Clin Exp Med (2016) 16:413–421
DOI 10.1007/s10238-015-0358-1

ORIGINAL ARTICLE

Decreased microRNA miR-181c expression in peripheral blood mononuclear cells correlates with elevated serum levels of IL-7 and IL-17 in patients with myasthenia gravis

Yong Zhang[1] · Mingfeng Guo[2] · Ning Xin[3] · Zhen Shao[1] · Xiuying Zhang[1] · Yanyan Zhang[1] · Jing Chen[1] · Shuangshuang Zheng[1] · Linlin Fu[4] · YuZhong Wang[5] · Dongmei Zhou[4] · Hao Chen[1] · Yan Huang[1] · Ruiguo Dong[1] · Chenghua Xiao[1] · Yonghai Liu[1] · Deqin Geng[1]

Received: 10 December 2014 / Accepted: 5 May 2015 / Published online: 12 May 2015

Abstract miR-181c is a newly identified negative regulator of immune cell activation. In this study, we aimed to investigate the expression and functional role of miR-181c in myasthenia gravis (MG). miR-181c showed significant downregulation in peripheral blood mononuclear cells (PBMCs) from MG patients compared with healthy controls, with lower expression in generalized patients than in ocular ones. MG patients also had increased serum IL-7 and IL-17 levels. Additionally, serum IL-7 level presents a positive correlation with the serum IL-17 level. miR-181c levels were negatively correlated with serum levels of IL-7 and IL-17 in either generalized patients or ocular patients. A luciferase reporter assay revealed that miR-181c could directly bind to the 3′-UTR of interleukin-7. Forced expression of miR-181c led to decreased IL-7 and IL-17 release in cultured PBMCs, while depletion of miR-181c increased the secretion of these two proinflammatory cytokines. The results from our study suggested for the first time that miR-181c was able to negatively regulate the production of proinflammatory cytokines IL-7 and IL-17 in MG patients, and it is a novel potential therapeutic target for MG.

Keywords MicroRNAs · Myasthenia gravis · MiR-181c · IL-7 · IL-17

Introduction

Myasthenia gravis (MG) is considered the classic organ-specific, autoantibody-mediated, human autoimmune disease, and its incidence has been increasing lately. The disorder is caused by antibodies binding to components in the neuromuscular junction [1], impairing neuromuscular transmission. Ptosis and diplopia are frequent onset symptoms. Respiratory muscles can also be affected and lead to myasthenic crisis. The pathogenesis of MG is unclear, but inflammatory cytokines and abnormal immune activation have been suggested to play an important role in MG pathogenesis [1–3].

miRNAs are small noncoding RNAs that can regulate gene expression by binding to complementary target mRNAs and thus inhibit their translation. miRNAs have been identified be critical for fine-tuning many biological processes and offer the prospect of multiple targets [4, 5]. Emerging data suggest that single miRNA species can

Yong Zhang, Mingfeng Guo and Ning Xin have contributed equally to this work.

✉ Yong Zhang
zy20037416@163.com

✉ Ruiguo Dong
dongruiguoxyfy@163.com

1 Department of Neurology, Affiliated Hospital of Xuzhou Medical College, Xuzhou, Jiangsu, China

2 Department of Emergency Medicine, Huai'an First People's Hospital, Nanjing Medical University, Huai'an, Jiangsu, China

3 Department of Neurology, The Second Affiliated Hospital of Soochow University, Soochow University, Suzhou, Jiangsu, China

4 Department of Pathogenic Biology and Lab of Infection and Immunology, Xuzhou Medical College, Xuzhou, Jiangsu, China

5 Department of Neurology, Affiliated Hospital of Jining Medical University, Jining, Shandong, China

profoundly alter the phenotype and outcome of immune responses [6–8].

