Int J Clin Exp Med 2016;9(2):2333-2342
www.ijcem.com /ISSN:1940-5901/IJCEM0017210

Original Article

Effects of miRNA-4804 on proliferation and cytokine secretion function by targeting chemokine-like receptor 1 in human monocytic cell line

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Received October 1, 2015; Accepted December 30, 2015; Epub February 15, 2016; Published February 29, 2016

Abstract: Objective: This study aimed to investigate the effect of miRNA-4804 on the proliferation and cytokine secretion function of human monocytes by targeting chemokine-like receptor 1 (CMKLR1). Methods: The expression levels of miRNA-4804 and cytokine mRNA in human monocytic cell line (THP1) were detected by real-time quantitative PCR. THP1 cell proliferation and surface molecules were detected by CCK8 and flow cytometry. The expression of cytokines in the cell culture supernatant was detected by enzyme-linked immunosorbent assay. The regulatory effect of miRNA-4804 on the candidate target gene CMKLR1 was determined by dual-luciferase reporter assay. Western blot was used to detect the expression of CMKLR1 protein. Results: Compared with the control group, the proliferation and expression of HLA-DR and CD86 molecules of THP1 cell induced with IFN-γ were significantly inhibited by miRNA-4804 mimics and significantly enhanced by miRNA-4804 inhibitors. The expression of IL-6, TNF-α, and IL-12 significantly increased, whereas IL-10 expression decreased in the culture supernatant of THP-1 cells upon IFN-γ stimulation. This alteration in cytokine expression could be antagonistic and promoted after treatment with miRNA-4804 mimics and miRNA-4804 inhibitors. Dual-luciferase reporter assay and Western blot showed that miRNA-4804 inhibited CMKLR1 expression. The expression of CMKLR1 mRNA was inhibited by miRNA-4804 in the THP1 cell line. Conclusion: MiRNA-4804 could regulate the proliferation and cytokine secretion of monocytes by targeting CMKLR1, and it may be involved in the regulation of immune homeostasis in chronic hepatitis B.

Keywords: MicroRNA-4804, monocyte, cell proliferation, cytokine, chemokine-like receptor1

Introduction

Hepatitis B virus (HBV) infection is one of the major public health problems worldwide, despite the availability of modern vaccination and antiviral treatment modalities. About 240 million people have chronic HBV infection, and approximately 650,000 people die annually from complications of this viral infection [1]. Chronic inflammatory and fibrous changes in the liver during HBV infection are mainly propelled by complex interactions between the virus and the host immune system [2]. The host immunological response, particularly the T cell response, plays a key role in the pathogenesis and progression of the disease rather than from direct viral cytopathic effect [3]. Chronic hepatitis B (CHB) has become a treatable and controllable disease but it is not as curable as hepatitis C virus infection. Therefore, new immunotherapeutic and antiviral approaches to control persistent HBV infection are of urgent need.

MicroRNAs (miRNAs) are endogenous non-coding RNAs that regulate gene expression at the post-transcriptional level [4]. MiRNAs have been demonstrated to be involved in cell differentiation, development, metabolism, and development of immune and inflammatory responses [5]. Abnormalities in miRNA have been observed in many human diseases, such as cancer, viral infection, and CHB. Recent studies have revealed that miRNAs play significant roles in the pathogenesis of HBV infection [6, 7]. HBV infection can alter expression patterns of cellular miRNA through the products of HBV replication and expression [8]. Cellular miRNA then regulates the expression and replication of HBV [9]. Some miRNAs (miRNA-146 and miRNA-548) might modulate the immune response of CHB infection and contribute to the persistent infection of HBV [10, 11].

Table 1. The primer sequences of IL-6, TNF-α, IL-12, IL-10 and GAPDH

Gene name	Primer sequences
IL-6 sense:	5' AAGCCAGAGCTGTGCAGATG3'
IL-6 antisense:	5' TTCCCCCACACCAAGTTGAG3'
TNF-α sense:	5' GCTGCACTTTGGAGTGATCG3'
TNF-α antisense:	5' TCACTCGGGGTTCGAGAAGA3'
IL-12 sense:	5' GGAGGCCTGTTTACCATTGGA3'
IL-12 antisense:	5' CTCCCATTAGTTATGAAAGAG3'
IL-10 sense:	5' CGAGATGCCTTCAGCAGAGT3'
IL-10 antisense:	5' CTCAGACAAGGCTTGGCAAC3'
GAPDH sense:	5' AAAGCCTGCCGGTGACTAAC3'
GAPDH antisense:	5' TAGGAAAAGCATCACCCGGAG3'

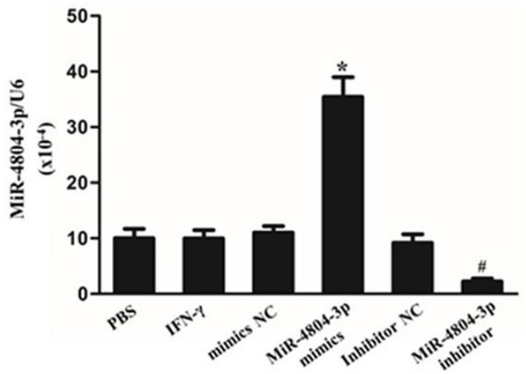

Figure 1. Expression of THP1 cell after transfection of miRNA-4804. Note: *$P<0.01$ and #$P<0.05$ vs. PBS.

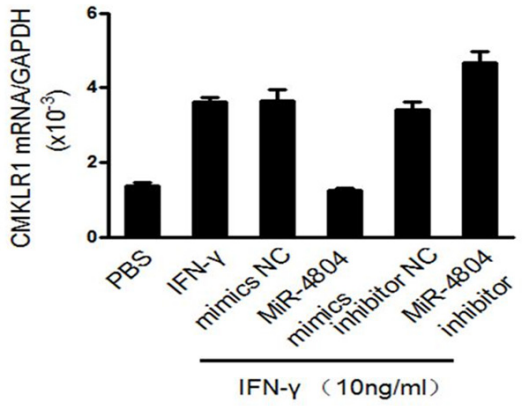

Figure 2. Effect of miRNA-4804 on the expression of CMKLR1 in THP1 cells.

