-802 Mimic

The miR-802 mimic was purchased from Thermo Fisher Scientific. Lipofectamine™ 3000 (Invitrogen, Carlsbad, CA, USA) was used to transfect miR-802 mimics into cells. Following transfection with miR-802 mimics or control mimic, endogenous miR-802 level, cell proliferation and invasion were assessed.

Luciferase Assay

The potential targets of miR-802 were analyzed by TargetScan (www.targetscan.org/vert_72/). Wild-type and mutant 3 -UTR fragments of REDD1 gene were cloned into pGL3 luciferase reporter vector (Promega, Madison, WI, USA), yielding pGL3-Report-REDD1. For luciferase assays, HEK 293T cells (2×10^4 cells/ml) were seeded into six-well plates and cotransfected with reporter plasmids and miR-802 mimics after 16 h of culture using Lipofectamine™ 3000. Dual Luciferase Assay Kit (Promega) was adopted to measure the luciferase activity after 48 h of transfection.

CCK-8 Assay

Hep3B and Huh-7 cell lines (2×10^3 cells/well) were planted into each well of 96-well microplates after transfection with control mimic or miR-802 mimics (50 nM and 100 nM). The cell viability was measured using Cell Counting Kit-8 (CCK-8) assay according to the manufacturer's instructions (KJ800; Dojindo Laboratories, Kumamoto, Japan). The optical density was read on a 96-well microplate reader at 450 nm.

Transwell Assay

Cells invasion activity was examined by Transwell assay. Hep3B and Huh-7 cell lines were withdrawn of FBS for 24 h for homogenization. Then the cells (2×10^4 cells/ml) were seeded into the upper chambers of the 24-well Transwell plate with 8-μm pore polycarbonate membrane (Corning, New York, NY, USA). The lower chamber was coated with Matrigel, and DMEM medium containing 10% FBS was added. Following incubation for 24 h, the cells that invaded the lower surface were fixed with 100% methanol and stained with 0.05% crystal violet. The stained cells were quantified by counting five randomly selected fields per filter under a microscope.

Enzyme-Linked Immunosorbent Assay (ELISA)

The level of interferon- (IFN-) in cells was measured by ELISA kits (Hushang Biological Technology Co., Ltd., Shanghai, P.R. China) according to the manufacturer's instructions. Samples were centrifuged, and the supernatant culture medium was collected for analysis.

Western Blotting

The expression levels of REDD1 and programmed cell death 1 (PD-1) were detected using Western blot analysis as previously described[12]. The following antibodies were used: anti- -actin, anti-REDD1, and anti-PD-1 (Cell Signaling Technology, Boston, MA, USA).

Data Analysis

Quantitative data were expressed as mean ± SD. Student's *t*-test, chi-square tests, and one-way ANOVA were used for comparison of mean between the groups. Statistical analyses were performed using SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). The Kaplan–Meier method was applied for survival analysis, and the statistical significance between the groups was evaluated using the log-rank/Mantel–Cox test. A value of $p < 0.05$ was considered significant.

RESULTS

The Association Between Blood miR-802 Expression and Clinicopathological Features of HCC Patients

To investigate the expression pattern and clinicopathological features of miR-802 in HCC patients, a total of 172 peripheral blood samples were collected from HCC patients. They were all confirmed as eligible for this

Table 1. Association Between Blood miR-802 Expression and the Clinicopathological Features of Patients With Human Hepatocellular Carcinoma

Variables	Low miR-802	High miR-802	*p* Values
Age (years)			0.352
50	41	38	
>50	49	44	
Sex			0.531
Male	66	61	
Female	24	21	
Portal vein tumor thrombi			0.0269*
Absent	54	29	
Present	36	53	
Histopathological grading			0.00852**
Well/moderately	55	23	
Poorly	35	59	
Tumor size			0.00674**
5.0	59	22	
>5.0	31	60	
Tumor number			0.00496**
Single	74	18	
Multiple	16	64	

HCC patients were segregated into miR-802 high and low expression groups. Statistical analyses were performed with the chi-square test.
*$p < 0.05$, **$p < 0.01$.

