Biochemical and Biophysical Research Communications 464 (2015) 881–887

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Regulation of tumorigenesis and metastasis of hepatocellular carcinoma tumor endothelial cells by microRNA-3178 and underlying mechanism

Wei Li, Shiqiang Shen*, Shanmin Wu, Zubing Chen, Chao Hu, Ruichen Yan

Department of General Surgery, Renmin Hospital of Wuhan University, Wuhan, 430060, Hubei Province, China

ARTICLE INFO

Article history:
Received 8 July 2015
Accepted 11 July 2015
Available online 13 July 2015

Keywords:
MiR-3178
Hepatocellular carcinoma
Tumor vascular endothelial cells
Hepatic sinusoidal endothelial cells
Proliferation
Apoptosis
Cell cycle
Invasion
Migration
Angiogenesis
EGR3

ABSTRACT

This study explored the effects of microRNA-3178 (miR-3178) on hepatocellular carcinoma (HCC) tumor endothelial cells (TECs) and on the target mRNA. Real-time polymerase chain reaction (PCR) was performed to detect the differential expression of miR-3178 in hepatic sinusoidal endothelial cells (HSECs) and HCC TECs. Furthermore, HCC TECs were transfected with miR-3178 mimic/inhibitor or their respective negative controls. The expression of miR-3178 before and after transfection was confirmed through RT-PCR. The effects of miR-3178 on the proliferation, apoptosis, cell cycle, invasion, migration, and angiogenesis of HCC TECs were also investigated through methyl thiazol tetrazolium assay, flow cytometry, matrigel invasion assay, transwell migration assay, and tube formation assay. Early growth responsive gene 3 (EGR3), as the putative target of miR-3178, was detected through RT-PCR and Western blot. Compared with HSECs, HCC TECs had lower miR-3178 expression levels ($P < 0.001$). MiR-3178 mimic inhibited proliferation, arrested cell cycle in G1 phase, and increased apoptosis. The numbers of migrated and invaded cells and capillary-like structures were significantly less in the mimic group than in the other groups. MiR-3178 mimic significantly decreased the mRNA and protein expression levels of EGR3. By contrast, miR-3178 inhibitor induced opposite effects. We conclude that miR-3178 was lowly expressed in HCC TECs, and miR-3178 mimic specifically inhibited the proliferation, migration, invasion, and angiogenesis and promoted the apoptosis and G1 phase arrest of HCC TECs in vitro through the inhibition of EGR3 expression. Thus, miR-3178 might be a critical target in HCC therapy.

1. Introduction

Hepatocellular carcinoma,a highly malignant tumor, is the fifth common malignancy and the second highest fatal neoplasm worldwide [1]. Given its insidious onset, HCC is usually diagnosed at advanced stages when tumors are in high grade and rapidly progressing [2]; about 70% of HCC patients eventually die because of the lack of effective treatments to prevent disease progression. Thus, new agents must be developed to treat HCC.

Angiogenesis is a multi-step process wherein vascular endothelial cells form new capillaries through budding. This process accelerates tumor growth and helps tumor cells escape and enter the circulation. Given its high propensity for vascular invasion, advanced HCC is usually treated using anti-angiogenetic approaches, such as transcatheter arterial chemoembolization [3]. However, some HCC patients do not benefit from anti-angiogenetic treatments [4]. Furthermore, tumor and normal endothelial cells show many differences at the molecular and functional levels. Thus, the selective inhibition or direct damage of HCC TECs may provide an effective and safe treatment for HCC.

MicroRNAs (miRNAs) are small adjustment RNAs recently discovered in epigenetics. These molecules can inhibit target gene expression at the transcription level and play essential roles in many normal biological processes involving cell growth, differentiation, apoptosis, and stress resistance [5,6]. At present, many miRNAs with abnormal expression are found in HCC, and some specific miRNAs are reportedly associated with the metastasis, recurrence, and prognosis of HCC [7–9]. Increasing evidence indicates that miRNAs regulate angiogenesis [10]. However, the effect of miRNAs on HCC TECs remains unclear. We hypothesized that

* Corresponding author.
E-mail address: shenshiqiang2014@hotmail.com (S. Shen).

http://dx.doi.org/10.1016/j.bbrc.2015.07.057
0006-291X/

miRNAs regulate the biological processes of HCC TECs and thus influence the blood vessel formation of HCC. In our previous study, we found 15 differentially expressed miRNAs between HSECs and HCC TECs by using HmiOA v4 Human miRNA OneArray® Chip [11]. Among these miRNAs, miR-3178 showed a significantly low expression ($\log2 = -0.932224$, $P = 0.000313$) and was not reported in other studies. In the present study, we further investigated the role of miR-3178 in HCC TECs.

2. Materials and methods

2.1. Cell culture

Human HCC TECs and HSECs were originally obtained from Shanghai Xinran Biotechnology (Shanghai, China) and cultured in DMEM (Dulbecco's Modified Eagle's Medium) high-glucose medium supplemented with10% fetal bovine serum (FBS) in humidified air at 37 °C with 5% CO_2.

2.2. Synthetic miRNA transfection

HCC TECs were divided into five groups: the CON group that consisted of normal TECs without mimic or inhibitor transfection; the nc-mimic group that consisted of normal TECs transfected with negative control mimic; the mimic group that consisted of normal TECs transfected with mimic; the nc-inhibitor group that consisted of normal TECs transfected with negative control inhibitor; and the inhibitor group that consisted of normal TECs transfected with inhibitor. All synthetic miRNAs were purchased from GenePharma Co., Ltd. (Shanghai, China). HCC TECs were transfected with miR-3178 mimic, nc-mimic, miR-3178 inhibitor, and nc-inhibitor at a final concentration of 20 nmol/L by using Lipofectamine 2000 (Invitrogen Life Technologies) in accordance with the manufacturer's instructions.