miR-181c is a newly identified negative regulator of immune cell activation. For example, miR-181c was downregulated in the process of CD4+ T lymphocyte cell activation [9]. Furthermore, miR-181c acts as a suppressor of human PBMC CD4+ T cell activation by inhibiting the translation of IL-2 [9], a helper (Th)1-type cytokine which plays a pathological role in experimental autoimmune myasthenia gravis (EAMG) [10, 11]. Another study demonstrated that TNF-α, one of the most typical proinflammatory cytokines, is also a direct target of miR-181c, and ectopic expression of miR-181c could alleviate microglia-mediated neuronal apoptosis by suppressing TNF-α production [12]. Thus, miR-181c may function as a novel regulator in the inflammatory response. However, its possible role in MG has not been determined yet. Thus, in the present study, we investigated the possible pathogenic role of miR-181c and its mechanisms in MG.

Materials and methods

Study population

Twenty-two patients with MG were enrolled in this study from the Department of Neurology at Affiliated Hospital of Xuzhou Medical College. The diagnosis of MG was made based on the following criteria: typical history and signs of fluctuating weakness of voluntary muscles, presence of serum antiacetylcholine receptor antibodies (AchR Ab), definite clinical improvement on injection of the cholinesterase inhibitor, edrophonium, and decremental pattern on repetitive nerve stimulation [13]. The patients were ranked according to the classification of MG (Osserman) [14]. All patients were classified into one of two groups: ocular MG (12 patients) or generalized MG (10 patients). All patients were seropositive for anti-AchR antibodies. Thymic abnormalities were found in five patients (three had a thymoma and had undergone a thymectomy, and the other two had hyperplastic thymus diagnosed by computed tomography). The patients did not have other autoimmune diseases, ongoing infection, and malignancies. None of the patients had received any immunomodulatory drugs within the past 3 months. The control group consisted of 20 healthy subjects with no inflammatory diseases. Our study received prior approval by local ethic committee, and informed consent was obtained from each subject. The details of the MG patients are summarized in Table 1.

PBMC isolation

About 15 ml of venous blood from each subject was collected into heparinized vacuum tubes. After centrifugation at 3000 rpm for 15 min, the PBMCs were isolated by Ficoll gradient separation with lymphocyte isolation agent (Beijing Solarbio Science and Technology Co., Ltd.). Serum samples were obtained and stored at −80 °C.

miR-181c real-time quantitative RT-PCR

Total RNA from PBMCs of MG patients and the controls was extracted using TRIzol (Invitrogen, USA), and then total RNA was reverse-transcribed to cDNA by using MMLV reverse transcriptase (Epicentre) and looped antisense primer mix. The expression levels of miR-181c were quantified using the stem-loop miRNA qRT-PCR Primer Set (Shanghai Integrated Biotech Solutions, Shanghai, China) according to the manufacturer's instructions. Quantitative real-time PCRs were performed using an Applied Biosystems 7900 Fast Real-Time PCR system with an SYBR Green kit (Shanghai Integrated Biotech Solutions, Shanghai, China). Real-time PCR cycle conditions included the following steps: miR-181c, denaturation at 95 °C for 3 min, followed by 45 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 40 s, and extension at 72 °C for 25 s. To normalize the expression levels of target miRNAs, U6 was used as reference. The relative amounts of miR-181c were calculated using the comparative $C_t(2^{-\Delta\Delta C_t})$ method. hsa-miR-181c sense: 5′ AAC ATTCAACCTGTCGGTGAGT 3′; hsa-miR-181c antisense: 5′ GCAACATTCAACCTGTCGGTG 3′; U6 snRNA sense: 5′ ATTGGAACGATACAGAGAAGATT 3′; U6 snRNA antisense: 5′ GGAACGCTTCACGAATTTG 3′.