study. The expression of miR-802 in 172 blood samples was examined by RT-qPCR. As shown in Table 1, miR-802 expression level was notably related to portal vein tumor thrombi ($p = 0.0269$), histopathological grading ($p = 0.00852$), tumor size ($p = 0.00674$), and tumor number ($p = 0.00496$), but not to age ($p = 0.352$) or sex ($p = 0.531$).

miR-802 Was Upregulated in HCC Tumor Tissue and Peripheral Blood Samples

To further understand the correlation between miR-802 and survival outcome in HCC cases, peripheral blood samples were collected from 172 HCC patients and 60 nontumor healthy persons. Tumor tissues and tumor adjacent tissues were obtained from 48 HCC patients. miR-802 expression level was remarkably increased in HCC peripheral blood samples compared with that in nontumor healthy person ($p < 0.001$) (Fig. 1A). Then the HCC patients were divided into two groups: a high expression level of blood miR-802 group ($n = 65$, fold change 4.0) and a low expression level of blood miR-802 group ($n = 50$, fold change 2.0). The average value of normal control was regarded as baseline 1. The survival curves of the two groups were determined by Kaplan–Meier survival analysis. The results showed that median survival time of the patients with high level of blood miR-802 is much shorter than those with low level of blood miR-802 ($p < 0.05$) (Fig. 1B). This result suggested that low expression of blood miR-802 indicated a favorable outcome in HCC patients.

At the same time, the miR-802 expression levels in tumor tissues and tumor adjacent tissues were measured. The result showed that miR-802 expression was upregulated in tumor tissues compared with tumor adjacent tissues ($p < 0.05$) (Fig. 2A). HCC patients were divided into two

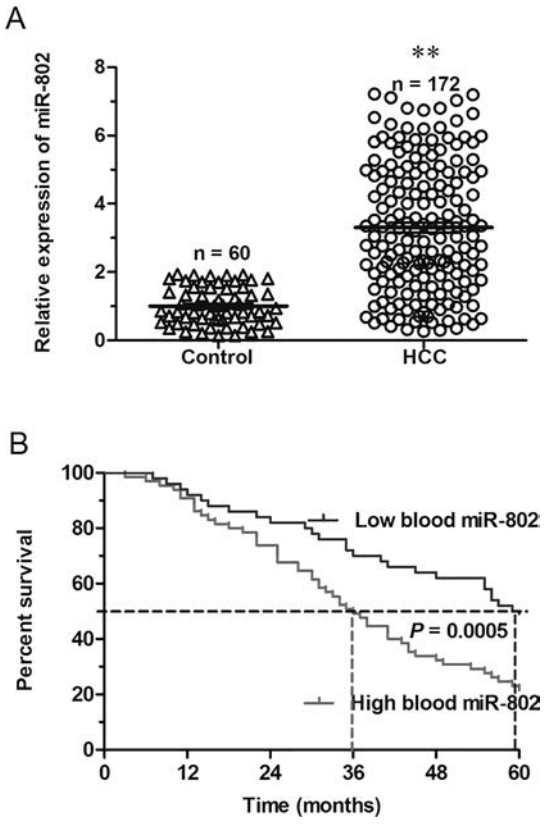

Figure 1. The association between blood miR-802 expression and survival time in hepatocellular carcinoma (HCC) patients. (A) Blood miR-802 was significantly upregulated in HCC patients ($n = 172$) compared to normal controls ($n = 60$). $^{**}p < 0.01$. (B) Survival time for 115 HCC patients who were divided into low blood miR-802 group ($n = 50$, fold change 2.0) and high blood miR-802 group ($n = 65$, fold change 4.0). The average value of normal control was regarded as baseline 1. Patients with a low level of blood miR-802 expression had significantly longer survival time ($p = 0.0005$) than patients with a high level of blood miR-802 expression.

