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Original Article

MicroRNA-200a inhibits epithelial-mesenchymal transition in human hepatocellular carcinoma cell line

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Abstract: Objective: Our study investigated the role of microRNA (miR)-200a and its molecular targets in hepatocellular carcinoma (HCC) cells. Methods: An inhibitor of miR-200a was transiently transfected into the hepatocellular carcinoma cell line, MHCC-97L. The effect of this transfection on mRNA levels of epithelial-mesenchymal transition (EMT)-related genes was measured by fluorescence-based quantitative real-time polymerase chain reaction (qRT-PCR). Further, protein levels of EMT-related genes, cell proliferation and apoptosis-related markers were assessed by Western blot analysis in these transfected cells. MTT and wound-healing assay were used to evaluate the proliferation and migration of MHCC-97L cells in presence and in absence of miR-200a inhibitor. Results: Compared with miR-NC control group, qRT-PCR results in anti-miR-200a group revealed a significant reduction in the mRNA levels of E-cadherin, with a concomitant increasing in vimentin mRNA level (all $P < 0.05$). Western blot results showed higher E-cadherin and Caspase-3 protein expressions in anti-miR-200a group compared to miR-NC group ($P < 0.05$). In addition, vimentin and Ki-67 protein expression was found sharply decreased in anti-miR-200a group compared to miR-NC group ($P < 0.05$). Consistent with this, wound-healing and MTT assay showed that migration and proliferation capacity of MHCC-97L cells in anti-miR-200a group is significantly increased compared with miR-NC group (both $P < 0.05$). Conclusion: Our study reveals an important role of miR-200a in inhibiting EMT, proliferation and migration in HCC cells, suggesting the possibility of miR-200a-based therapeutics in HCC.

Keywords: MicroRNA-200a, epithelial-mesenchymal transition, hepatocellular carcinoma, MHCC-97L, migration, proliferation

Introduction

Hepatocellular carcinoma (HCC) accounts for 70-85% of the primary liver cancers and is the third most frequent cause of cancer-related deaths in the world [1, 2]. Approximately, 90% of HCC is related to liver cirrhosis in its origin, with a wide range of risk factors such as hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol abuse, nonalcoholic steatohepatitis, autoimmune hepatitis, primary biliary cirrhosis, and carcinogen exposure [3, 4]. Therapy for HCC includes surgical resection and liver transplantation, but this is only effective in a small fraction of HCC patients whose tumors are diagnosed early [4]. Importantly, more than 70% of HCC patients remain at a high risk for tumor recurrence several years after surgical resection or liver transplantation, mainly due to intrahepatic dissemination of primary lesions, resulting in the poor prognosis for HCC [5]. In the last decade, various genetic and epigenetic alterations have been linked to multiple steps in hepatocarcinogenesis [6]. Using this knowledge, targeted molecular therapies are currently being developed for patients with metastatic or relapsed HCC [7]. However, current targeted therapies are inadequate in improving the outcomes in HCC patients as the molecular mechanisms of HCC are still obscure, and are likely to be very complicated [8]. Therefore, it is important to unravel novel molecular pathways involved in the development of HCC, as this may pave an alternative path for HCC therapy.

Table 1. The designed and synthesized transfection sequences for transient transfection of MHCC-97L cells in the anti-miR-200a group and the miR-NC group

Group	Sequence
anti-miR-200a	ACAUCGUUACCAGACAGUGUUA
miR-NC	CAGAUUUUGUGUAGUACAA

The anti-miR-200a and miR-NC sequences were designed and synthesized by Zimmer Company (Shanghai, China).

Table 2. Primer sequence of *miR-200a*, *U6*, *E-cadherin*, *vimentin*, *Tiam1*, *SNAI1*, *MMP2*, *TIMP2*, and GAPDH gene for fluorescence-based quantitative real-time polymerase chain reaction

Gene	Primer sequence
miR-200a	F: 5'-CGTAACACTGTCTGGTAACGATGT-3'
U6	F: CTCGCTTCGGCAGCACA-3'
E-cadherin	F: 5'-CGGTGGTCAAAGAGCCCTTACT-3'
	R: 5'-TGAGGGTTGGTGCAACAACGTCGTTA-3'
Vimentin	F: 5'-TGAGTACCGGAGACAGGTCGAG-3'
	R: 5'-TAGCAGCTTCAACGCAAAGTTC-3'
Tiam1	F: 5'-AAGACGTACTCAGGCCATGTCC-3'
	R: 5'-GACCCAAATGTCGCAGTCAG-3'
SNAI1	F: 5'-ACAAGCACCACCGAGTCCG-3'
	R: 5'-CCCTCCCTCCACACAGAAAT-3'
MMP2	F: 5'-GAGAACCAAAGTCTGAAGAG-3'
	R: 5'-GGAGTGAGAATGCTGATTAG-3'
TIMP2	F: 5'-GATGCACATCACCCTCTGTG-3'
	R: 5'-GTGCCCGTTGATGTTCTTCT-3'
GAPDH	F: 5'-ACAGTCAGCCGCATCTTCTT-3'
	R: 5'-GACAAGCTTCCCGTTCTCAG-3'

Primers were designed using Primer 5.0.