2.3. Quantitative RT-PCR

2.3.1. Identification of miR-3178 expression

After 72 h of transfection, total RNAs were extracted using Trizol reagent in accordance with the manufacturer's protocol (Invitrogen) and were reverse-transcribed into cDNA by using Primerscript™ First-Strand cDNA Synthesis Kit with hsa-miR-3178 or RUN6B-specific RT primers (TaKaRa). The expression of miRNA-3178 was normalized to the endogenous U6 small nuclear RNA.

2.3.2. Gene expression assays

qPCR of EGR3 was performed using the standard TaqMan miRNA assay on an ABI ViiA7 RT-PCR detection system. The reaction was conducted at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 60 s. Homo actin was used as an internal control.

2.4. Detection of HCC TEC proliferation through methyl thiazol tetrazolium (MTT) assay

After transfection, cell proliferation over a 72 h period was measured. The cells were seeded in a 96-well culture plate at a density of 10^3 cells per well. The cells were washed twice with PBS buffer and then cultured with serum-free DMEM for 12 h. To each well, 100 μL of MTT solution was added (5 mg/mL, 0.5% MTT) and then incubated for 4 h. Subsequently, the supernatant was discarded, and 150 μL of dimethyl sulfoxide was added to each well. The absorbance value of each well at OD 490 nm was measured using a Multilabel Counter Microplate Reader. Cell proliferation ability was expressed as cell proliferation rate (%) = (average A_{490} value of experimental groups − average A_{490} value of control groups)/(average A_{490} value of 0 h − average A_{490} value of control groups) × 100.

2.5. Detection of HCC TEC apoptosis through flow cytometry

After 72 h of transfection, the cells were washed with PBS. The supernatant was discarded, and the cells were resuspended in PBS. To each sample, 500 μL of binding buffer, 5 μL of AnnexinV, and 5 μL of propidium iodide (PI) were added in the dark for 15 min at room temperature. Subsequently, 400 μL of incubation buffer was added and transferred into flow cytometry tubes for detection within 1 h.

2.6. Detection of cell cycle through flow cytometry

After 48 h of transfection, the cells were washed twice with PBS. To each tube, 250 μL of pancreatic enzyme, 200 μL of trypsin inhibitor, and 50 μg/mL RNaseA were added. The precooled PI (50 μg/mL) was added to each tube after 10 min, stained in the dark at 4 °C for 30 min, and then transferred into flow cytometry tubes for detection within 3 h.

2.7. Detection of invasion and migration through transwell assay

After 48 h of transfection, migration assay was performed in 24-well transwell chambers (8 μm; Corning Inc., Corning, NY, USA). The suspended cells were seeded in the upper chamber, and 600 μL of DMEM containing 10% serum was added to the lower chamber. After incubation for 24 h at 37 °C, the cells on the upper surface of the membrane were removed, and then the migrated cells were fixed with 95% ethanol and stained with 0.1% crystal violet for 20 min. The chambers were removed after rinsing for several times, and the cells on the surface of the bottom of the chamber were wiped out. The membrane was stripped off with tweezers and inverted to dry. The number of migrated cells was determined by counting five random fields on each membrane. The invasion assay protocol was similar to that of the migration assay, but the upper chambers were first covered with 1 mg/mL Matrigel precooled with serum-free DMEM at 4 °C.

2.8. Detection of HCC TEC angiogenesis through tube formation assay

The formation of capillary-like structures was observed in vitro. Matrigel was incubated at 4 °C overnight and mixed with precooling serum-free DMEM in 1:2 ratio in a 96-well plate. Then, HCC TECs (2×10^4 cells per well) were seeded onto Matrigel-coated wells. Tube formation was observed every 2 h under an inverted fluorescence microscope (Olympus IX51) equipped with an Olympus Qcolor 3 digital camera. Tube formation was determined by counting 10 random fields on each well. All images were processed with Image Pro Plus (Media Cybernetics).

2.9. Detection of protein expression through Western blot assay

The cells were treated with RIPA lysis buffer on ice for 20 min. Protein concentration was determined using the BCA Kit. Allocation of 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gels was performed on the basis of protein concentration. Cell lysates were run on SDS-PAGE gels and transferred to polyvinylidene fluoride (PVDF) membranes. PVDF membranes were blocked with TBST containing 5% skimmed milk powder at room temperature for 2 h and then incubated overnight with primary antibody at 4 °C. PVDF membranes were washed with TBST and then incubated with HRP-conjugated secondary antibodies for

2 h at room temperature. Finally, the proteins were visualized using an Odyssey CLx infrared imaging system.

2.10. Statistical analysis

Experimental data were analyzed by SPSS 19.0 software. Values were presented as the mean ± standard deviation (SD). Data of the two groups were compared by *t* test. *P* value < 0.05 indicated that the difference was significant.

3. Results

3.1. Identification of differential miR-3178 expression in HSECs and HCC TECs through RT-PCR

The expression of miR-3178 was significantly lower in HCC TECs (0.31 ± 0.03) than in HSECs (1.04 ± 0.08) ($P < 0.001$; Fig. 1A). The results are consistent with our previous microarray results, proving the reliability of our microarray.