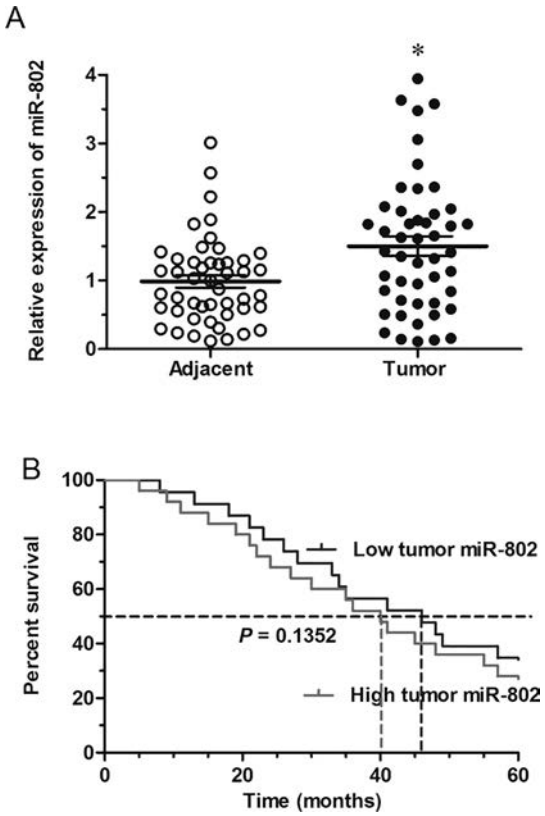

Figure 2. The association between tissue miR-802 expression and survival time in HCC patients. (A) miR-802 was upregulated in tumor tissue when compared with adjacent tissue in HCC patients ($n = 48$). $^{*}p < 0.05$. (B) Survival time for 48 HCC patients who were divided into low tumor expression miR-802 group ($n = 23$, fold change < 1.0) and high tumor expression miR-802 group ($n = 25$, fold change 1.0). The average value of miR-802 expression in 48 tumor tissues was considered as baseline 1. There is no obvious difference in the survival time between the low tumor miR-802 group and high tumor miR-802 group.

groups: a high expression of tumor miR-802 group ($n = 25$, fold change 1.0) and a low expression of tumor miR-802 group ($n = 23$, fold change < 1.0). The average value of 48 tumor tissue miR-802 expression was considered as baseline 1. However, there were no significant differences between high and low level of tumor miR-802 group at the 50% survival rate ($p = 0.1352$) (Fig. 2B). This result indicated that the expression level of miR-802 in tissue has no significant association with the prognosis of HCC patients.

miR-802 Has No Influence on the Cell Viability and Invasion Ability of Hep3B and Huh-7 Cell Lines

The expression level of miR-802 in HCC cell lines and normal hepatocytes was analyzed by qPCR. Compared with the normal hepatic HL-7702 and THLE-3 cell lines, there were no remarkable changes in the expression of miR-802 in HCC SMMC-7721, MHCC97, Bel-7402, Hep3B, and Huh7 cell lines (Fig. 3A). This indicated that the expression of miR-802 in normal hepatic cells and HCC cancer cells had no statistical difference.

To further explore the value of miR-802 in cell proliferation and invasion, the expression level of miR-802 in both Hep3B and Huh7 cell lines was detected after transfection with control mimic or different concentrations of miR-802 mimics (10 nM, 50 nM, and 100 nM). As shown in Figure 3B and E, it is obvious that the transfection of miR-802 mimics elevated the expression level of miR-802 in both Hep3B and Huh7 cell lines in a