Bioinformatic analysis

miR-181c target genes were computationally evaluated using common prediction algorithms provided at TargetScan, miRanda, and miRWalk software searching for conserved sites that matched the seed region of the microRNA, obtained from miRBase database. NCBI and UTRdb genome browsers provided information of human IL-7 gene (NM_000880.3).

miRNA mimics, inhibitor, and plasmids

Both human miR-181c mimics and its control, and human miR-181c inhibitor and its control were synthesized by Shanghai Integrated Biotech Solutions Co., Ltd. The 3′-UTR segments of human IL-7 mRNA (GenBank accession

Table 1 General information of study subjects

Information	MG patients	Ocular MG	Generalized MG (IIa and IIb)	HCs
Number of cases	22	12	10	20
Age (years)	50.86 ± 15.68	47.58 ± 16.90	54.80 ± 13.89	51.30 ± 12.86
Female/male	11/11	5/7	6/4	11/9
Thymoma (undergone thymectomy)	3	3/1	3/2	–
Thymoma/thymic hyperplasia	2	2/1	2/1	–

MG myasthenia gravis, *HCs* healthy controls

The age between two groups (MG/HCs) had no significant differences ($p > 0.05$), and the group ratios were not significantly different ($p > 0.05$)

number NM_000880.3) containing the putative miR-181c binding sequence were inserted into the PmeI and NotI sites of psiCHECK2 vector (Promega, USA), downstream of the stop codon of the firefly luciferase reporter gene. The mutation on miR-181c binding sites in human IL-7 3′-UTRs was generated using the Quick Change XL Site-Directed Mutagenesis kit (Stratagene, La Jolla, CA, USA). The mutant had seven consecutive base pairs replaced at the 3′ region of the site. Wild-type and mutant inserts were confirmed by sequencing.

Luciferase reporter assay

HEK293 cells (1.5×10^5) in 24-well plates were cotransfected with 200 ng firefly luciferase reporter vector containing the target site (psiCHECK2-IL-7 3′-UTR or psiCHECK2-IL-7 3′-UTR-mut), miR-181c mimics (50 nM) or miR-181c mimic negative control (50 nM) using Lipofectamine 2000 (Invitrogen). The cells were harvested for luciferase assays 48 h after transfection. The Dual Luciferase Reporter Assay System (Promega) was used to measure the reporter activity according to the manufacturer's protocol.

PBMC isolation, culture, and transfection

PBMCs were isolated as described above. After being washed twice in phosphate buffered saline (PBS), PBMCs were suspended in RPMI 1640 supplemented with 5 % calf bovine serum (CBS, Life Technologies, Inc.) and 1 % penicillin–streptomycin (Life Technologies, Inc.). The cell suspension was adjusted to a concentration of 2×10^5 cells/ml and cultured for 24 h in a 24-well plastic culture plate in a final volume of 1 ml at 37 °C in 5 % CO 2/95 % air. Subsequently, the PBMCs were transfected with miR-181c mimic or scramble negative control, and miR-181c inhibitor or scramble negative control using HiPerFect Transfection Reagent (QIAGEN, Germany) according to the manufacturer's instructions. To determine the transfection efficiency, PBMCs cultured for 48 h were used to detect the miR-181c expression by real-time PCR. After 48 h of transfection, the supernatants were collected for further analysis of the proinflammatory cytokines IL-7 and IL-17.

Cytokine measurement by enzyme-linked immunosorbent assay (ELISA)

IL-7 and IL-17 levels of serum and cultured supernatants were measured with ELISA kits (eBioscience, USA) according to the manufacturer's instructions.

Statistical analysis

Data are expressed as mean ± SD. Differences between two groups were determined by the two-tailed Student's *t* test, and multiple comparisons were determined by the ANOVA. Serum IL-7 and IL-17 levels, PBMC miR-181c and serum IL-7 levels, and PBMC miR-181c and serum IL-17 levels were correlated by parametric Pearson correlation analysis. *p* values <0.05 were considered statistically significant.