3.2. Detection of miR-3178 expression in HCC TECs before and after transfection through RT-PCR

After transfection, no significant difference in miR-3178 expression was detected between the CON group (1.04 ± 0.14) and the nc-mimic (0.99 ± 0.09) or nc-inhibitor group (1.02 ± 0.11) ($P > 0.05$). The mimic group (365.98 ± 49.49) showed significantly higher miR-3178 expression than the other groups ($P < 0.001$), whereas the inhibitor group (0.19 ± 0.04) showed significantly lower miR-3178 expression than the other groups ($P < 0.001$; Fig. 1B). The results prove the effectiveness of RNA interference reagent in upregulating or downregulating miR-3178 expression.

3.3. Detection of HCC TEC proliferation through MTT assay

After transfection, we observed HCC TEC proliferation for 3 d. At 24, 36, 48, and 72 h, no significant difference in the proliferation rate of HCC TECs was observed between the CON group and the nc-mimic or nc-inhibitor group ($P > 0.05$). The proliferation rate of HCC TECs in the mimic group gradually decreased over time after transfection and achieved the lowest rate after 72 h. The proliferation rate of HCC TECs was lower in the mimic group than in the other groups at corresponding time points after transfection. The proliferation rate in the inhibitor group gradually increased over time after transfection and achieved the highest rate at 72 h. The proliferation rate of HCC TECs was higher in the inhibitor group than in the other groups at corresponding time points after transfection (Fig. 1C).

3.4. Detection of HCC TEC apoptosis through flow cytometry

In accordance with the principle of double staining Annexin V/PI, cells in the second and fourth quadrants represent late and early stages of apoptosis, respectively. At 72 h after transfection, no significant difference in the apoptotic rate of HCC TECs was detected between the CON group (6.43 ± 1.23)% and the nc-mimic group (6.41 ± 1.56)% or the nc-inhibitor group (6.54 ± 1.53)% ($P > 0.05$). The mimic group (18.19 ± 2.25)% showed significantly higher apoptotic rate than the other groups ($P < 0.01$), whereas the inhibitor group (3.2 ± 0.69)% showed significantly lower apoptotic rate than the other groups ($P < 0.05$; Fig. 2A and C). The results suggest that miR-3178 upregulation significantly promoted apoptosis in HCC TECs.

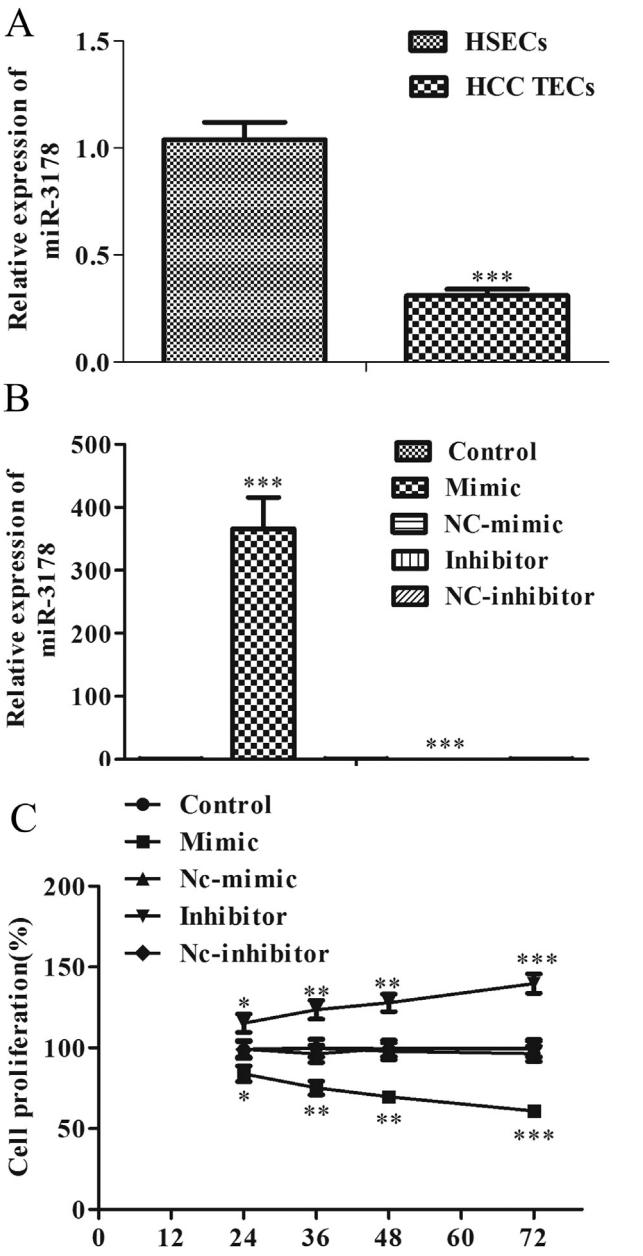

Fig. 1. MiR-3178 expression regulates HCC TECs proliferation. (A) The relative mRNA expression of miR-3178 in HSECs and HCC TECs was measured using RT-PCR. (B) The expression of miR-3178 in HCC TECs was measured through RT-PCR by comparing the transfection of 20 nmol/L miR-3178 mimic, nc-mimic, inhibitor, or nc-inhibitor. (C) Proliferation of HCC TECs at 24, 36, 48, and 72 h was determined through MTT assay. Values are expressed as mean ± SEM of 3 independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ and $^{***}P < 0.001$.