Most cancers are epithelial in origin and undergo a multi-step process which includes loss of epithelial characteristics, such as polarized cell organization, and gain of mesenchymal features such as increased cell motility and invasion of surrounding tissues, and the entire process is collectively referred to the epithelial-mesenchymal transition (EMT) [9]. EMT results in metastatic dissemination of tumors and the acquisition of therapeutic resistance in epithelial-derived tumors [10]. EMT is characterized both by down-regulation of epithelial markers, such as the intercellular adhesion protein E-cadherin, and up-regulation of mesenchymal markers such as fibronectin, vimentin, N-cadherin and collagen [11]. E-cadherin is a pivotal protein in cell polarity and epithelial organization, therefore, loss of E-cadherin protein is one of the hallmarks of EMT [12].

Aberrant expression of microRNAs (miRs) plays an important role in cancer progression [13]. As such, identification of aberrantly expressed miRs in specific cancer types, termed as the miRNA signature, is a crucial step in the elucidation of miR-mediated oncogenic pathways [14]. Accordingly, miR signature in HCC prominently includes altered expression of miR-200 family members [15]. In humans, the miR-200 family is composed of five members organized into two clusters: miR-200a/b/429 on chromosome 1 and miR-200c/141 on chromosome 12 [16]. The miR-200a is encoded on a 7.5 kb polycistronic primary miRNA transcript [17]. The miR-200a is an important regulator in specifying the epithelial phenotype through preventing the expression of transcriptional repressors [18]. To date, molecular pathways regulated by miR-200a have not been fully explored in HCC [19]. Greater understanding of miR-200a-regulated molecular pathways might contribute to the development of better prognosis and diagnosis of HCC [3]. Therefore, our study investigated the functional role of miR-200a and its molecular targets in HCC.

Materials and methods

Cell transfection and grouping

Twenty-four hours before transfection, MHCC-97L cells in logarithmic phase (proved by Zhongnan Hospital of Wuhan University) were detached with trypsin (Gibco) and counted. Cell concentration was adjusted to 2×10^5 cells/ml, and 200 μl of this cell suspension was inoculated into 6-well plates containing 2 ml fresh culture medium. After culture in antibiotic-free DMEM medium containing 10% FBS for 24 hours, and cell confluency reaching 80%, the cells were transfected using Lipofectamine™2000 transfection kit (Invitrogen) in accordance with the protocol of manufactory. MHCC-97L cells were divided into 2 groups: anti-miR-200a group and miR-NC group. The anti-miR-200a and miR-NC sequences were designed and synthesized by Zimmer Company (Shanghai, China), as shown in **Table 1**. Transfected cells were incubated for 6 hours at 37°C in a 5% CO_2 thermostatic incubator. After change of the solution, cell transfection was

Table 3. Comparison of expression level of miR-200a between anti-miR-200a group and miR-NC group after cell transfection by fluorescence-based quantitative real-time polymerase chain reaction

Group	$2^{-\Delta\Delta Ct}$	*t*	*P*
miR-NC	1	16.92	0.000
anti-miR-200a	0.352 ± 0.080		

$2^{-\Delta\Delta Ct}$, multiple proportions of gene expression between anti-miR-200a group and miR-NC group.

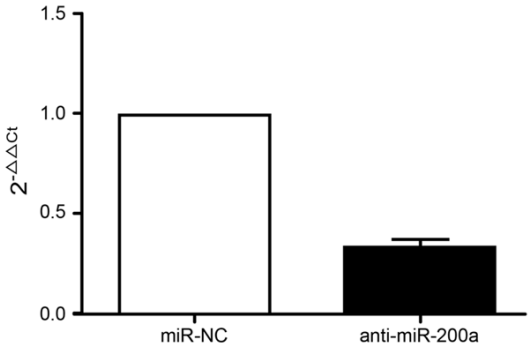

Figure 1. The expression of miR-200a in anti-miR-200a group and miR-NC group after cell transfection by fluorescence-based quantitative real-time polymerase chain reaction. The $2^{-\Delta\Delta Ct}$ stands for multiple proportions of gene expression between anti-miR-200a group and miR-NC group.

observed and photographed under confocal laser scanning microscope with exciting light adjusting to fluorescence wavelength of cy3.