Results

Expression of miR-181c was significantly downregulated in MG patients

To explore the role of miR-181c in the pathogenesis of MG, we separated the PBMCs from the patients and healthy controls, and real-time quantitative RT-PCR was used to detect the expression of miR-181c. As shown in Fig. 1, expression of miR-181c in generalized MG or ocular MG patients was significantly lower than in healthy controls ($p < 0.001$). Furthermore, in two types of patients with MG, the expression levels of miR-181c were also different. There is a significant decrease in PBMC miR-181c levels from patients with generalized MG compared to patients with ocular MG ($p < 0.05$).

Interleukin-7 is a direct target of miR-181c

miRNAs exert their function by posttranscriptional regulation of protein-coding mRNAs. To get an insight into the functions of miR-181c, we predicted its target mRNAs using computational algorithms based on a systematic analysis of the structural requirements for target site function (Targetscan, miRanda, and miRWalk; Fig. 2a). One of the targets predicted by three algorithms was IL-7, a key cytokine for T cell development and survival [15]. The 3′-UTR of the IL-7 transcript contains an evolutionarily conserved binding site for miR-181c (Fig. 2a). The IL-7 3′-UTR containing a wild-type or mutated binding site for miR-181c was cloned into luciferase reporter constructs to experimentally verify the IL-7 transcript as a target of miR-181c. Using a luciferase reporter assay, we found that wild-type IL-7 3′-UTR luciferase activity was significantly ($p < 0.01$) suppressed by cotransfected miR-181c mimics in comparison with nontargeting miRNA (Fig. 2b). Moreover, significantly ($p < 0.01$) lower luciferase activity was detected in the cells transfected by the wild-type IL-7 construct relative to the constructs containing the mutated miR-181c seed region (Fig. 2b), indicating that the IL-7 transcript is a direct target of miR-181c.

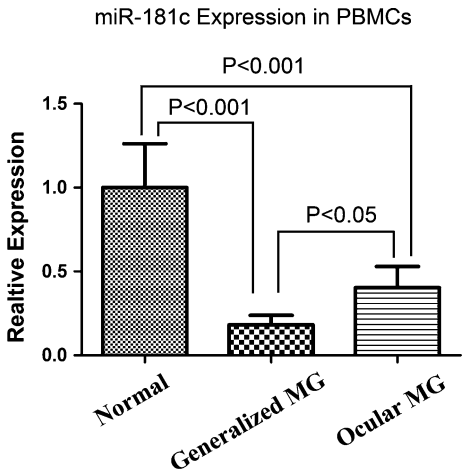

Fig. 1 Expression level of miR-181c in PBMCs. miR-181c was recently identified to be important regulators of inflammatory responses, especially in the mammalian immune system. To confirm the roles of miR-181c in myasthenia gravis (MG), we detected the expression of miR-181c in separated PBMCs from MG patients and the healthy donors. Our results demonstrated that miR-181c was significantly downregulated in generalized MG or ocular MG patients than in normal controls ($p < 0.001$), with lower expression in generalized patients than in ocular ones ($p < 0.05$). Data are representative of three independent experiments with similar results and represent mean ± SD

Serum levels of IL-7 and IL-17 were significantly increased in patients with MG

Considering that miR-181c was downregulated in MG PBMCs and IL-7 is a target for miR-181c, we analyzed IL-7 concentration in serum from MG patients. Recent research indicated that IL-7 drives Th17 cytokine production [16], and we also analyzed IL-17 concentration in serum from MG patients. As shown in Fig. 3a, b, the levels of IL-7 and IL-17 were significantly higher in GMG patients (175.7 ± 23.86 pg/ml; 24.11 ± 5.21 pg/ml) and OMG (134.12 ± 19.02; 17.03 ± 3.18) patients than in healthy controls (77.48 ± 17.93 pg/ml; 9.04 ± 1.89 pg/ml). Furthermore, the levels of IL-7 and IL-17 were significantly