3.5. Detection of cell cycle through flow cytometry

After 48 h of transfection, no significant difference in the cell population at the G0/G1 phase was observed between the CON group (55.12 ± 4.13) % and the nc-mimic (54.08 ± 4.72) % or nc-inhibitor group (55.09 ± 4.65) % ($P > 0.05$). The mimic group (69.12 ± 4.87) % showed significantly higher G0/G1 phase cell population than the other groups ($P < 0.05$). The inhibitor group (41.79 ± 3.88) % showed significantly lower G0/G1 cell population than the other groups ($P < 0.05$). No significant difference in the percentage of S phase cells was detected between the CON group (24.23 ± 2.13) % and the nc-mimic (24.78 ± 2.24) % or nc-inhibitor

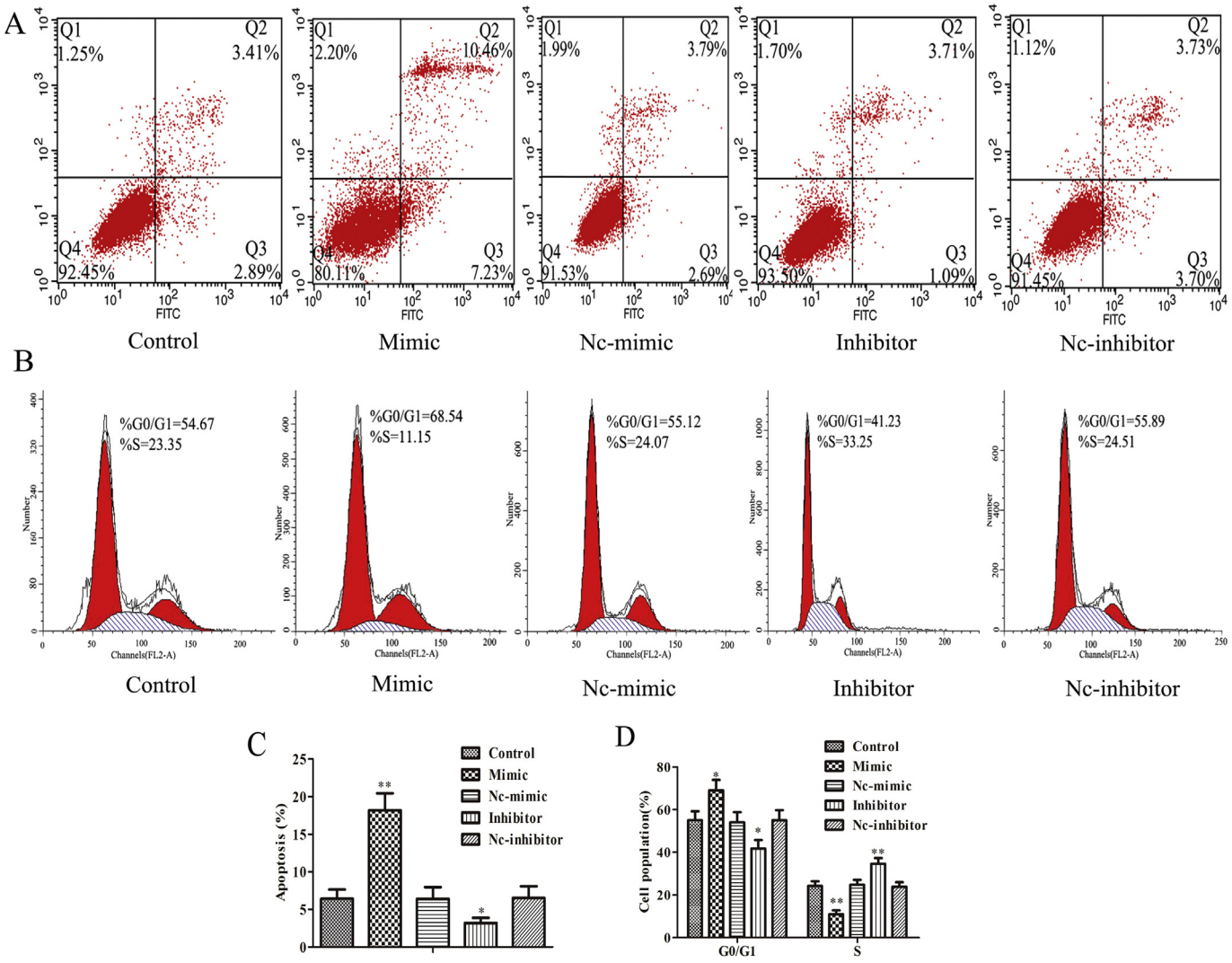

Fig. 2. MiR-3178 regulates apoptosis and cell cycle of HCC TECs. (A and C) Cell apoptosis was analyzed through flow cytometry. The Q1 quadrant contains cells undergoing necrosis, the Q2 quadrant contains cells undergoing late-stage apoptosis, the Q3 quadrant contains cells undergoing early-stage apoptosis, and the Q4 quadrant contains viable cells. (B and D) Cell cycle was analyzed through flow cytometry. Values are expressed as mean ± SEM of 3 independent experiments.$^{*}P < 0.05$ and $^{**}P < 0.01$.

group (23.88 ± 2.09) % ($P > 0.05$). The mimic group (10.98 ± 1.76) % showed significantly lower S phase cell population than the other groups ($P < 0.01$), whereas the inhibitor group (34.65 ± 2.62) % showed significantly higher S phase cell population than the other groups ($P < 0.01$; Fig. 2B and D). The results indicate that miR-3178 upregulation can induce cell cycle arrest at the G0/G1 phase and reduce S phase cell population.