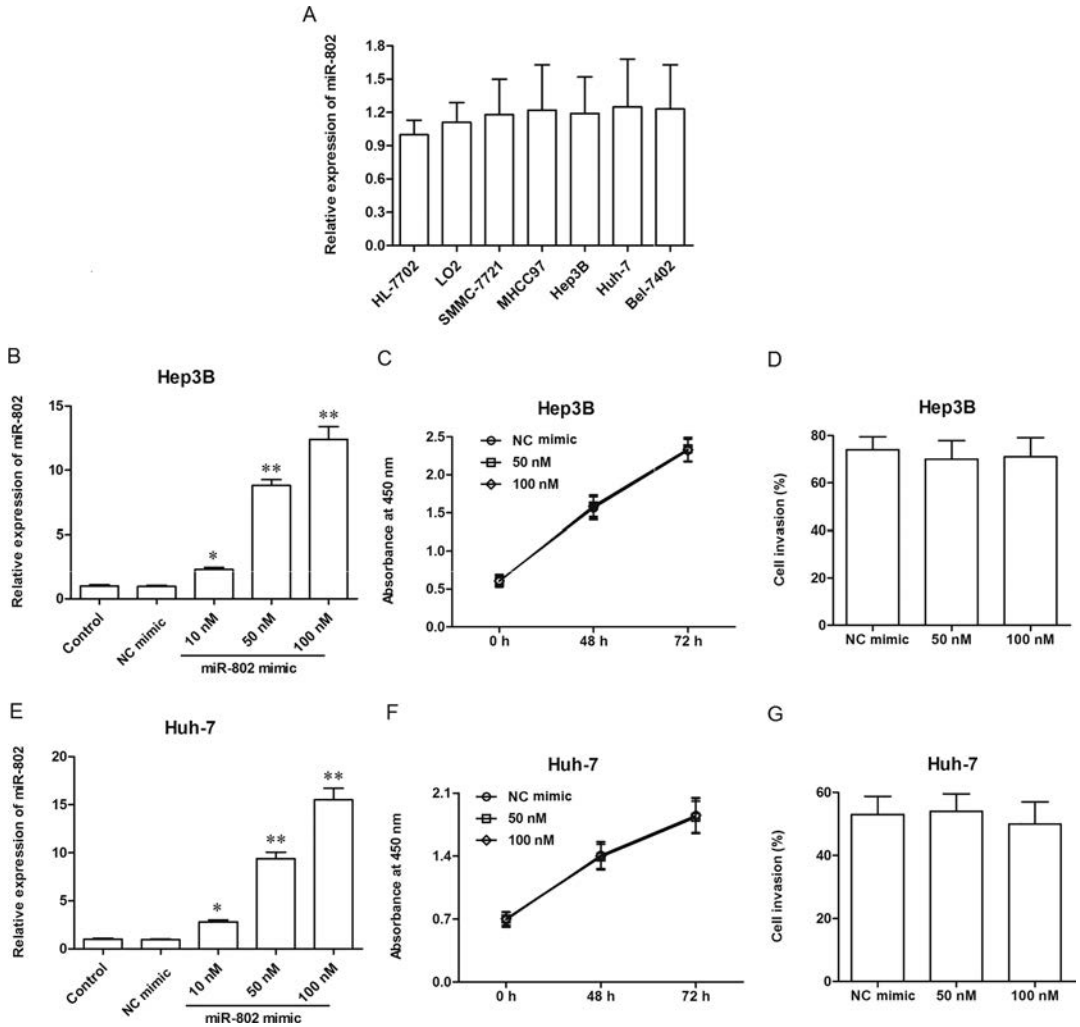

Figure 3. Expression of miR-802 in HCC cells and normal hepatocytes, and the effect of miR-802 on Hep3B and Huh-7 cell lines in cell viability and invasion ability. (A) There were no significant differences in miR-802 expression between normal hepatocytes (HL-7702 and THLE-3 cell lines) and HCC cell lines (SMMC-7721, MHCC97, Bel-7402, Hep3B, and Huh7 cell lines). The expression level of miR-802 in cells was tested by RT-qPCR. (B and E) A high expression of miR-802 was detected in Hep3B and Huh7 cell lines after the transfection with miR-802 mimics compared with transfection with NC mimics or control group. *$p < 0.05$, **$p < 0.01$, compared with the control group or NC siRNA group. (C and F) High level of miR-802 exerts no influence on the cell viability in Hep3B and Huh7 cell lines. (D and G) miR-802 has no effect on the cell invasion in Hep3B and Huh7 cell lines.

dose-dependent manner, compared with the control and control mimic group. Next, to observe the influence of miR-802 on cell viability and invasion activity, 50 nM and 100 nM of miR-802 mimics were transfected into Hep3B and Huh7 cells. It was found that there was no difference between the three groups (control mimic group, 50 nM transfected group, and 100 nM transfected group) at 0 h, 48 h, and 72 h in cell viability (Fig. 3C and F). Similarly, there was no statistical difference in cell invasion ability following transfection with miR-802 mimics (Fig. 3D and G). These results suggested that miR-802 did not affect tumor progress through regulating cell proliferation and invasion.