MiRNA-4804 is a highly expressed miRNA in the peripheral blood mononuclear cells (PBMCs) of CHB patients that were screened using miRNA microarrays in a previous study [12]. The functions and molecular mechanisms of miRNA-4804 have not yet been reported. The expression gene of hsa-miR-4804-3p is located in 5q13.2 (72174418-72174490) of the human chromosome [13]. In the present study, THP-1 cell lines were selected to investigate the effect of miRNA-4804 on cell proliferation and secretion of cytokine by flow cytometry, real-time quantitative PCR and enzyme-linked immunosorbent assay (ELISA). The target genes of miRNA-4804 were predicted using bioinformatics software. The regulatory effect of miRNA-4804 on the candidate target gene chemokine-like receptor 1 (CMKLR1) was determined by dual-luciferase reporter assay and Western blot.

Materials and methods

Culture and transfection of THP-1 cell line

THP1 is a human monocytic cell line derived from an acute monocytic leukemia patient. THP-1 cell lines (Shanghai Institute for Biological Science, Shanghai, China) were cultured in RPMI 1640 with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37°C with 5% carbon dioxide. The miRNA-4804 mimic, miRNA-4804 inhibitor, and unrelated sequence positive and negative controls were purchased from GenePharma (Shanghai GenePharma Co., Ltd., Shanghai, China). Recombinant human IFN-γ (PeproTech Inc., Rocky Hill, NJ, USA) was used at a final concentration of 10 ng/mL to induce the THP-1 cells for 48 h. Cells were transfected using lipofectamine transfection reagent (Life Technology, Carlsbad, CA, USA) and harvested 48 h later.

RNA extraction and quantitative detection of miRNA-4804 and CMKLR1

Total RNA was isolated using TRIzol and miRNeasy mini kits (QIAGEN, Duesseldorf, Germany) according to the manufacturer's instructions. MiRNA-4804 and CMKLR1 in THP1 were detected by quantitative RT-PCR. The primers were synthesized by Shanghai Biotechnology Co., Ltd. The primer sequences of U6 and hsa-miR-4804-3p were F: 5'-ATTGGAACGATACAGAGAAGATT-3', R: 5'-GGAACGCTTCACGAATTTG-3'; and GSP: 5'-GCCCTGCTTAACCTTGCCCT-3', R: 5'-TGCAGGGTCCGAGGT-3', respectively. In brief, 1 μL of cDNA, 10 μL of SYBR Green I, 1 μL of 20 μM specific PCR primer F, and 1 μL of 20 μM specific PCR primer R were mixed with water to a total volume of 20 μL. The mixture was carefully glued using parafilm and briefly centrifuged. U6 small nuclear RNA mol-

Table 2. Effect of miRNA-4804 on proliferation of THP1 cell

Groups	A450
Blank	0.875±0.054
IFN-γ	1.695±0.057*
Mimcs NC+ IFN-γ	1.621±0.026*
MiR-4804- mimics + IFN-γ	0.788±0.077#
Inhibitor NC+ IFN-γ	1.619±0.042*
MiR-4804- inhibitor + IFN-γ	1.985±0.008*

Note: *$P<0.05$ vs. Blank groups; #$P<0.05$ vs. IFN-γ groups.

ecule was used as an internal control. The reactions for U6 and hsa-miR-4804-3p were performed as follows: 95°C for 2 min, followed by 40 cycles of 95°C for 10 s and 60°C for 40 s. The primer sequences of CMKLR1 (human NM_0001142343) was 5'-TGGTCTACAGCATCGTCTGC-3' (forward) and 5'-AACAGGAAATCTGCCACTGC-3' (reverse). In brief, 5.0 μL of Master Mix (2×), 0.3 μL of 10 μM PCR specific primer F, 0.3 μL of 10 μM specific PCR primer R, and 1 μL of cDNA were mixed with water to a total volume of 10 μL. Beta-actin was used as an internal control. The reactions for CMKLR1 and beta-actin were performed as follows: 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 60 s.

Proliferation assay of THP-1 cell

The proliferation of THP-1 cells was determined using a Cell Counting Kit (CCK)-8 (Dojindo Laboratories, Kumamoto, Japan). THP-1 cells (cell concentration of 1×10^6/mL) were placed in 96-well plates. Each hole contained 0.1 mL of cells. The cells were divided into six groups: (1) control group (blank): THP-1 cells were treated with PBS; (2) IFN-γ-induced group: THP-1 cells were treated with 10 ng/mL IFN-γ; (3) mimic NC group: THP-1 cells were transfected with an unrelated sequence; (4) miRNA-4804 mimic group: THP-1 cells were transfected with miRNA-4804 mimics; (5) inhibitor NC group: THP-1 cells were transfected with single-stranded RNA; and (6) MiRNA-4804 inhibitor group: THP-1 cells were transfected with miRNA-4804 inhibitors. Subsequently, 10 ng/mL IFN-γ was added after transfection for 6 h, and cells were induced for 48 h. About 10 mL of CCK solution was added to each cell at the end of IFN-γ induction. The culture plate was incubated for 4 h in the incubator. The absorbance at 450 nm was measured using a microplate reader.

Expression of HLA-DR and CD antigens on the surface of THP-1 cells detected by flow cytometry

THP1 cells were cultured for 48 h, washed with cold PBS, and centrifuged at 1500 rpm for 10 min. CD14 mAb labeled with FITC and CD86, CD80, HLA-DR, and IgG labeled with PE (BD Comp., Franklin Lakes, NJ, USA) were added, gently mixed, and incubated at 4°C for 30 min in the dark. The cells were washed twice with cold PBS. The expression levels of CD80, CD86, and HLA-DR were detected by flow cytometry with CD14 gating.