3.6. Measurement of HCC TEC invasion through transwell invasion assay

After transfection, no significant difference in the number of invaded cells was detected between the CON group (41 ± 2.1) and the nc-mimic group (39 ± 1.6) or the nc-inhibitor group (42 ± 2.4) ($P > 0.05$). The mimic group (20 ± 1.1) showed significantly lesser number of invaded cells than the other groups ($P < 0.001$), whereas the inhibitor group (61 ± 2.8) showed significantly greater number of invaded cells than the other groups ($P < 0.001$; Fig. 3A and C). Therefore, miR-3178 probably negatively regulated the invasion of HCC TECs.

3.7. Measurement of HCC TEC migration through transwell migration assay

After transfection, no significant difference in cell migration was observed between the CON group (54 ± 2.5) and the nc-mimic group (57 ± 2.9) or the nc-inhibitor group (60 ± 2.8) ($P > 0.05$). The mimic group (29 ± 1.5) had significantly lesser number of migrated HCC TECs than the other groups ($P < 0.001$), whereas the inhibitor group (83 ± 3.7) showed significantly greater number of migrated cells than the other groups ($P < 0.001$; Fig. 3B and D). Thus, miR-3178 probably negatively regulated the metastasis of HCC TECs.

3.8. Detection of HCC TEC angiogenesis through tube formation assay

After transfection, no significant difference in the number of capillary-like structures was found between the CON group (18 ± 0.83) and the nc-mimic group (16 ± 0.89) or the nc-inhibitor group (17 ± 0.79) ($P > 0.05$). The mimic group (8 ± 0.43) showed significantly lesser number of capillary-like structures than the

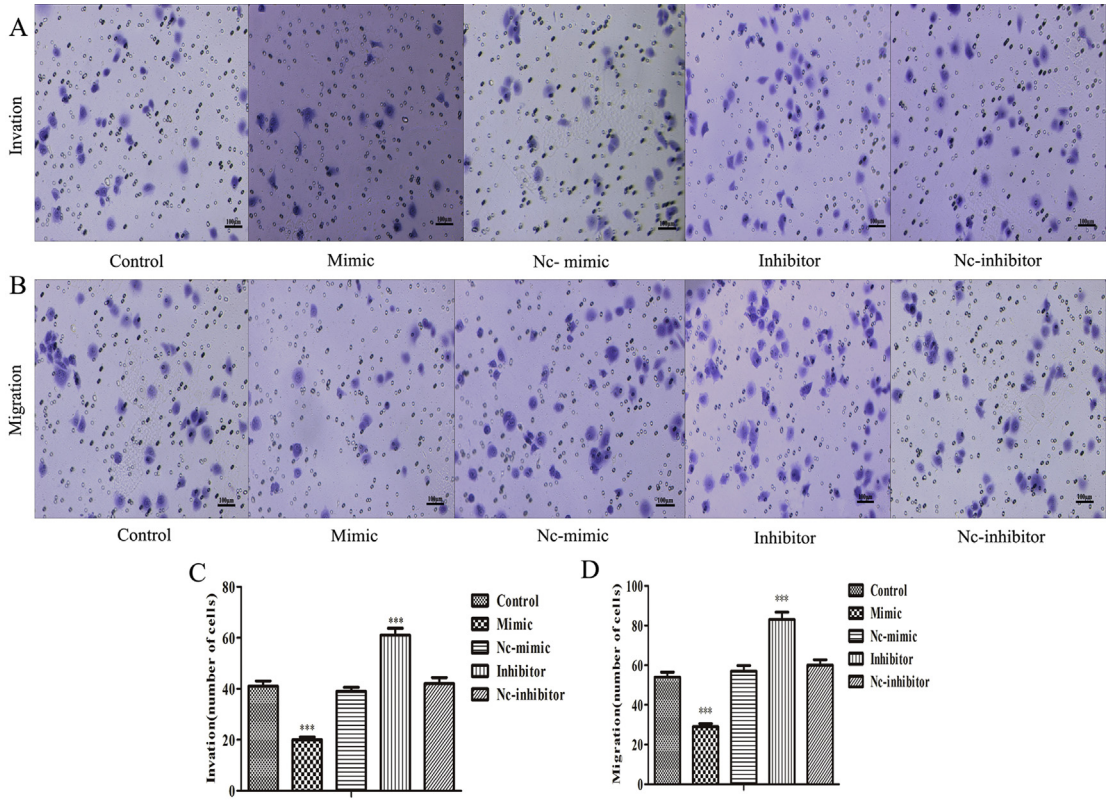

Fig. 3. MiR-3178 regulates the invasion and migration of HCC TECs. (A and C) Invasion, measured by transwell invasion assays, was inhibited by miR-3178 upregulation and promoted by miR-3178 downregulation in HCC TECs. (B and D) Migration, measured by transwell migration assays, was inhibited by miR-3178 upregulation and promoted by miR-3178 downregulation in HCC TECs (Original magnification, ×100). $^{***}P < 0.001$.

other groups ($P < 0.05$), whereas the inhibitor group (27 ± 1.37) showed significantly greater number of capillary-like structures than the other groups ($P < 0.01$; Fig. 4A and C). The results indicate that miR-3178 upregulation can inhibit the angiogenesis of HCC TECs.