Fluorescence-based quantitative real-time polymerase chain reaction (qRT-PCR)

MHCC-97L cells in anti-miR-200a and miR-NC group were collected, and the total RNA was subsequently extracted with RNAiso Plus reagent according to the manufacturer's instructions (TaKaRa). Reverse transcription and RT-PCR were carried out with extracted RNA as template by using SYBR PrimeScript RT-PCR Enzyme Kit in accordance with the protocol of manufacturer (TaKaRa). PCR primers were designed using Primer 5.0 (**Table 2**). The reverse primers in miR-200a and U6 PCR reaction were universal primers (Uni-miR qPCR Primer). The reverse transcription was conducted in a 25 μl reaction solution containing total RNA, miRNA Primer Script RT Enzyme Mix. The total volume of the miR-200a fluorescence-based qPCR reaction system was 20 μL, and the PCR conditions were as follows: predenaturation at 95°C for 10 s, followed by 40 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 20 s, and extension at 72°C for 10 s. The RT-PCR melting curve analysis of amplification products was performed at 95°C for 15 s, at 60°C for 1 min, at 95°C for 30 s, and at 60°C for 15 s. The total volume of the fluorescence-based qPCR reaction system of EMT-related genes (*E-cadherin*, *Vimentin*, *Tiam1*, *SNAI1*, *MMP2*, *TIMP2*) was 20 μL, and the PCR conditions were as follows: predenaturation at 95°C for 10 s, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 5°C for 30 s, and extension at 72°C for 30 s. The melting curve analysis of EMT amplification products was performed at 95°C for 15 s, at 60°C for 1 min, at 95°C for 30 s, and at 60°C for 15 s. The concentration and purity of RNA were assessed by ultraviolet spectrophotometer, and the integrity of RNA was detected by agarose gel electrophoresis (2% agarose gel). Folds = $2^{-\Delta\Delta Ct}$, which means multiple proportions of gene expression between anti-miR-200a group and miR-NC group. $\Delta\Delta Ct$ = [Ct (target gene) - Ct (GAPDH)]$_{\text{anti-miR-200a group}}$ - [Ct (target gene) - Ct (GAPDH)]$_{\text{miR-NC group}}$. The experiment was repeated for 3 times.

Western blot

Transfected MHCC-97L cells were collected 48 hours after transfection, and total protein was extracted using RIPA buffer. The electrophoretic separation of proteins was conducted by using SDS-PAGE gel (BioDev-Tech. Co., Ltd, Beijing, China) at a constant voltage of 80 V, and the proteins were then electrically transferred to a PVDF membrane (Millipore, Co., America). After blocking with BSA for 1 hour, mouse anti-human E-cadherin, vimentin, Ki-67 monoclonal antibodies (1:100, Beijing Zhongshan Golden Bridge Biotechnology Co., Beijing, China), and mouse anti-human Caspase-3 monoclonal antibody (1:100, Bioss Company, Beijing, China) were added, followed by incubation overnight at 4°C. On the following day, the membrane was washed by PBST after 30 min rewarming to room temperature. Horse-radish peroxidase labeled secondary antibody (1:100, Beijing Zhongshan Golden Bridge Biotechnology Co., Beijing, China) was added, the proteins were incubated at room temperature for 1 hour using GAPD as an internal control. The color

Table 4. Comparison of mRNA expression levels of epithelial-mesenchymal transition-related genes (*E-cadherin*, *Vimentin*, *Tiam1*, *SNAI1*, *MMP2*, *TIMP2*) between anti-miR-200a group and miR-NC group by fluorescence-based quantitative real-time polymerase chain reaction

Gene	Group	$2^{-\Delta\Delta Ct}$	*t*	*P*
E-cadherin	miR-NC	1.000	12.25	0.000
	anti-miR-200a	0.799 ± 0.013		
Vimentin	miR-NC	1.000	31.85	0.000
	anti-miR-200a	1.740 ± 0.015		
Tiam1	miR-NC	1.000	1.310	0.227
	anti-miR-200a	0.927 ± 0.098		
SNAI1	miR-NC	1.000	1.938	0.070
	anti-miR-200a	0.934 ± 0.052		
MMP2	miR-NC	1.000	1.643	0.120
	anti-miR-200a	0.950 ± 0.051		
TIMP2	miR-NC	1.000	1.223	0.239
	anti-miR-200a	1. 020 ± 0.054		

$2^{-\Delta\Delta Ct}$, multiple proportions of gene expression between anti-miR-200a group and miR-NC group.

development was performed with the chemiluminiscence method, and fluorescence images were collected using a gel imaging system. Relative expression of target protein = gray value of target band/gray value of internal reference of the same sample.