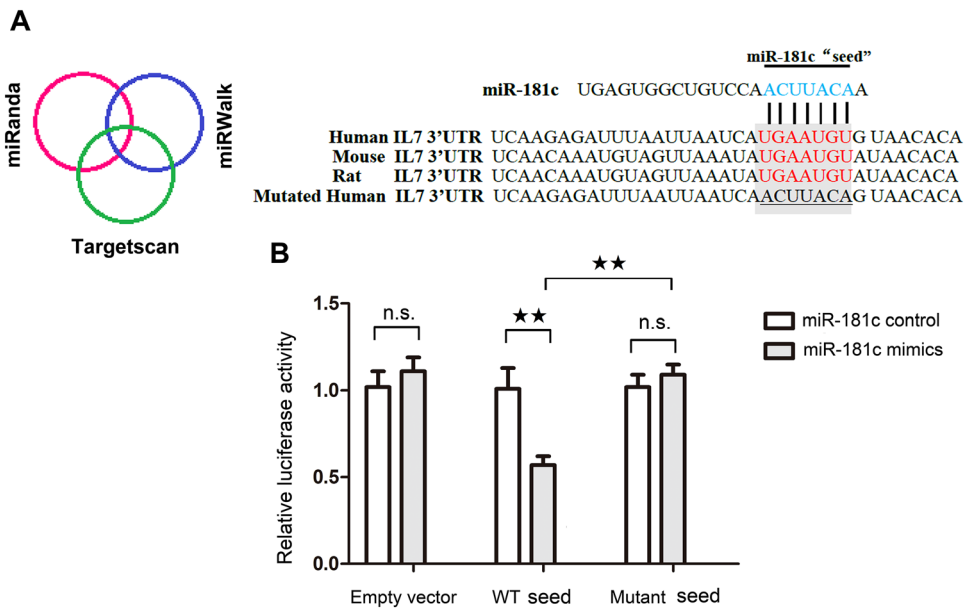

Fig. 2 miR-181c targets IL-7 for translational suppression. **a** Seed match and sequence alignment of IL-7 3′-UTR and miR-181c. *Gray boxes* indicate evolutionary conservation of miR-181c target site. The *underlined part* indicates positions of nucleotide mismatches introduced to the miR-181c binding site. **b** 3′-UTR luciferase reporter assay with wild-type (WT) or mutated IL-7 3′-UTR or no 3′-UTR (empty vector) cotransfected with miR-181c mimics or nontargeting miRNA (control). Relative luciferase activity for three separate transfections is shown. $^{**}p < 0.01$, *n.s.* not significant