3.9. Identification of EGR3 mRNA expression in HCC TECs through RT-PCR

After transfection, no significant difference in EGR3 mRNA expression was noted between the CON group (1.02 ± 0.10) and the

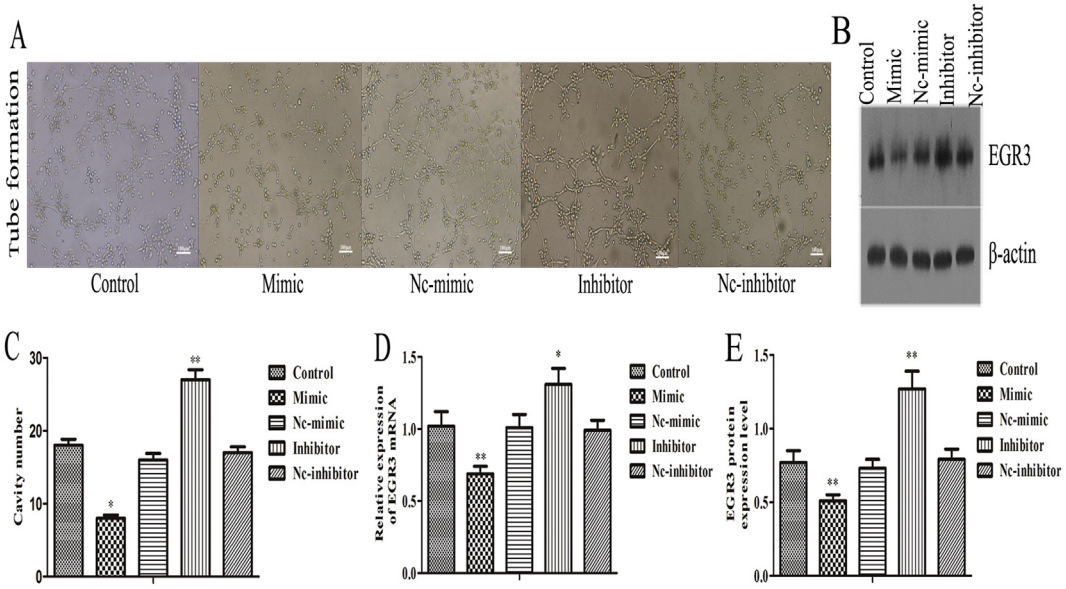

Fig. 4. MiR-3178 regulates angiogenesis of HCC TECs, and EGR3 is the functional target of miR-3178. (A and C) Tube formation of HCC TECs was determined by assaying the numbers of branch nodes (Original magnification, ×100). (B and E) Western blot analysis of EGR3 protein expression. β-actin was used as a loading control. (D) RT-PCR of EGR3 mRNA expression. Values are expressed as mean ± SEM of 3 independent experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$.

nc-mimic (1.01 ± 0.09) or nc-inhibitor group (0.99 ± 0.07) ($P > 0.05$). The mimic group (0.69 ± 0.05) showed significantly lower EGR3 expression than the other groups ($P < 0.01$), whereas the inhibitor group (1.31 ± 0.11) showed significantly higher EGR3 expression than the other groups ($P < 0.05$; Fig. 4D). The results indicate the silencing effect of miR-3178 on EGR3 mRNA.

3.10. Identification of EGR3 protein expression in HCC TECs through Western blot analysis

After transfection, no significant difference in EGR3 protein expression was detected between the CON group (0.77 ± 0.08) and the nc-mimic (0.73 ± 0.06) or nc-inhibitor group (0.79 ± 0.07) ($P > 0.05$). The mimic group (0.51 ± 0.04) showed significantly lower EGR3 protein expression than the other groups ($P < 0.01$), whereas the inhibitor group (1.27 ± 0.12) showed significantly higher EGR3 protein expression than the other groups ($P < 0.01$; Fig. 4B and E). The results indicate the silencing effect of miR-3178 on EGR3 protein expression.

4. Discussion

MiRNAs are highly conserved noncoding small RNAs with a length of 20–24 nt, and they widely exist among eukaryotes. These molecules play important roles in the translation of target mRNAs by binding to the 3′-untranslated region (3′UTR) of mRNAs. Thus far, nearly 2000 human miRNA sequences have been registered [12–14].

Furthermore, cancer-specific miRNAs have been found in various types of cancers and considered as potential tumor biomarkers [15,16]. Recent research has shown that the abnormal expression of miRNAs is closely related to the formation of HCC [17]. Another research reported that miRNAs regulate angiogenesis [18]. These differentially expressed miRNAs may be new anti-angiogenesis targets for HCC [19].

TECs differ from vascular endothelial cells in terms of morphological, structural, growth, and immunological characteristics [20]. In the present study, HCC TECs were closely arranged in typical “filamentous rod-shaped” morphology. However, HSECs were arranged regularly and showed “cobblestone-like” morphology. Cancer has generally low miRNA expression [21]. MiR-3178 has lower expression in human pancreatic cancer tissues than in healthy control tissues [22]. In the current study, RT-PCR results validated that miR-3178 expression was significantly down-regulated in HCC TECs. This result is consistent with the results of previous microarray analysis. Mimic is a partially double-stranded RNA that can simulate the endogenous precursor of miRNAs [23]. MiR mimics have become promising treatments against cancer or diseases involving gene/protein upregulation [24]. Designing of miRNA mimics and inhibitors is important to study the function of miRNAs. We transfected miR-3178 mimic and inhibitor into HCC TECs to establish stable cell lines with high or low miR-3178 expression. Subsequently, RT-PCR was used to identify miR-3178 expression before and after transfection. Results showed that miR-3178 mimic significantly increased miR-3178 expression, whereas miR-3178 inhibitor significantly reduced miR-3178 expression. This result confirms the successful transfection of miR-3178 mimic and inhibitor.