The Expression of CD8, CD28, and REDD1 Were Negatively Correlated With miR-802

To verify whether miR-802 participated in the regulation of T cells, the expressions of CD8, CD28, and REDD1 were examined in this study ($n = 65$). An inverse correlation was found between CD8 and miR-802 ($r_s = -0.1106$; $r^2 = 0.7454$; $p < 0.001$) in peripheral blood mononuclear cells (Fig. 4A). The same correlations were found between CD28 and miR-802 ($r_s = -0.1344$; $r^2 = 0.7700$; $p < 0.001$), REDD1 and miR-802 ($r_s = -0.2986$; $r^2 = -0.9477$; $p < 0.001$) in mononuclear cells isolated from HCC patients' blood samples (Fig. 4B and C). CD8 is a transmembrane glycoprotein, serving as a coreceptor for the TCR[16]. A cytotoxic T cell with CD8 is called $CD8^+$ T cell, and $CD8^+$ T cell is a kind of cytotoxic T cell that can recognize and kill tumor cells[17]. CD28 is a protein expressed on T cells that can provide costimulatory signals for production of various interleukins through activating T cells[18]. REDD1 is a small protein, also known as protein DNA-damage-inducible transcript 4 (DDIT4), which is encoded by the *DDIT4* gene[19]. A recent report suggested that REDD1 participated in the proliferation and survival of T cells[20].

miR-802 Directly Targets REDD1

According to the above results, we next investigated whether and how miR-802 influenced the expression of REDD1. In general, miRNAs regulate cell processes by controlling the expression of their target genes. We first predicted the target gene(s) of miR-802 by Targetscan software and found that REDD1 was a potential target gene of miR-802 (Fig. 5A). In order to prove that REDD1 is a target of miR-802, the Luc-3 -UTR-WT and Luc- 3 -UTR-MUT (mutated on the putative miR-802 sites) vectors of REDD1 were constructed (Fig. 5B). Luciferase assay showed that miR-802 mimics inhibited the luciferase activity of Luc- 3 -UTR-WT, but not Luc-3 -UTR-MUT (Fig. 5C). This result suggested that miR-802 may bind with REDD1 3 -UTR. In a further study, miR-802 mimics were transfected into $CD8^+CD28^+$ cells. From the result of Western blotting, miR-802 mimics also impaired the expression of REDD1 in $CD8^+CD28^+$ cells (Fig. 5D). Hence, it is demonstrated that REDD1 is a target of miR-802, and it was suppressed by miR-802 in mRNA and protein levels.

miR-802 Inhibited the Expression of PD-1 and IFN-γ

To further explore the relationship between miR-802 and immune T cells, the expression of PD-1, IFN- , and $CD8^+CD28^+$ cell numbers were researched in this study. IFN- is a dimerized soluble cytokine mainly produced by T cells, which is critical for antitumor immunity[21]. As shown in Figure 6A, it is obvious that overexpression of miR-802 induced an augmented level of PD-1. Furthermore, control mimic or miR-802 mimics (10 nM and 50 nM) were transfected into $CD8^+CD28^+$ cells. The $CD8^+CD28^+$ cell number was reduced by miR-802 mimics (Fig. 6B). At the same time, a declined level of IFN- was found in $CD8^+CD28^+$ cells following the transfection of miR-802 mimics (Fig. 6C). These results indicated that miR-802 could regulate the function of immune T cells through upregulating the expression of