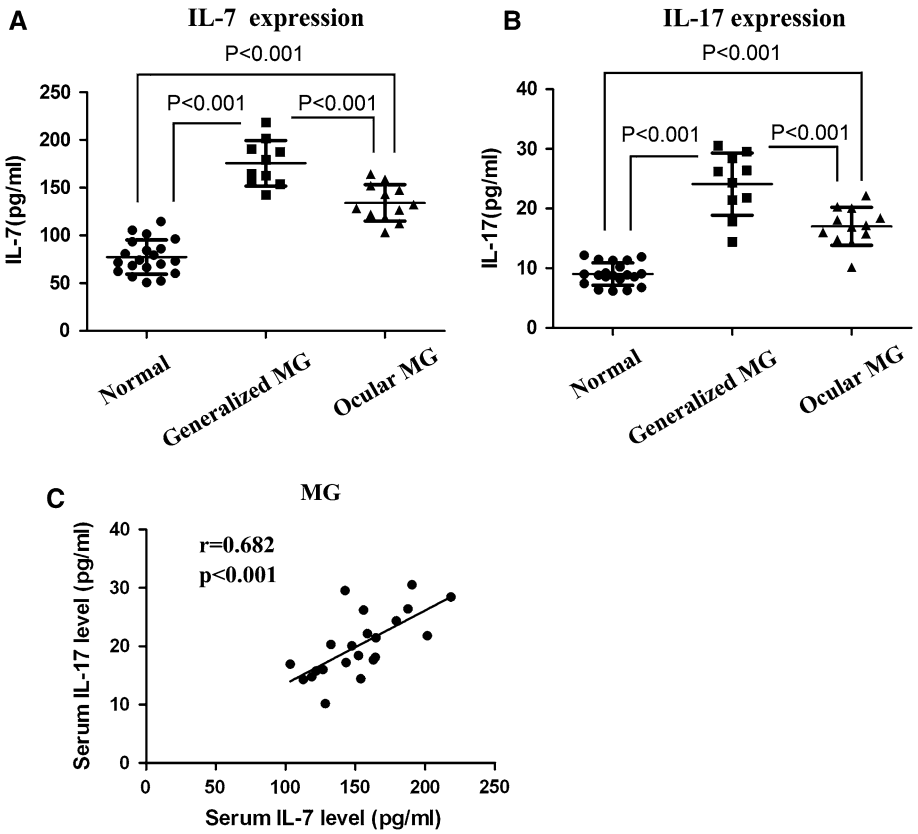

Fig. 3 Serum levels of IL-7 and IL-17 were increased in patients with MG. Considering that miR-181c was downregulated in MG PBMCs and IL-7 is a target for miR-181c, we analyzed IL-7 concentration in serum from MG patients. Recent research indicated that IL-7 drives Th17 cytokine production. We also analyzed IL-17 concentration in serum from MG patients. **a**, **b** The levels of IL-7 and IL-17 were significantly higher in GMG patients (175.7 ± 23.86 pg/ml; 24.11 ± 5.21 pg/ml) and OMG patients (134.12 ± 19.02; 17.03 ± 3.18) than in healthy controls (77.48 ± 17.93 pg/ml; 9.04 ± 1.89 pg/ml). ($p < 0.001$; $p < 0.001$). Furthermore, the levels of IL-7 and IL-17 were significantly higher in GMG patients than in OMG patients ($p < 0.001$; $p < 0.001$). **c** We observed a significant positive correlation between serum IL-7 and IL-17 levels in all MG patients ($r = 0.682$ Pearson's correlation, $p < 0.001$). Data are representative of three independent experiments with similar results and represent mean ± SD

higher in GMG patients than in OMG patients. Importantly, we observed a significant positive correlation between serum IL-7 and IL-17 levels ($r = 0.682$ Pearson's correlation, $p < 0.001$) in the whole cohort of patients with MG (Fig. 3c).

Correlation between miR-181c and IL-7 and correlation between miR-181c and IL-17 in MG patients

To comprehend the relationship between miR-181c and IL-7, and the relationship between miR-181c and IL-17, the correlation between the PBMC miR-181c expression levels and serum IL-7 and IL-17 levels in MG patients was analyzed. We observed a significant inverse correlation between miR-181c and serum IL-7 levels ($r = -0.799$, $p < 0.001$) in the whole cohort of patients with MG (Fig. 4a). Meantime, a significative inverse correlation between miR-181c and serum IL-17 levels was also found in the whole cohort of MG patients ($r = -0.751$, $p < 0.001$) (Fig. 4b).

Effect of miR-181c overexpression or silencing on the cytokine response in cultured PBMCs from patients with MG

As IL-7 and IL-17 have been found to play an important role in immune regulation, a further in vitro study was designed to examine whether the miR-181c had an effect on the production of these two cytokines. We measured the concentrations of IL-7 and IL-17 by ELISA in supernatants of cultured PBMCs after miR-181c and its inhibitor treatment. As shown in Fig. 5a, the miR-181 level was strongly increased 48 h after miR-181c mimic transfection ($p < 0.001$), and it was strongly decreased 48 h after miR-181c inhibitor transfection ($p < 0.01$). The expression levels of IL-7 and IL-17 were significantly downregulated in PBMCs transfected with miR-181c mimics compared