Cell proliferation was detected through MTT assay. The proliferation of HCC TECs was accelerated after miR-3178 inhibitor transfection and achieved the highest rate at 72 h. By contrast, the proliferation of HCC TECs was inhibited after miR-3178 mimic transfection and achieved the lowest rate at 72 h. The initial MTT assay results showed that miR-3178 mimic transfection at 72 h presented the most significant proliferation inhibition and was used as the time point for apoptosis detection. The results of flow cytometry showed that miR-3178 mimic transfection significantly increased the apoptotic rate of HCC TECs. By contrast, miR-3178 inhibitor transfection significantly decreased the apoptotic rate of these cells. Promoting the G_1/S phase is likely to prevent tumor proliferation. Cell cycle was detected through flow cytometry in our study. The mimic group showed significantly higher G0/G1 phase cell population and significantly lower S phase cell population than the other groups. By contrast, the inhibitor group showed significantly lower G0/G1 phase cell population and significantly higher S phase cell population than the other groups, these data indicate that miR-3178 can inhibit HCC TEC growth. These findings help explain the mechanism of the relationship between miR-3178 and HCC TEC proliferation. The results of the MTT assay and flow cytometry demonstrate that miR-3178 acts as a tumor suppressor gene during the occurrence and progression of HCC TECs.

Cancer cells lose their contacts and invade surrounding tissues and subsequently distant organs [25,26]. Therefore, we investigated the effects of miR-3178 on the metastasis of HCC TECs. The results of transwell invasion and migration assays showed that miR-3178 mimic decreased the number of invaded and migrated cells in vitro. By contrast, miR-3178 inhibitor increased the number of invaded and migrated cells in vitro. Our study demonstrates that miR-3178 upregulation decreases the aggressiveness and invasiveness of HCC TECs.

The angiogenic activity of HCC correlates with the risk of metastasis and prognostic significance [27]. Previous research reported that anti-angiogenesis might be highly efficient for the treatment of HCC by inhibiting angiogenesis in tumor tissues [28–30]. However, one study indicated that HCC TECs have stronger resistance to drugs than normal peritumoral vascular endothelial cells [31]. Another also suggested that TECs are more appropriate for screening anti-angiogenesis drugs than normal ECs [32]. In the present study, the results of tube formation assay showed that miR-3178 influenced HCC angiogenesis without contamination with hepatocytes. MiR-3178 mimic decreased the number of capillary-like structures in vitro, whereas miR-3178 inhibitor increased the number of capillary-like structures in vitro. These results confirm the role of miR-3178 on anti-angiogenesis in HCC TECs.

Considering that miRNAs negatively regulate gene expression at the transcription level, we speculated that miRNA-3178 regulates the biological behavior of HCC TECs by regulating the expression of some oncogenes. Target gene prediction software showed EGR3 as a possible candidate target. Current studies about EGR3 mainly concentrated on neural development and virus. Studies reported that vascular endothelial growth factor (VEGF) upregulates EGR3 expression in human umbilical vein endothelial cells (HUVECs) and that EGR3 knockdown in HUVECs reduces proliferation, migration, and angiogenesis [33,34]. These studies showed that EGR3 is a key factor of activated signaling pathways of VEGF in endothelial cells. The results of Western blot and RT-PCR showed that miR-3178 mimic decreased the mRNA and protein expression of EGR3, whereas miR-3178 inhibitor induced opposite effects. These results confirmed that miR-3178 significantly inhibited the expression of EGR3. Basing from the above mentioned data, we hypothesized that miR-3178 regulates the proliferation, apoptosis, cell cycle, invasion, migration, and angiogenesis of HCC TECs by inhibiting EGR3 expression. However, a single miRNA may have many targets; our study could only indicate that EGR3 is a potential target gene of miR-3178. In our future studies, luciferase reporter assay will be conducted to investigate the role of miRNA-3178 on EGR3. Moreover, we will construct a nude mouse model of human HCC TEC xenograft tumors and observe the influence of miRNA-3178 on xenograft tumor and tube formation.

In summary, our study demonstrated the following results. First, miR-3178 expression is significantly reduced in HCC TECs. Second, miR-3178 acts as a tumor suppressor to inhibit proliferation, migration, invasion, and angiogenesis; promote apoptosis; and arrest G1 phase in HCC TECs in vitro. Third, EGR3 may be a target gene of miR-3178. These findings indicate that our study has clinical relevance and that miR-3178 overexpression may have potential for HCC treatment.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.07.057.