Detection of cytokines in the culture supernatant of THP1 cells

The expression levels of IL-6, TNF-a, IL-12, and IL-10 in the culture supernatant of THP1 cells were detected using an ELISA kit (Wuhan Boshide Biological Engineering Co. Ltd., Wuhan, China) according to the manufacturer's instructions.

Detection of IL-6, TNF-a, IL-12, and IL-10 gene expression in THP1 cells

The mRNA expression levels of IL-6, TNF-α, IL-12, and IL-10 in THP1 cells were detected by quantitative fluorescence PCR. The primers were designed and synthesized by Shanghai Biological Engineering Co., Ltd. (**Table 1**). In brief, 1 μL of cDNA, 10 μL of SYBR Green I, 0.4 μL of 20 μM specific PCR primer F, and 0.4 μL of 10 μM specific PCR primer R were mixed with water to a total volume of 20 μL. GAPDH was used as an internal control. The reactions were performed as follows: 95°C for 30 s, followed by 40 cycles of 95°C for 5 s and 60°C for 34 s.

Prediction and analysis of miRNA-4804 target genes through GO and KEGG

The possible target genes of miRNA-4804 were predicted by Target Scan (http://www.targetscan.org) and miRDB (http://www.mirdb.org/). The intersection of these two databases was obtained. GO and KEGG analyses of possible target genes of miRNA-4804 were performed as previously described [14].

Construction and transfection of luciferase reporter plasmids

The 3'-untranslated region (UTR) fragment of CMKLR1 (1775-2037 nt, GenBank accession number NM_0001142343), which contains putative miRNA-4804 binding sequences (1906-

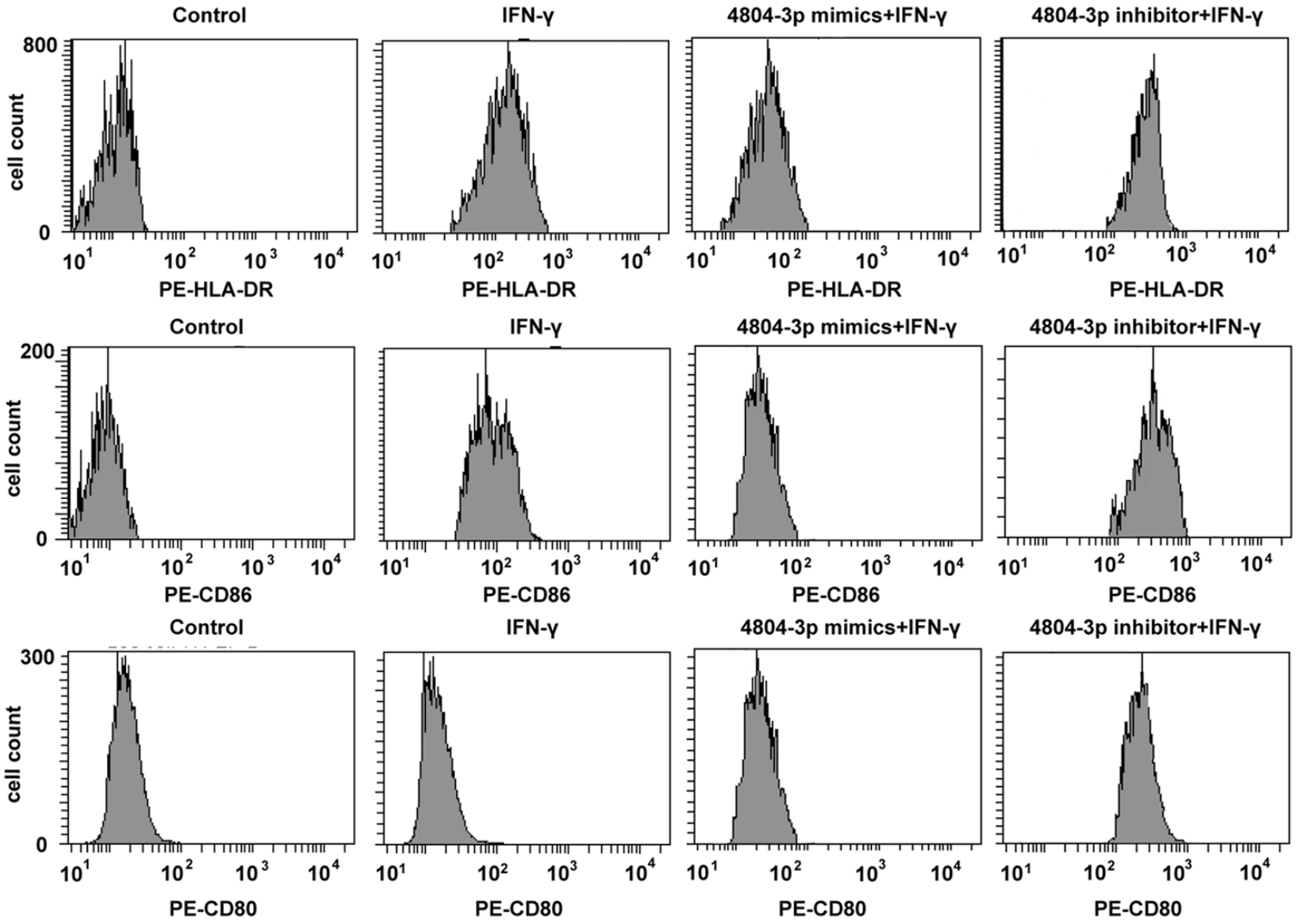

Figure 3. Expression of HLA-DR, CD80 and CD86 in THP-1 cells by flow cytomestry.