Wound-healing assay

After transfection for 18 hours, the wound-healing assay was carried out as described by Chun et al. [20]. There were 3 pores in each group (triplicates). The MHCC-97L cells were seeded in a 24-well plate at 1.5×10^4 cells/well and incubated. When the MHCC-97L cells in the monolayers were confluent, the "wound" was generated by scratching each well with a sterile glass-tipped pipette (10 μl) vertically. The scratch area was washed repeatedly with phosphate buffer solution (PBS). The culture medium contained 2% fetal bovine serum so as to minimize the influence on cell proliferation. At 0, 6, 12, 24 and 48 hours after scratching, the migration of MHCC-97L cells was observed and recorded.

Transwell assay

Eighteen hours after transfection, transwell inserts (24-well insert, Corning Co., Corning, America) was applied to determine the effect of miR-200a on the migration and invasion of MHCC-97L cells. Fibronectin (3 μg/ml) was added to the inserts, incubated at room temperature for 2 hours, and fibronectin was removed, the inserts were washed with PBS twice, and stored overnight at 4°C. Cell suspension (100 μl) was added to the upper inserts at 2×10^4/ml, while lower inserts were added with 600 μl cell culture medium (DMEM + 5% FBS). Twenty-four hours after incubation, the MHCC-97L cells that failed to migrate through membrane were wiped off by cotton bud. Paraform aldehyde (4%) fixation and DAP staining was performed. Under the fluorescence microscopy, 5 visual fields (×100) were randomly chosen to calculate the number of MHCC-97L cells and photograph. The procedure was performed in triplicates.

MTT assay

MHCC-97L cell suspension in each group was seeded in 96-well plates at 2,000 cells per well. Twelve hours after the cells were seeded, transient transfection was carried out. The anti-miR-200a group was added with the mixture containing 25 μl Opti-MEM, 0.25 μl anti-miR-200a, and 0.25 μl Lipofectamine™2000 per well. The miR-NC group contained a mixture containing 25 μl Opti-MEM, 0.25 μl negative control, and 0.25 μl Lipofectamine™2000 per well, and then added with culture medium to 100 μl. Twenty-four hours after transfection, the proliferation of MHCC-97L cells was detected: 20 μl 5 mg/ml MTT (Sigma Co, America) was added per well, the MHCC-97L cells were cultured continually at 37°C for 4 hours, the culture medium was removed carefully, 150 μl dimethylsulfoxide (DMSO) was added followed by incubation at room temperature for 10 min, the plates were shaken with micro-oscillator for 10 min for fully dissolution of crystal, blank control wells containing only culture medium were set as zero pores, absorbance (OD) value of each well was measured using a microplate reader at 490 nm at 24 and 48 hours after transfection, the proliferation of MHCC-97L cells was represented with corresponding OD ratio.

Statistical analysis

Data were presented with means ± standard deviation (SD). The one-way ANOVA was used