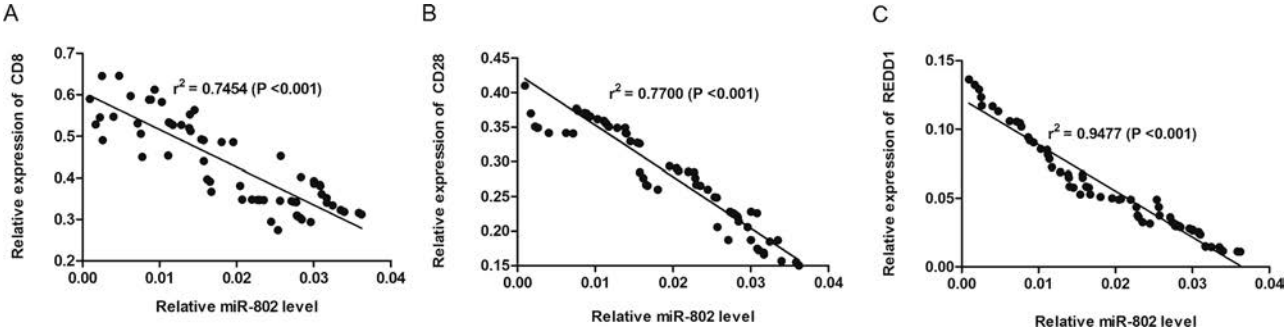

Figure 4. Correlation between miR-802 and the expression of CD8, CD28, and REDD1. The expressions of CD8, CD28, and REDD1 were negatively correlated with the transcript level of miR-802 in peripheral blood mononuclear cells ($n = 65$).

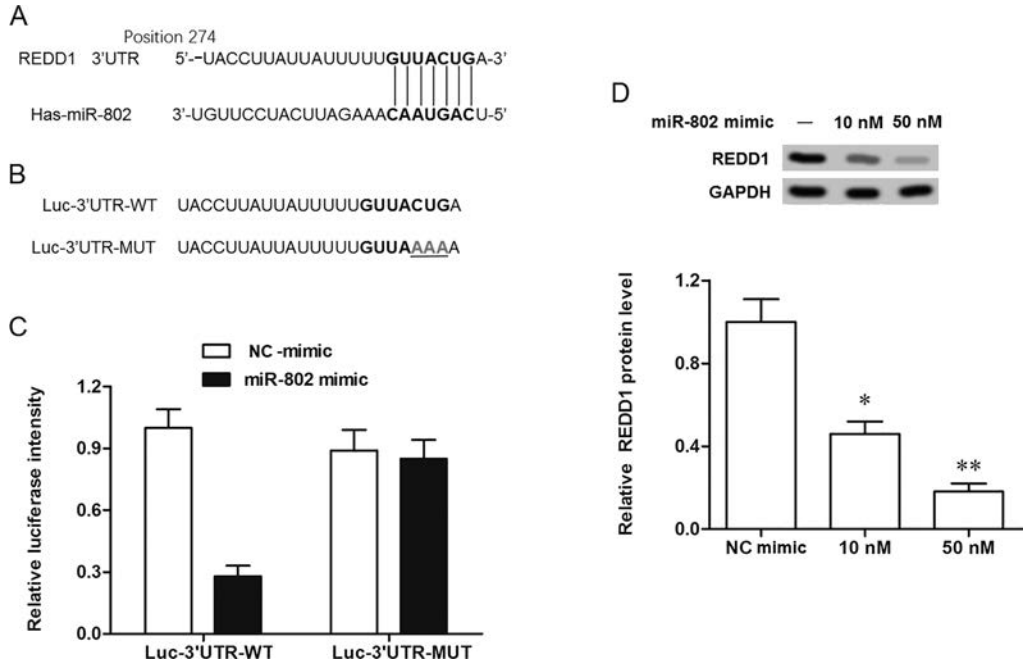

Figure 5. miR-802 directly targets REDD1. (A) The predicted binding site of miR-802 in the 3 -UTR of REDD1. (B) The mutant site (underlined) of REDD1 3 -UTR in luciferase reporter vector. (C) The luciferase activity of Luc-3 -UTR-WT was effectively inhibited by miR-802 mimics. HEK293 cells were transfected with miR-802 mimics or control mimics, then transfected with the luciferase constructs of Luc-3 -UTR-WT or Luc-3 -UTR-WUT. (D) Western blotting was used to analyze the expression of REDD1 in $CD8^+CD28^+$ cells following transfection with control mimic or miR-802 mimics. REDD1 expression was observably suppressed by miR-802. $*p < 0.05$, $**p < 0.01$.