Table 3. Effect of miRNA-4804 on expression of HLA-DR and CD in THP1 cell

Groups	HLA-DR	CD86	CD80
Blank	14.88±1.93	3.43±1.36	5.05±2.08
IFN-γ	21.61±3.14*	7.76±0.75*	5.86±1.93
MiR-4804- mimics + IFN-γ	15.85±1.34#	4.06±1.87#	5.34±2.12
MiR-4804-inhibitor+IFN-γ	24.42±4.97*	8.26±1.38*	11.31±2.63*
F	6.282	9.439	5.411
P	0.017	0.005	0.025

Note: *$P<0.05$ vs. Blank groups; #$P<0.05$ vs. IFN-γ groups.

1912 nt), was amplified with the primers. The PCR products were cloned into the firefly luciferase reporter vector psiCHECK (Promega Corporation, Madison, WI, USA) called psiCHECK-CMKLR1. Plasmids carrying the mutated sequence in the complementary sites for the seed regions of CMKLR1 were generated based on psiCHECK-CMKLR1 plasmids through site-specific mutagenesis called psiCHECK-CMKLR1-mut. Transfection was carried out using lipofectamine transfection reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. In brief, 5×10^4 293T cells in a 48-cell plate were transfected with the indicated miRNA-4804 mimic (Shanghai GenePharma, Shanghai, China) or plasmid DNA, and cells were then collected 48 h after transfection for assay. Luciferase activity was measured using a dual-luciferase reporter assay kit (Promega Corporation, Madison, WI, USA), and data were recorded using a multi-plate reader (GloMax, Promega, Madison, WI, USA).

Western blot

Western blot for CMKLR1 was performed as previously described [14]. The gray value of each specific band on the images was digitized using Image J analysis software (NIH, Bethesda, MD, USA). Protein gray values were divided by the internal reference GAPDH to correct errors. Results of a sample represent the relative protein content.

Statistical analysis

Data were expressed as the mean ± standard deviation. The *t*-test was used to compare results between two groups. One-way ANOVA and SNK-q tests were used for multiple comparisons. Data analysis was performed using SPSS17.0 statistical software (SPSS, Inc., Chicago, IL, USA). Statistical significance was set at $P < 0.05$.

Results

Expression of miRNA-4804 and CMKLR1 in THP1 cell lines

The expression levels of miRNA-4804 and CMKLR1 in THP1 cell lines were detected by quantitative fluorescence PCR. The results showed that miRNA-4804 expression in THP1 cells did not change significantly after stimulation with IFN-γ. MiRNA-4804 expression significantly increased after transfection with miRNA-4804 mimics but significantly decreased after transfection with miRNA-4804 inhibitors ($t = 6.523$, $P = 0.003$; $t = 4.387$, $P = 0.012$) (**Figure 1**). Compared with the control group, the expression of CMKLR1 significantly increased in THP1 cells induced with IFN-γ. The expression of CMKLR1 significantly decreased after transfection with miRNA-4804 mimics. CMKLR1 expression significantly increased after transfection with miRNA-4804 inhibitors ($F = 46.25$, $P < 0.001$) (**Figure 2**).

Effect of miRNA-4804 on the proliferation of THP1 cell lines

The effect of miRNA-4804 on the proliferation of THP1 cells was detected by CCK. The results showed that the proliferation of THP1 cells was significantly enhanced after stimulation with IFN-γ compared with that of the blank control. The proliferation of THP1 cells was significantly inhibited and enhanced after transfection with miRNA-4804 mimics and inhibitors, respectively, compared with that of the control group ($F = 285.3$, $P < 0.001$) (**Table 2**).

Effect of miRNA-4804 on the expression of HLA-DR and CD in THP-1 cells induced by IFN-γ

The effect of miRNA-4804 on the expression of HLA-DR and CD antigens in THP1 cells was detected by flow cytometry (**Figure 3**). The expression of HLA-DR and CD86 in THP1 cells was significantly enhanced compared with that in the control group, whereas the expression of CD80 was not changed after induction with IFN-γ. Compared with the control group, the expression levels of HLA-DR and CD86 were significantly inhibited, and CD80 expression demonstrated no obvious change after transfection with miRNA-4804 mimics. The expression of HLA-DR, CD86, and CD80 significantly increased after transfection with miRNA-4804 inhibitors ($F = 6.28$, $P = 0.017$) (**Table 3**).

Table 4. Effect of miRNA-4804 on expression of cytokines in THP1 cell

Groups	IL-6 (pg/ml)	TNF-α (pg/ml)	IL-12 (pg/ml)	IL-10 (pg/ml)
Blank	5.68±0.09	46.82±1.52	7.06±0.67	38.43±1.40
IFN-γ	86.65±2.72	242.69±18.80*	206.65±14.93*	8.81±0.77*
mimcs NC+ IFN-γ	80.25±2.42	225.62±7.60*	199.80±16.33*	9.42±0.96*
MiR-4804 mimics+IFN-γ	6.68±0.35#	50.78±2.40#	15.78±0.56#	148.52±15.47#
inhibitor NC+ IFN-γ	91.62±3.79	254.56±8.01*	217.42±17.23*	7.67±0.46*
MiR-4804 inhibitor+IFN-γ	181.98±9.74*	466.98±20.31*	561.38±20.49*	5.18±1.09*
F	627.20	486.77	602.79	233.82
P	<0.001	<0.001	<0.001	<0.001

Note: *$P<0.05$ vs. Blank groups; #$P<0.05$ vs. IFN-γ groups.