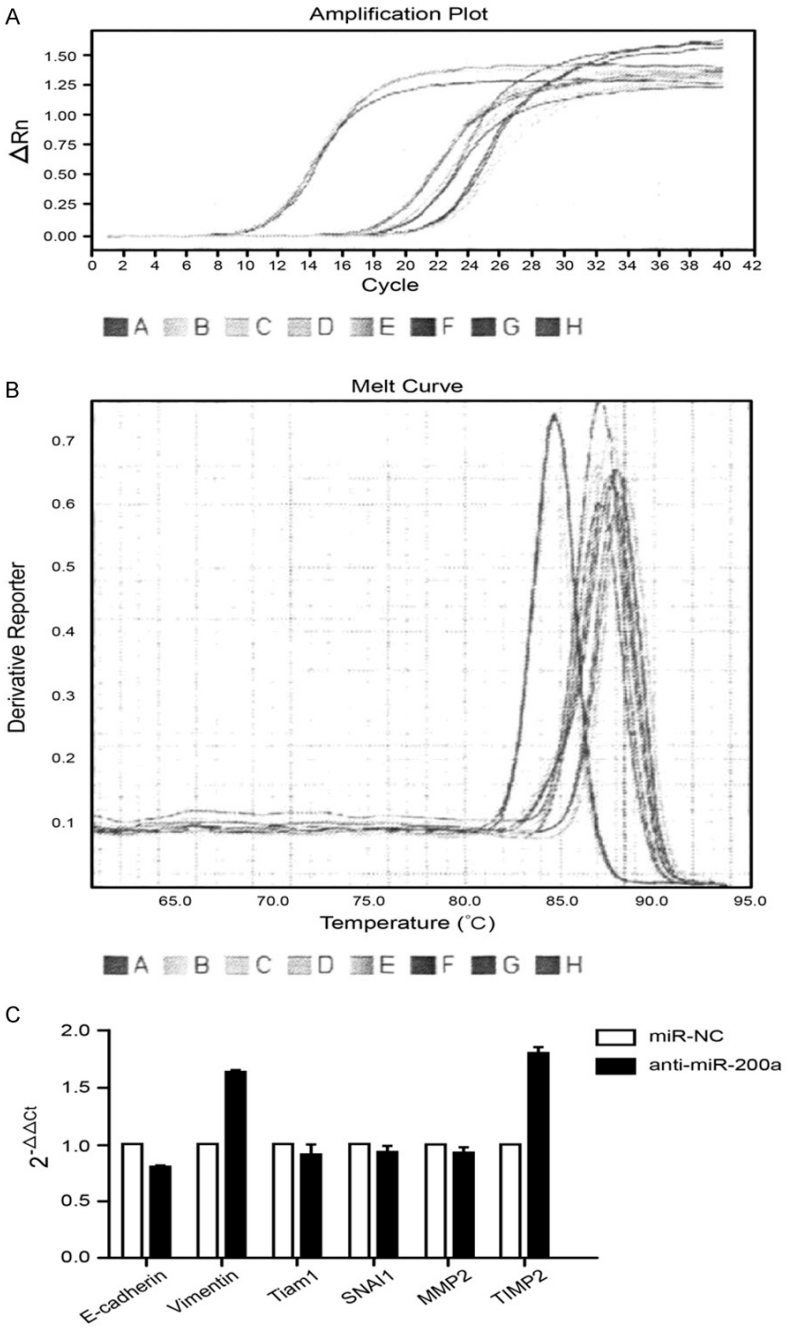

Figure 2. The mRNA expression levels of epithelial-mesenchymal transition-related genes (*E-cadherin*, *Vimentin*, *Tiam1*, *SNAI1*, *MMP2*, *TIMP2*) by fluorescence-based quantitative real-time polymerase chain reaction. A. Stands for amplification curve; B. Stands for solubility curve; C. Stands for the expression of epithelial-mesenchymal transition-related genes; and $2^{-\Delta\Delta Ct}$ stands for multiple proportions of gene expression between anti-miR-200a group and miR-NC group.

for pair-wise comparison of the mean of univariate sample, and the LSD test was applied to pair-wise comparison of the mean of multivariate sample. SPSS17.0 software (SPSS Inc., Chicago, IL, USA) was applied for data analysis. Statistical significance was set at *P* value less than 0.05.

Results

Detection of miR-200a and EMT-related genes expression

The qRT-PCR results revealed that the expression of miR-200a in the anti-miR-200a group reduced about 60% as compared with the miR-NC group after cell transfection (t = 17.45, $P < 0.05$) (**Table 3**; **Figure 1**). In contrast with the miR-NC group, the mRNA expression of *E-cadherin* in the anti-miR-200a group decreased significantly (t = 12.25, P = 0.000), while the mRNA expression of *Vimentin* increased (t = 31.85, P = 0.000). There was no observable differences in the mRNA expression of *Tiam1*, *SNAI1*, *MMP2* and *TIMP2* between the anti-miR-200a group and the miR-NC group (t = 1.10, P = 0.227; t = 1.938, P = 0.070; t = 1.643, P = 0.120; t = 1.223, P = 0.239, respectively), as shown in **Table 4** and **Figure 2**.

Protein expressions of EMT-related genes and proliferation and apoptosis-related genes

The Western blot results showed that E-cadherin and Caspase-3 protein expressions in the anti-miR-200a group were lower than the miR-NC group (E-cadherin:0.825±0.021vs.0.242±0.013,Caspase-3: 0.962 ± 0.015 vs. 0.480 ± 0.030, respectively). Significant increase in vimentin

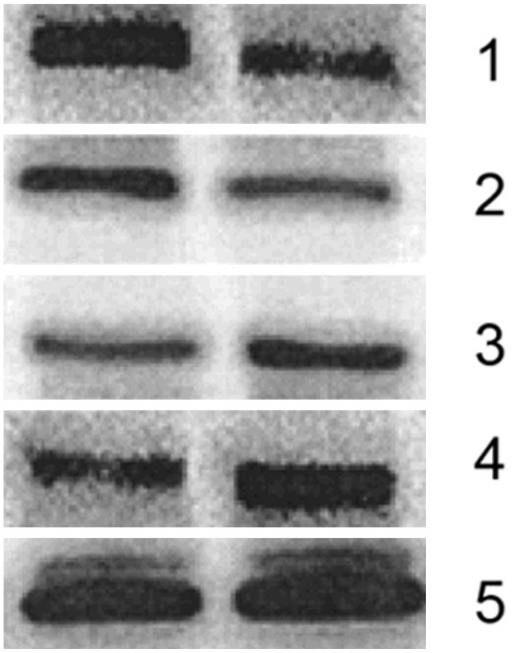

Figure 3. Protein expression levels of epithelial-mesenchymal transition-related genes and proliferation and apoptosis-related genes. 1 stands for GAPDH; 2 stands for E-cadherin; 3 stands for Caspase-3; 4 stands for Vimentin; and 5 stands for Ki-67.