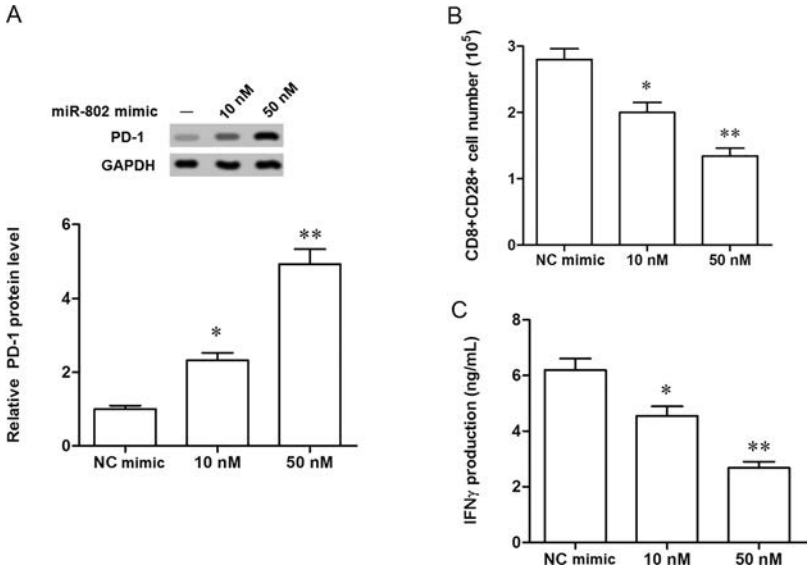

Figure 6. The examination of PD-1 and IFN- , and $CD8^+CD28^+$ cell number after transfection with miR-802 mimics in $CD8^+CD28^+$ cells. (A) PD-1 expression was observably upregulated by miR-802. (B) $CD8^+CD28^+$ cell number was inhibited by miR-802. (C) The secretion of IFN- was restrained after transfection with miR-802 mimics (10 nM and 50 nM). $*p < 0.05$, $**p < 0.01$.

PD-1, downregulating the secretion of IFN- , and reducing the number of $CD8^+$ $CD28^+$ cells.

DISCUSSION

During the last two decades, a number of miRNAs have been confirmed as oncogene or tumor suppressor in HCC[1]. Our findings implied that miR-802 expression is significantly correlated with the clinicopathological features of HCC patients. Through the collection and analysis of 172 HCC patients' clinical data, we found that the high expression of miR-802 in blood samples is markedly related to the formation of portal vein tumor thrombi, histopathological grading, tumor size, and tumor number. Moreover, miR-802 was remarkably upregulated in peripheral blood of the HCC patient group compared with the nontumor control group. A shorter survival time was found in HCC patients with high expression of blood miR-802.

miR-802 was downregulated in prostate cancer, breast cancer, gastric cancer, and tongue squamous cell carcinoma tissues and cell lines, and overexpression of miR-802 in these cell lines suppresses cell proliferation, migration, and invasion[11,12,22,23]. However, another study identified that miR-802 was upregulated in osteosarcoma tissues, and overexpression of miR-802 was able to promote cell proliferation[24]. In our study, miR-802 was upregulated in HCC tumor tissues, but the survival time displayed no difference between the high tumor miR-802 group and low tumor miR-802 group. Interestingly, there is no difference between normal hepatocytes and HCC cell lines in the expression of miR-802; even enforced expression of miR-802 exerted no effect on the proliferation and migration of Hep3B and Huh-7 cells.

As miR-802 has a higher content in blood plasma than in tumor cells[25], circulating miR-802 has been proposed as a biomarker of type 2 diabetes and drug-induced liver damage[26,27]. It is suggested that the miR-802 expressed in blood is more important than its expression in the tumor tissue. In this study, we found that a higher level of miR-802 usually means a lower level of CD8 and CD28 in vivo. $CD8^+CD28^+$ cell number was declined following the overexpression of miR-802 in vitro. $CD8^+$ cells are a kind of cytotoxic T cells that can recognize and kill tumor cells[17] and initiate the activation of T cells through binding to MHC I protein in the primary signal[16]. CD28 is a prototypic T-cell costimulatory receptor that is required in the secondary signal for the initiation of T-cell activation, and it could enhance T-cell function and survival through activating signal 1[18,28]. Furthermore, CD28 can stabilize mRNA of cytokines and enhance the activation of nuclear factor of activated T cells (NFAT)[29]. Hence, it is speculated that miR-802 participated in the activation of T cell-mediated immunity.