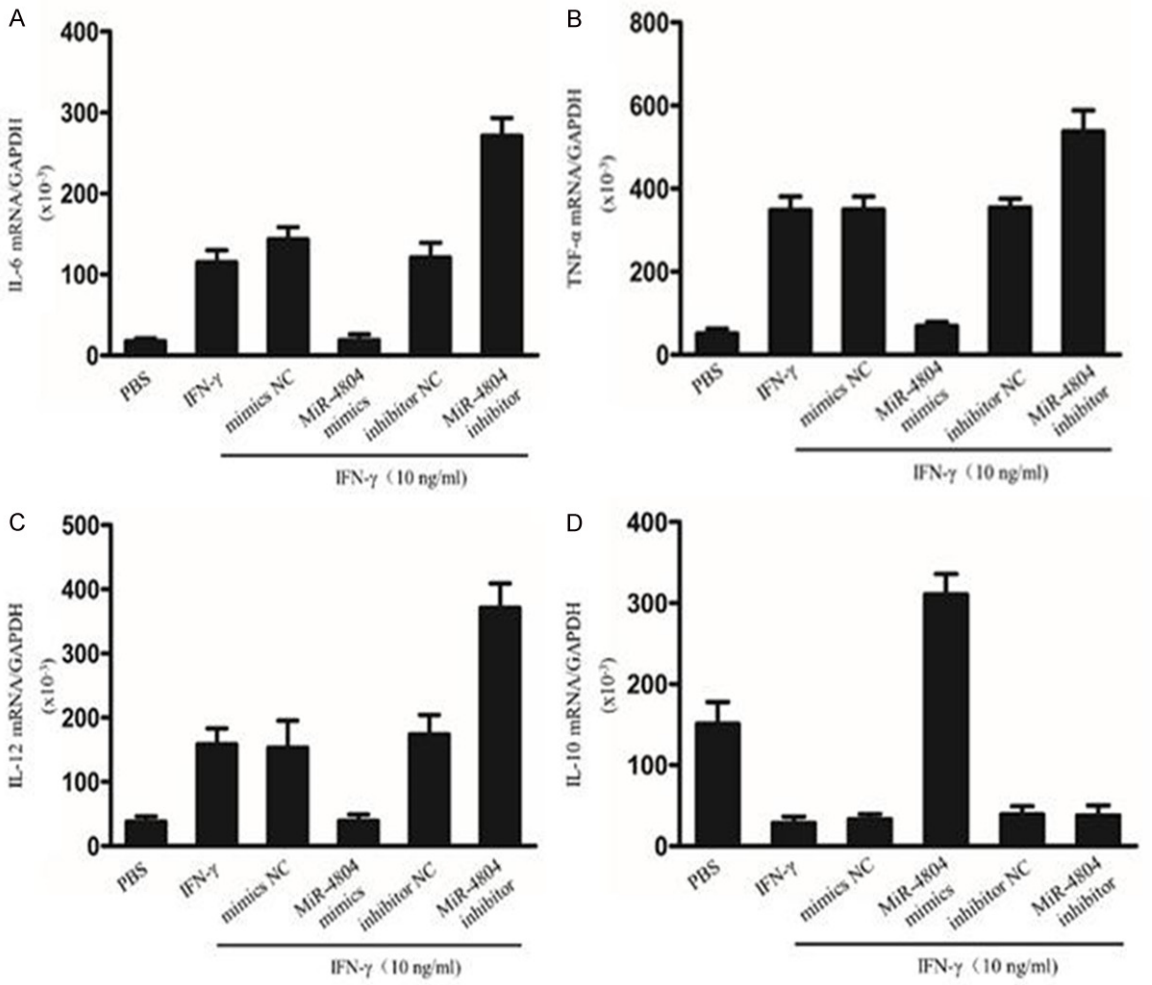

Figure 4. Effect of miRNA-4804 on expression of cytokines in THP1 cell. A: IL-6; B: TNF-α; C: IL-12; D: IL-10.

Effect of miRNA-4804 on the expression of cytokines in the culture supernatant of THP-1 cells

ELISA was used to detect the expression of IL-10, TNF-α, IL-12, and IL-6 in the culture supernatant of THP-1 cells. The expression of IL-6, TNF-α, and IL-12 in THP-1 cells significantly increased, whereas the expression of IL-10 significantly decreased after stimulation with IFN-γ. The role of secreted cytokines in THP-1 cells induced with IFN-γ was antagonistic after transfection with miRNA-4804 mimics, in which the expression of IL-6, TNF-α, and IL-12 was low,