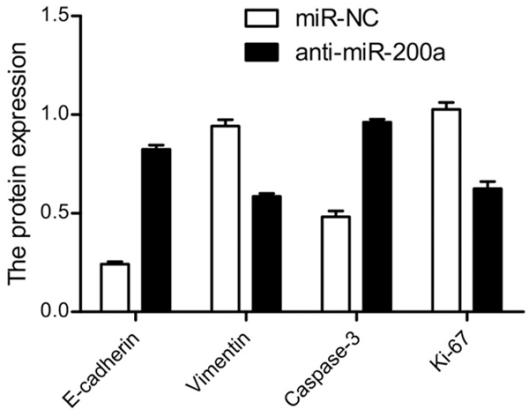

Figure 4. Comparison of protein expression levels of epithelial-mesenchymal transition-related genes and proliferation and apoptosis-related genes between anti-miR-200a group and miR-NC group.

and Ki-67 protein levels were found in the anti-miR-200a group as compared with the miR-NC group (vimentin: 0.586 ± 0.015 vs. 0.942 ± 0.032, Ki-67: 0.642 ± 0.036 vs. 1.027 ± 0.035, respectively).These differences between the anti-miR-200a group and the miR-NC group were statistically significant (all $P < 0.01$) (**Figures 3**, **4**).

Effect of down-regulated miR-200a on MHCC-97L cells migration

The wound-healing assay showed that the migration of MHCC-97L cells in the anti-miR-200a group was faster than the miR-NC group (as seen in **Figure 5**). Similarly, 24 hours after culture in transwell inserts, the number of MHCC-97L cells migrating through membrane was greater in the anti-miR-200a group compared to the miR-NC group (45.670 ± 6.779 vs.18.870 ± 4.642, $t = 9.786$, $P < 0.01$) (**Figure 6**).

Effect of down-regulated miR-200a on MHCC-97L cells proliferation

Twenty-four and forty-eight hours after transfection, cell proliferation of MHCC-97L cells was faster in the anti-miR-200a group in comparison with the miR-NC group (24 h: 0.767 ± 0.047 vs. 0.600 ± 0.131; 48 h: 1.078 ± 0.320 vs. 0.666 ± 0.180, respectively), as illustrated in **Table 5** and **Figure 7**. All the difference in MHCC-97L cells proliferation displayed statistical significance (all $P < 0.01$).

Discussion

In the human genome, miRs are a class of endogenous small non-coding RNA molecules (19-22 nucleotides) negatively regulating protein coding gene expression at the post-transcriptional level by repressing translation or promoting RNA degradation with some sequence specificity, which is an important conceptual breakthrough in the post genome sequencing era [21]. Recently, an increasingly growing body of evidence has revealed that many miRs express aberrantly in several types of human cancers, and they play crucial roles in tumorigenesis, progression and metastasis of human cancers [22, 23]. The miR-200 family is reported to be a group of evolutionarily conserved miRs, including 5 members (miR-200a, -200b, -200c, -141 and -429), all extensively participating in pathways inhibiting EMT in normal development [18]. However, the function of miR-200a in HCC setting is not well understood; therefore, improved understanding of related miRs is necessary for continued progress in the research of HCC.

Our present study revealed that the expression of anti-miR-200a down-regulated E-cadherin mRNA expression levels in MHCC-97L cells,