In addition, our results demonstrated that miR-802 directly targets REDD1, for the following reasons: (i) levels of REDD1 are declined with increasing expression of blood miR-802 in HCC patients, (ii) miR-802 suppressed the activity of REDD1-3 -UTR-WT luciferase reporter and did not influence the activity of REDD1-3 -UTR-WUT, (iii) miR-802 inhibited the expression of REDD1 protein. Therefore, it is speculated that miR-802 directly targets REDD1, leading to the degradation of REDD1 mRNA, downregulating the expression of REDD1. REDD1 is a serine/threonine kinase that is downregulated in a number of human cancers. Multiple studies have implicated its function in regulation of tumor suppression[30–32]. It contributes to cell apoptosis, mitochondrial energy metabolism, carbohydrate metabolism, and participates in the inhibition of the PI3K-Akt-mTOR signal pathway[33–36]. In addition, inactivation of REDD1 induces ROS dysregulation and promotes tumorigenesis[37]. Both PI3K-Akt-mTOR signals and ROS play vital roles in T-cell activation and proliferation[38,39]. What is more, REDD1 has been reported to be upregulated during T-cell activation, while knockout of REDD1 in mice will weaken the proliferation and survival of T cells[20]. Therefore, it is suggested that miR-802 directly targets REDD1, diminishing the host immune response through reducing the viability and function of T cells.

It has been reported that PD-1 was significantly upregulated in HCC, and its expression was correlated with the local recurrence rate, poor prognosis, and the stage of HCC[40,41]. In this study, overexpression of miR-802 led to a remarkable increase in PD-1. PD-1 is a cell surface membrane protein that binds two ligands, PD-L1 and PD-L2[42]. PD-1 is a member of the CD28/CTL4 family; CD28 produces positive signals that promote and sustain T-cell responses, whereas PD-1 limits this response[43]. The balance between CD28 and PD-1 (i.e., stimulatory and inhibitory signals) determines the ultimate response of T cells[29]. After PD-1-targeted therapies, the number of peripheral blood CD8 T cell was increased[42].

PD-1 was regarded as an immune checkpoint, activated in HCC tumor tissue for evasion from host immunity, inhibition proliferation, and cytokine production of T cells[4,44]. Immune checkpoint blockade is an effective cancer therapy that has been reported in recent decades. To date, there have been five anti-PD-1 antibodies and three anti-PD-L1 antibodies reported[4]. Among them, anti-PD-1 antibodies CT-011 and nivolumab have been initiated in a phase I/II trial in advanced HCC[4,45]. Strikingly, increased production of IFN- is an important consequence of immune checkpoint blockade[4]. In this study, downregulation of IFN- was observed after overexpression of miR-802. IFN- is a cytokine expressed by lymphocyte cells[46]. In tumors, IFN- could suppress tumor growth and increase MHC I and II expressions and

antigen presentation. In addition, it has an important role in suppressing tumor metastasis by altering the extracellular matrix and tumor architecture[47]. Hence, miR-802 may upregulate the expression of PD-1 and downregulate the secretion of IFN- . It is speculated that interference of miR-802 would downregulate PD-1 and upregulate the secretion of IFN- .

In conclusion, according to the analyses above, high expression of blood miR-802 indicated a poor outcome of HCC patients. Our results suggested that circulation of miR-802 upregulated the expression of PD-1 and downregulated the expression of REDD1, $CD8^+CD28^+$cell number, and IFN- . The underlying mechanism is possibly that miR-802 caused the disorder of stimulatory and inhibitory signals in the tumor microenvironment. Overexpression of PD-1 led to the HCC tumor tissue evasion from host immunity, and the function of T cells was inhibited. Therefore, it is suggested that blood miR-802 may act as a novel prognostic indicator and a new therapeutic target for HCC.

ACKNOWLEDGMENT: The authors declared no conflicts of interest.

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