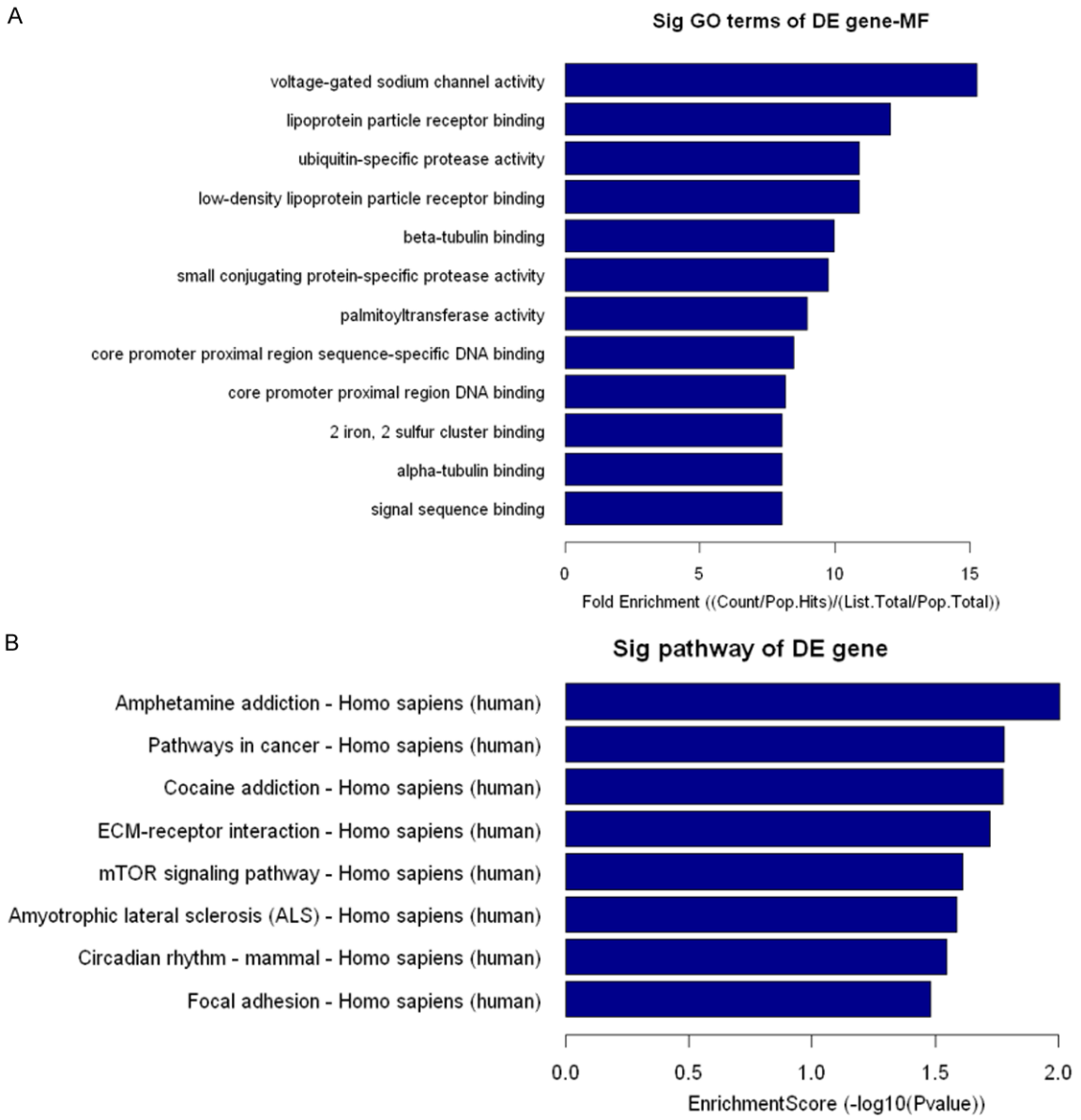

Figure 5. Molecular function of Go analysis of miRNA-4804 target gene (A); Pathway analysis of miRNA-4804 target gene (B).

and IL-10 expression was high. By contrast, the expression levels of IL-6, TNF-a, and IL-12 in THP-1 cells were enhanced, and the expression of IL-10 in THP-1 cells was inhibited after transfection with miRNA-4804 inhibitors ($P < 0.001$) (**Table 4**).

Effect of miRNA-4804 on the expression of the cytokine genes in THP-1 cells

Quantitative fluorescence PCR was used to detect the mRNA expression of IL-10, TNF-α, IL-12, and IL-6 in THP1 cells stimulated by IFN-γ. The mRNA expression of IL-6, TNF-α, and IL-12 was significantly enhanced, whereas the expression of IL-10 mRNA significantly decreased in THP-1 cells after stimulation with IFN-γ. Treatment with miRNA-4804 mimics inhibited the expression levels of IL-6, TNF-a, and IL-12, and promoted IL-10 expression in THP-1 cells. However, treatment with miRNA-4804 inhibitors promoted the expression of IL-6, TNF-α, and IL-12, and inhibited the expression of IL-10 in THP-1 cells induced with IFN-γ ($F = 124.18, 115.89, 215.03, 267.26$, respectively; $P < 0.001$) (**Figure 4**).

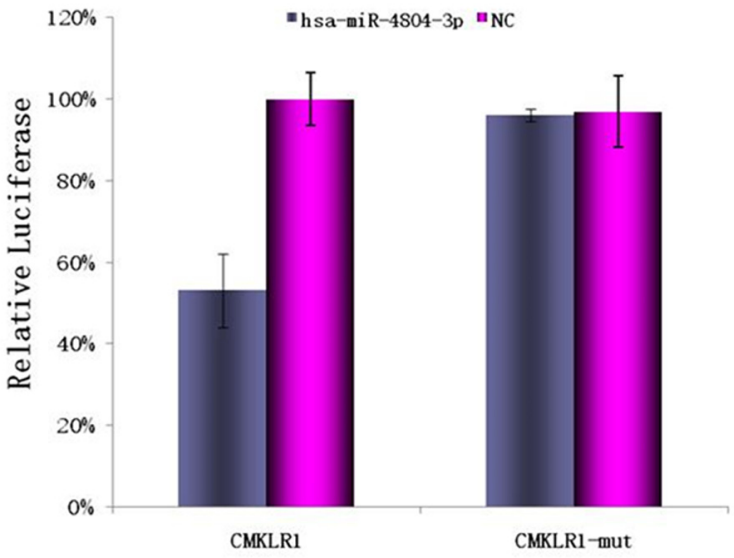

Figure 6. The regulatory effect of miR-4804 on target gene CMKLR1 in 293T cells.

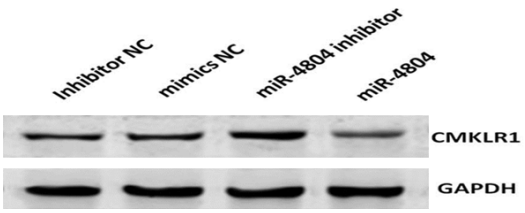

Figure 7. The effect of miR-4804 on the expression of CMKLR1 protein in THP1 cells.

Prediction and analysis of miRNA-4804 target genes

The target genes of miRNA-4804 were analyzed using TargetScan and miRDB database. The number of target genes of miRNA-4804 was 2206 and 143 in TargetScan and miRDB, respectively. Their intersection was 110. CMKLR1 is one of the potential target genes. Sequence analysis showed one binding site of miRNA-4804 in the 3'-UTR sequence of the CMKLR1 gene. GO analysis showed that miRNA-4804 was involved in voltage-gated sodium channel activity, lipoprotein particle receptor binding activity, and ubiquitin-specific protease activity. Pathway analysis demonstrated that miRNA-4804 was involved in cancer, extracellular matrix-receptor interaction, mTOR signal transduction, and focal adhesion (**Figure 5**).