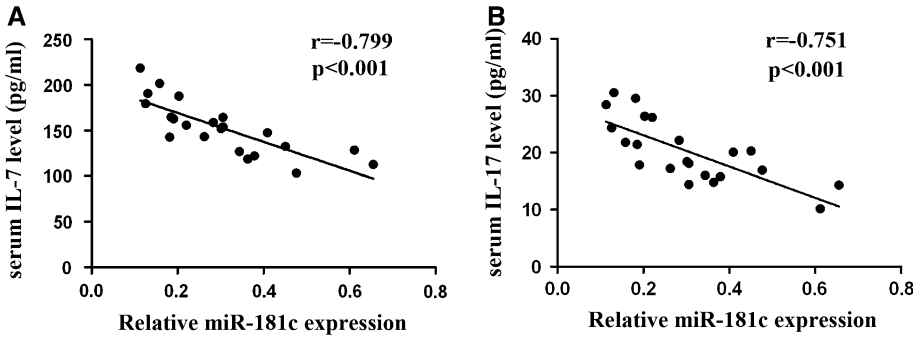

Fig. 4 Correlation between miR-181c and IL-7 and correlation between miR-181c and IL-17 in MG patients. **a** Inverse correlation between PBMC miR-181c and serum IL-7 expression in the whole cohort of patients with MG ($r = -0.799$, $p < 0.001$). **b** Inverse correlation between PBMC miR-181c and serum IL-17 expression in the whole cohort of patients with MG ($r = -0.751$, $p < 0.001$). The correlation between the miR-181c and IL-7 and correlation between the miR-181c and IL-17 were determined by Pearson analysis and linear regression