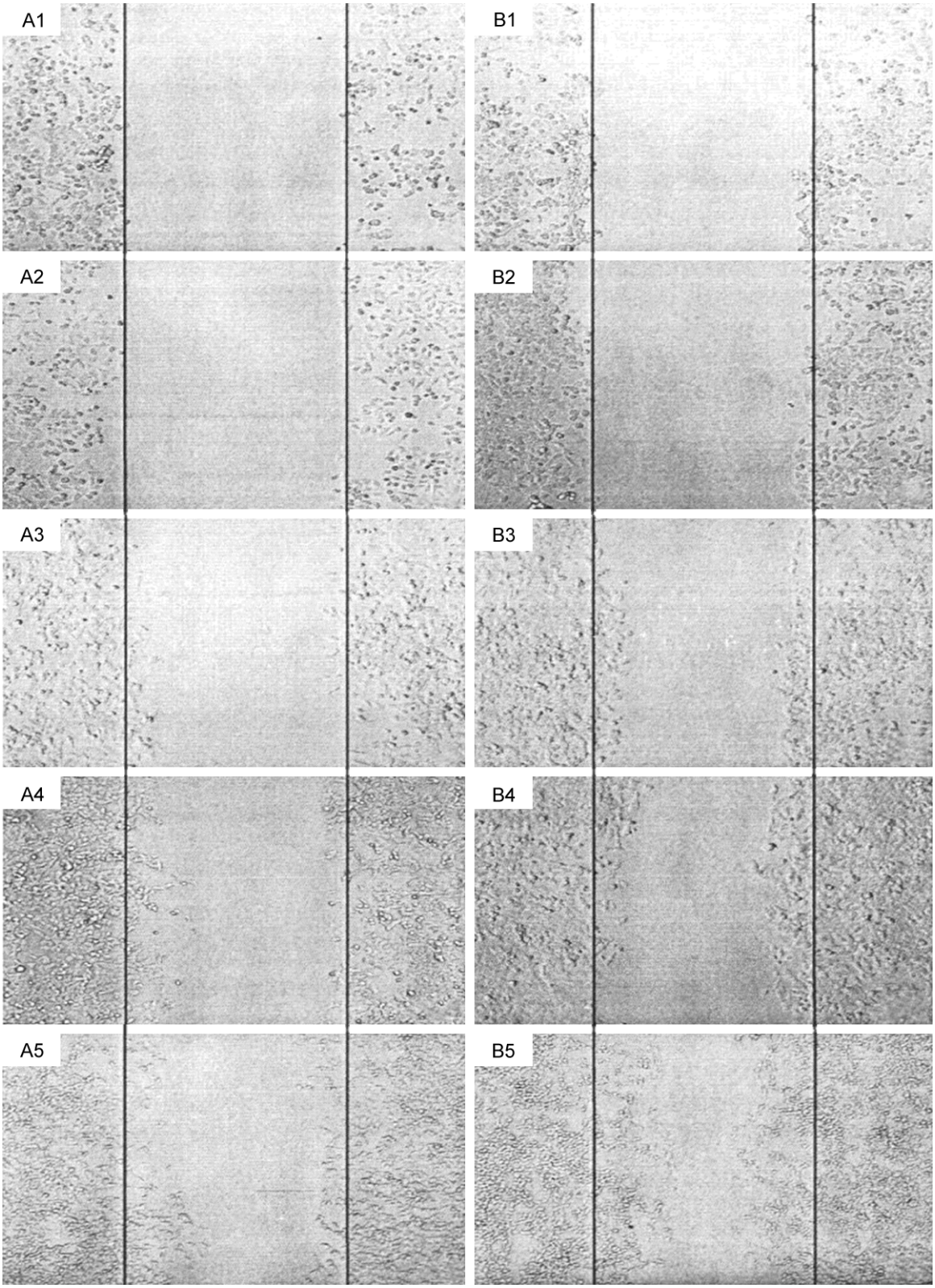

Figure 5. Wound-healing assay results showing faster migration of MHCC-97L cells in the anti-miR-200a group as compared with the miR-NC group. A1-A5: Stands for anti-miR-200a group (0 h, 6 h, 12 h, 24 h, 48 h); and B1-B5: Stands for miR-NC group (0 h, 6 h, 12 h, 24 h, 48 h).

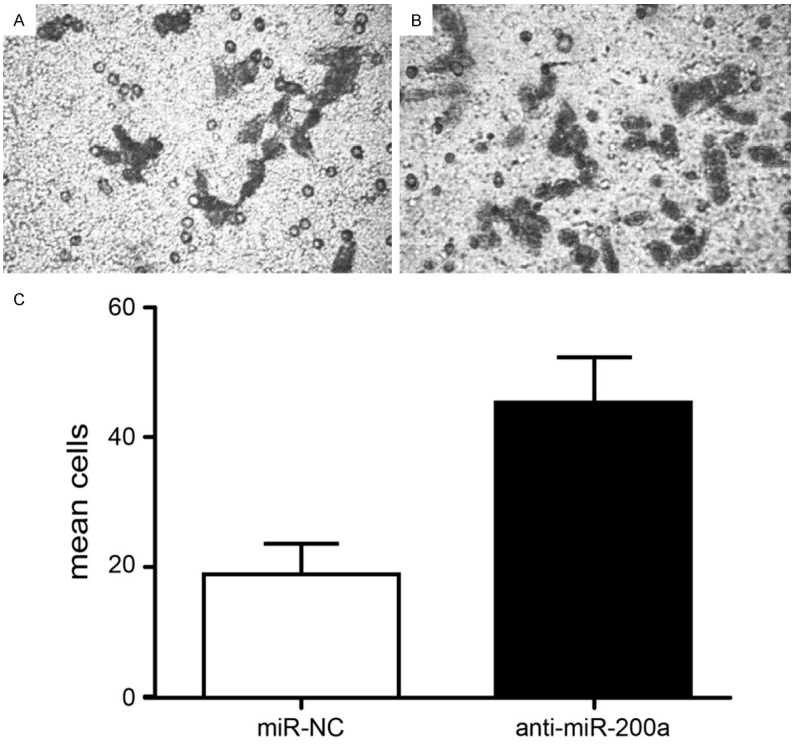

Figure 6. Change of MHCC-97L cells motility in anti-miR-200a group and miR-NC group by Transwell assay showing more MHCC-97L cells migrating through membrane in the anti-miR-200a group 24 hours after culture in transwell inserts. A. Stands for miR-NC group, × 400; B. Stands for anti-miR-200a group, × 400; and C. Stands for the number of migrated MHCC-97L cells.