Regulation of miR-4804 on the target gene CMKLR1

The regulatory effect of miR-4804 on the 3'-UTR of the target gene CMKLR1 was detected by dual-luciferase reporter gene assay. The results showed that the ratios of hsa-miR-4804-3p/NC in wild-type CMKLR1 and the control group were 53%+9.1% and 100%+6.4%, respectively (t = 7.42, P = 0.0019). The ratios of hsa-miR-4804-3p/NC in the CMKLR1 mutant strain and the control group were 96%+1.5% and 97%+8.8% (t = 0.19, P = 0.878) (**Figure 6**).

Effects of miR-4804 on the expression of CMKLR1 protein

The effect of miR-4804 on the expression of CMKLR1 protein was detected by Western blot. The values of CMKLR1/GAPDH in inhibitor NC, mimic NC, miRNA-4804 inhibitor, and miRNA-4804 mimic were 0.50, 0.51, 0.67 and 0.36, respectively (**Figure 7**).

Discussion

Monocytes are one of the most active cells in vivo, and they play an important role in innate immune response. Macrophages and dendritic cells are developed from monocytes under the condition of inflammation. HLA-DR, CD80, and CD86 are abundantly expressed during monocyte maturation. Some studies have showed that macrophages and dendritic cells play a defective role in CHB patients [15, 16]. However, Tavakoli et al reported that peripheral blood dendritic cells are phenotypically and functionally intact in chronic hepatitis B virus infection [17]. The present study found that THP1 cell proliferation was significantly enhanced after IFN-γ stimulation, and miRNA-4804 could inhibit the proliferation of monocytes induced with IFN-γ. The expression of HLA-DR and CD86 on the surface of THP1 cells was significantly enhanced after IFN-γ stimulation. The expression of HLA-DR and CD86 was significantly inhibited by miRNA-4804 agonist and significantly increased by miRNA-4804 inhibitors. These results suggested that miRNA-4804 may inhibit the proliferation and expression of

surface molecules of monocytes. Previous studies have found that miRNA-4804 is elevated in PBMCs of CHB patients. MiRNA-4804 might lead to the differentiation and maturation of monocytes by inhibiting the proliferation and surface molecular expression of monocytes, thereby affecting the innate immune response to HBV infection in patients.

Monocytes are stimulated by different stimuli, such as lipopolysaccharide and IFN-γ, which can secrete various cytokines. Studies have shown that alterations in miRNA expression can regulate the innate immune response in HBV infection through Toll-like receptor and the NF-KB pathway [18]. The present study demonstrated that the expression of IL-12, TNF-a, and IL-6 significantly increased, whereas the expression of IL-10 significantly decreased after stimulation with IFN-γ in THP-1 cells. MiRNA-4804 could inhibit the expression of IL-6, TNF-a, and IL-12 and promote the expression of IL-10 in THP1 cells induced by IFN-γ. The results of these genes were similar to those of the proteins. IL-6, TNF-a, and IL-12 are important contributing factors in the inflammatory and immune response, whereas IL-10 is an anti-inflammatory and immune inhibitory factor [19]. The elevation of miRNA-4804 in CHB patients may affect the immune inflammatory reaction by regulating monocyte secretory function and play an important role in the immune homeostasis mechanism. However, the specific regulatory mechanism remains unclear and requires further study.

CMKLR1 is a highly conserved protein that is mainly expressed on the surface of plasmacytoid dendritic cells, macrophages, and monocyte cells in humans. Its ligand is the chemerin, and it is mainly expressed in liver and lung tissues [20]. Previous studies have shown that chemerin can raise CMKLR1 expression in macrophages through an adjacent secretory pathway and accumulates in liver tissues; chemerin is also involved in the liver's inflammatory response and insulin resistance [21, 22]. In the current study, the expression of CMKLR1 in monocytes was upregulated with IFN-γ. This result was similar to the findings reported by Wittamer [23]. Dual-luciferase reporter assay and Western blot showed that miRNA-4804 could inhibit the expression of CMKLR1 mRNA and protein. The expression of CMKLR1 mRNA was inhibited by miRNA-4804 in the THP1 cell line. These results indicated that CMKLR1 could be the potential target gene of miRNA-4804. Activated CMKLR1 may be directly involved in the regulation of inflammatory responses [24]. Thus, miRNA-4804 might regulate the inflammatory response in CHB by targeting CMKLR1. A recent report showed that the expression levels of chemerin and CMKLR1 significantly increase in the liver of patients with chronic hepatitis C [25]. However, the expression of chemerin and CMKLR1, as well as the effects of their interaction, in CHB patients must be explored in future investigations.

In conclusion, the results suggested that miRNA-4804 influenced the proliferation of monocytes and the secretion of cytokines by regulating the expression of CMKLR1, which may play a role in the immune homeostasis of CHB infection.

Acknowledgements

General Program of Jiangsu Province Health Department (H201362). Social development project of Taizhou City (TS201531).

Disclosure of conflict of interest

None.

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References

[1] WHO, The World Health Organization. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. 2015-03-01.

[2] Tsai HT, Tsai TH, Lu TM, Yang CC. Immunopathology of hepatitis B virus infection. Int Rev Immunol 2008; 27: 427-446.

[3] Maini MK, Schurich A. The molecular basis of the failed immune response in chronic HBV: therapeutic implications. J Hepatol 2010; 52: 616-619.

[4] Zhu S, Pan W, Qian Y. MicroRNA in immunity and autoimmunity. J Mol Med (Berl) 2013; 91: 1039-1050.

[5] Lee HM, Nguyen DT, Lu LF. Progress and challenge of microRNA research in immunity. Front Genet 2014; 5: 178.