References

[1] M. Schwartz, S. Roayaie, M. Konstadoulakis, Strategies for the management of hepatocellular carcinoma, Nat. Clin. Pract. Oncol. 4 (2007) 424–432.
[2] R. Siegel, J. Ma, Z. Zou, A. Jemal, Cancer statistics, CA Cancer J. Clin. 64 (2014) 9–29.
[3] J.M. Llovet, A.M. Di Bisceglie, J. Bruix, et al., Design and endpoints of clinical trials in hepatocellular Carcinoma, J. Natl. Cancer Inst. 100 (2008) 698–711.
[4] M.R. Mancuso, R. Davis, S.M. Norberg, et al., Rapid vascular regrowth in tumors after reversal of VEGF inhibition, J. Clin. Investig. 116 (2006) 2610–2621.
[5] J. Winter, S. Diederichs, MicroRNA biogenesis and cancer, Methods Mol. Biol. 676 (2011) 3–22.
[6] E.A. Gibb, C.J. Brown, W.L. Lam, The functional role of long non-coding RNA in human carcinomas, Mol. Cancer 10 (2011) 38.
[7] C. Braconi, T. Patel, MicroRNA expression profiling:A molecular tool for defining the phenotype of hepatocellular tumors, Hepatology 47 (2008) 1807–1809.
[8] J.L. Mott, MicroRNAs involved in tumor suppressor and oncogene path ways :implications for hepatobiliary neoplasia, Hepatology 50 (2009) 630–637.
[9] S. Giordano, A. Columbano, MicroRNAs:new tools for diagnosis,prognosis,and therapy in hepatocellular carcinoma? Hepatology 57 (2013) 840–847.
[10] Y. Suárez, W.C. Sessa, MicroRNAs as novel regulators of angiogenesis, Circ. Res. 104 (2009) 442–454.
[11] Zhong-hui Cui, Shi-qiang Shen, Zu-bing Chen, et al., Growth inhibition of hepatocellular carcinoma tumor endothelial cells by miR-204-3p and underlying mechanism, World J. Gastroenterol. 20 (2014) 5493–5504.
[12] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[13] L. He, G.J. Hannon, MicroRNAs: small RNAs with a big role in gene regulation, Nat. Rev. Genet. 5 (2004) 522–531.
[14] M. D'Anzeo, L. Faloppi, M. Scartozzi, et al., The role of micro-RNAs in hepatocellular carcinoma:from molecular biology to treatment, Molecules 19 (2014) 6393–6406.
[15] X. Hu, J.K. Schwarz, J.S. Lewis Jr., et al., A microRNA expression signature for cervical cancer prognosis,, Cancer Res. 70 (2010) 1441–1448.
[16] M. Lin, W. Chen, J. Huang, et al., MicroRNA expression profiles in human colorectal cancers with liver metastases, Oncol. Rep. 25 (2011) 739–747.
[17] C.M. Wong, A.K. Kai, F.H. Tsang, et al., Regulation of hepatocarcinogenesis by microRNAs, Front. Biosci. (Elite Ed.) 5 (2013) 49–60.
[18] L. Zhang, M. Zhou, G. Qin, et al., MiR-92a regulates viability and angiogenesis of endothelial cells under oxidative stress, Biochem. Biophys. Res. Commun. 446 (2014) 952–958.
[19] C. He, D.J. Klionsky, Regulation mechanisms and signaling pathways of autophagy, Annu. Rev. Genet. 43 (2009) 67–93.
[20] R. Kerbel, J. Folknmn, Clinical translation of angiogenesis inhibitors, Nat. Rev. Cancer 2 (2002) 727–739.
[21] J. Lu, G. Getz, E.A. Miska, et al., MicroRNA expression profiles classify human cancers, Nature 435 (2005) 834–838.
[22] Mao-Song Lin, Wei-Chang Chen, Jun-Xing Huang, et al., Aberrant expression of microRNAs in serum may identify individuals with pancreatic cancer, Int. J. Clin. Exp. Med. 7 (2014) 5226–5234.
[23] Z. Wang, The guideline of the design and validation of miRNA mimics, Methods Mol. Boil 676 (2011) 211–223.
[24] P. Trang, P.P. Medina, J.F. Wiggins, et al., Regression of murine lung tumors by the let-7 microRNA, Oncogene 29 (2010) 1580–1587.
[25] R. Kalluri, R.A. Weinberg, The basics of epithelial-mesenchymal transition, J. Clin. Investig. 119 (2009) 1420–1428.
[26] C.L. Chaffer, R.A. Weinberg, A perspective on cancer cell metastasis, Science 331 (2011) 1559–1564.
[27] A.I. Shchyogolev, E.A. Dubova, U.N. Tumanova, Vascularization of hepatocellular carcinoma tissue depends on its differentiation degree, Bull. Exp. Biol. Med. 153 (2012) 490–494.
[28] N.N. Rahbari, A. Mehrabi, N.M. Mollberg, et al., Hepatocellular carcinoma: current management and perspectives for the future, Ann. Surg. 253 (2011) 453–469.
[29] A.X. Zhu, D.G. Duda, D.V. Sahani, et al., HCC and angiogenesis: possible targets and future directions, Nat. Rev. Clin. Oncol. 8 (2011) 292–301.
[30] A. Villanueva, J.M. Llovet, Targeted therapies for hepatocellular carcinoma, Gastroenterology 140 (2011) 1410–1426.
[31] Y.Q. Xiong, H.C. Sun, W. Zhang, et al., Human hepatocellular carcinoma tumor -derived endothelial cells manifest increased angiogenesis capability and drug resistance compared with normal endothelial cells, Clin. Cancer Res. 15 (2009) 4838–4846.
[32] A.C. Dudley, M. Klagsbrun, Tumor Endothelial Cell Abnormalities, Humana Press, 2008, pp. 73–84.
[33] J. Suehiro, T. Hamakubo, T. Kodama, et al., Vascular endothelial growth factor activation of endothelial cells is mediated by early growth response-3, Blood 115 (2010) 2520–2532.
[34] D. Liu, I. Evans, G. Britton, et al., The zinc-finger transcription factor,early growth response 3, mediates VEGF- induced angiogenesis, Oncogene 27 (2008) 2989–2998.