Table 5. Comparison of proliferation of MHCC-97L cells between anti-miR-200a group and miR-NC group at 24 and 48 hours after transfection by MTT assay

	miR-NC	anti-miR-200a	*t*	*P*
24 h	0.600 ± 0.131	0.767 ± 0.047	3.600	0.002
48 h	0.666 ± 0.180	1.078 ± 0.320	3.366	0.004

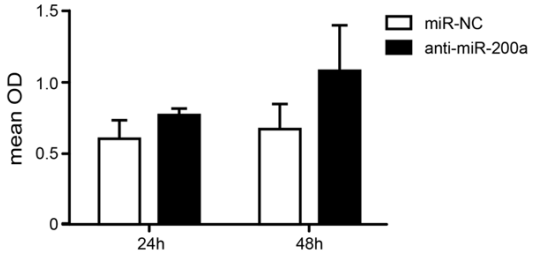

Figure 7. Mean optical density value detected by MTT assay showing faster proliferation of MHCC-97L cells in anti-miR-200a group as compared with miR-NC group at 24 and 48 hours after transfection.

while vimentin mRNA expression was concomitantly up-regulated in these cells compared to the miR-200a group. In addition, the protein expression of E-cadherin and Caspase-3 decreased obviously, while vimentin and Ki-67 protein expression increased significantly in anti-miR-200a group compared with the miR-200a group, implying that MHCC-97L cells lose epithelial features while simultaneously gain the mesenchymal characteristics required for the EMT during cancer metastasis. To this end, we hypothesized that miR-200a dysregulation might be implicated in the malignant transformation of HCC cells through regulating the expression of E-cadherin, vimentin, caspase-3 and Ki-67. However, the exact underlying mechanism of how down-regulated miR-200a expression increased E-cadherin and reduced vimentin expression in HCC cells remains unclear. Based on the functional characterization of miR-200a, it is plausible that increased expression of miR-200a might lead to negative regulation of its gene targets (ZEB1, ETS1 and FLT1), which in turn regulate the expression of E-cadherin and vimentin to trigger an EMT switch in HCC cells [15]. Similarly, Gregory et al. observed at a molecular level that miR-200a suppresses the expression of the E-cadherin transcriptional repressors ZEB1 and ZEB2, transcriptions factors that induce the transition from an epithelial phenotype to a mesenchymal phenotype [24]. Those findings of our study were also in line with a previous study reporting up-regulation of miR-200a in HCC cell lines compared with in normal hepatic cells and in HCC tissues compared with the adjacent non-cancerous hepatic tissues [3]. Therefore, it could be concluded that up-regulated miR-200a might be intimately correlated to cancer metastasis of HCC through promoting EMT.

The proliferation and migration capacity of MHCC-97L cells in the anti-miR-200a group were both increased compared to the miR-

200a group, suggesting that miR-200a might also inhibit proliferation and migration of MHCC-97L cells and protect patients from metastatic behavior of HCC. Thus, our study clearly showed that dysregulated miR-200a in HCC cells might result in aberrant expression of EMT-related genes, such as E-cadherin, Caspase-3, vimentin and Ki-67, triggering an EMT switch in HCC. Furthermore, it has been revealed that during EMT, loss of epithelial markers and gain in the expression of mesenchymal markers may facilitate cells to undergo changes in cell morphology, which is also accompanied with enhanced cell motility and migration [25]. In summary, proliferation and migration of MHCC-97L cells increased as the expression of miR-200a reduced, through effecting the expression of EMT-related genes and promoting EMT in HCC. As well as, Kim et al. have demonstrated that EMT is a key process in tumor progression and metastasis, and stimulated EMT by related genes or miRs including *p53* and miR-200 family might generate the acceleration of proliferation and migration in HCC cells [26].

To conclude, our present study suggests that the down-regulation of miR-200a in HCC cell line promotes EMT in vitro, partly through the regulation of E-cadherin, Caspase-3, vimentin and Ki-67 signaling pathways, giving rise to aggressive tumors. These findings underlined the importance of miR-200a in inhibiting EMT in hepatoma cell line and its role in proliferation and migration of HCC cells, suggesting that miR-200a-based prevention and therapeutics in HCC might be clinically beneficial.

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