[6] Xie KL, Zhang YG, Liu J, Zeng Y, Wu H. MicroRNAs Associated With HBV Infection And HBV-

related HCC. Theranostics 2014; 4: 1176-1192.
[7] Pan XB, Ma H, Jin Q, Wei L. Characterization of microRNA expression profiles associated with hepatitis B virus replication and clearance in vivo and in vitro. J Gastroenterol Hepatol 2012; 27: 805-812.
[8] Kohno T, Tsuge M, Murakami E, Hiraga N, Abe H, Miki D, Imamura M, Ochi H, Hayes CN, Chayama K. Human microRNA hsa-miR-1231 suppresses hepatitis B virus replication by targeting core mRNA. J Viral Hepat 2014; 21: e89-97.
[9] Li N, Chen MQ, Cheng Q. Identification of microRNA mediating type I interferon pathway in chronic hepatitis B patients. Chin J Viral Dis 2012; 2: 107-112.
[10] Wang S, Zhang X, Ju Y, Zhao B, Yan X, Hu J, Shi L, Yang L, Ma Z, Chen L, Liu Y, Duan Z, Chen X, Meng S. MicroRNA-146a feedback suppresses T cell immune function by targeting Stat1 in patients with chronic hepatitis B. J Immunol 2013; 191: 293-301.
[11] Winther TN, Bang-Berthelsen CH, Heiberg IL, Pociot F, Hogh B. Differential plasma microRNA profiles in HBeAg positive and HBeAg negative children with chronic hepatitis B. PLoS One 2013; 8: e58236.
[12] Xing T, Xu H, Yu W, Wang B, Zhang J. Expression profile and clinical significance of miRNAs at different stages of chronic hepatitis B virus infection. Int J Clin Exp Med 2015; 8: 5611-5620.
[13] Persson H, Kvist A, Rego N, Staaf J, Vallon-Christersson J, Luts L, Loman N, Jonsson G, Naya H, Hoglund M, Borg A, Rovira C. Identification of new microRNAs in paired normal and tumor breast tissue suggests a dual role for the ERBB2/Her2 gene. Cancer Res 2011; 71: 78-86.
[14] Xing T, Xu H, Yu W, Wang B, Zhang J. MiRNA-548ah, a Potential Molecule Associated with Transition from Immune Tolerance to Immune Activation of Chronic Hepatitis B. Int J Mol Sci 2014; 15: 14411-14426.
[15] Zhang Z, Chen D, Yao J, Zhang H, Jin L, Shi M, Zhang H, Wang FS. Increased infiltration of intrahepatic DC subsets closely correlate with viral control and liver injury in immune active pediatric patients with chronic hepatitis B. Clin Immunol 2007; 122: 173-180.
[16] Op Den Brouw ML, Binda RS, Van Roosmalen MH, Protzer U, Janssen HL, van der Molen RG, Woltman AM. Hepatitis B virus surface antigen impairs myeloid dendritic cell function: a possible immune escape mechanism of hepatitis B virus. Immunology 2009; 126: 280-289.
[17] Tavakoli S, Mederacke I, Herzog-Hauff S, Glebe D, Grün S, Strand D, Urban S, Gehring A, Galle PR, Böcher WO. Peripheral blood dendritic cells are phenotypically and functionally intact in chronic hepatitis B virus (HBV) infection. Clin Exp Immunol 2008; 151: 61-70.
[18] Jiang X, Kanda T, Wu S, Nakamura M, Miyamura T, Nakamoto S, Banerjee A, Yokosuka O. Regulation of microRNA by hepatitis B virus infection and their possible association with control of innate immunity. World J Gastroenterol 2014; 20: 7197-7206.
[19] Ferrari C. HBV and the immune response. Liver International 2015; 35 Suppl 1: 121-128.
[20] Zabel BA, Ohyama T, Zuniga L, Kim JY, Johnston B, Allen SJ, Guido DG, Handel TM, Butcher EC. Chemokine-like receptor 1 expression by macrophages in vivo: regulation by TGF-beta and TLR ligands. Exp Hematol 2006; 34: 1106-1114.
[21] Döcke S, Lock JF, Birkenfeld AL, Hoppe S, Lieske S, Rieger A, Raschzok N, Sauer IM, Florian S, Osterhoff MA, Heller R, Herrmann K, Lindenmüller S, Horn P, Bauer M, Weickert MO, Neuhaus P, Stockmann M, Möhlig M, Pfeiffer AF, von Loeffelholz C. Elevated hepatic chemerin mRNA expression in human non-alcoholic fatty liver disease. Eur J Endocrinol 2013; 169: 547-557.
[22] Krautbauer S, Wanninger J, Eisinger K, Hader Y, Beck M, Kopp A, Schmid A, Weiss TS, Dorn C, Buechler C. Chemerin is highly expressed in hepatocytes and is induced in non-alcoholic steatohepatitis liver. Exp Mol Pathol 2013; 95: 199-205.
[23] Wittamer V, Franssen JD, Vulcano M, Mirjolet JF, Le Poul E, Migeotte I, Brézillon S, Tyldesley R, Blanpain C, Detheux M, Mantovani A, Sozzani S, Vassart G, Parmentier M, Communi D. Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. J Exp Med 2003; 198: 977-985.
[24] Mariani F, Roncucci L. Chemerin/chemR23 axis in inflammation onset and resolution. Inflamm Res 2015; 64: 85-95.
[25] Kukla M, Adamek B, Waluga M, Zalewska-Ziob M, Kasperczyk J, Gabriel A, Mazur W, Sobala-Szczygieł B, Bułdak RJ, Zajęcki W, Kępa L, Ziora K, Żwirska-Korczala K, Wiczkowski A, Hartleb M. Hepatic chemerin and chemokine-like receptor 1 expression in patients with chronic hepatitis C. Biomed Res Int 2014; 2014: 517820.