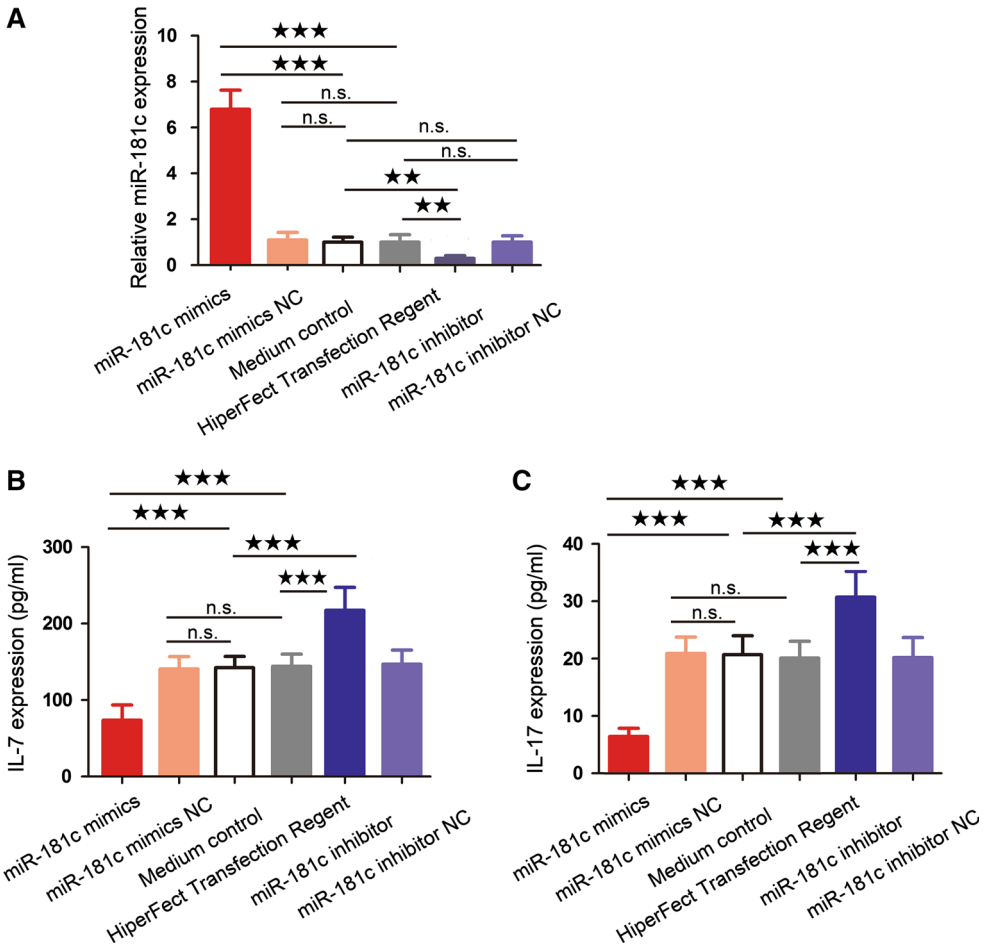

Fig. 5 IL-7 and IL-17 expressions after transfection with miR-181c mimic/inhibitor. **a** Real-time PCR analysis of miR-181c expression in MG ($n = 10$) PBMCs transfected by miR-181c mimics, miR-181c mimic negative control (miR-181c mimic NC), miR-181c inhibitor, miR-181c inhibitor negative control (miR-181c inhibitor NC), and no miR-181c fragments, respectively (**$p < 0.01$; ***$p < 0.001$). **b**, **c** IL-7 and IL-17 levels in culture supernatant were significantly downregulated after miR-181c mimic transfection (***$p < 0.001$), while they were significantly upregulated after miR-181c inhibitor transfection (***$p < 0.001$). Data are representative of three independent experiments with similar results and represent mean ± SD

with cells transfected with miR-181c mimic negative control and blank control/HiPerFect transfection reagent control ($p < 0.001$) (Fig. 5b, c), and these were significantly upregulated in cells transfected with miR-181c inhibitor compared with cells transfected with miR-181c inhibitor negative control and blank control/HiPerFect transfection reagent control ($p < 0.001$) (Fig. 5b, c). Meanwhile, there was no significant difference between cells that were transfected with miR-181c mimic negative control, miR-181c inhibitor negative control, and blank control.

Discussion

The major findings of our study were: (1) miR-181c expression was decreased in PBMCs from MG patients; (2) serum IL-7 and IL-17 levels were increased in MG patients; (3) miR-181c expression was negatively correlated with serum IL-7 and IL-17 levels; and (4) knockdown of miR-181c resulted in increased IL-7 and IL-17 release in cultured PBMCs from MG patients, and its overexpression led to less IL-7 and IL-17 release. Taken together, our results indicate that miR-181c might be involved in the pathogenesis of MG.

Abnormal miR-181c expression in PBMCs has been reported in multiple sclerosis (MS) [17], a prototypic autoimmune inflammatory disorder of the central nervous system. More importantly, miR-181c levels were found to be significantly downregulated in cerebrospinal fluid (CSF) of patients with secondary progressive MS (SPMS) compared with those with relapsing–remitting MS (RRMS) [18]. Toll-like receptors (TLRs) are a family of receptors through which the innate immune system of the host recognizes the presence of invading pathogens, and inappropriate TLR signaling may result in the autoimmune diseases [19]. A recent study identified an important role for the miR-181c-TLR4 pathway in hypoxic microglial activation and neuroinflammation. miR-181c was found to inhibit TLR4 expression by binding to its 3′-UTR, thus inhibiting NF-kB activation and the production of downstream proinflammatory mediators [20]. Hence, miR-181c is likely to be of importance in neuroimmunological diseases.

To address the function of miR-181c that is specifically downregulated in PBMCs from MG patients, experiments were performed to identify the target gene for miR-181c. Among the potential direct mRNA targets of miR-181c, a number of targets have been identified in murine and in human systems, such as IL-7, IL-1A, IL-1B, TNF-α, IL-25, and IL-16. In this study, we focused on IL-7 as a target gene of miR-181c in view of its role in promoting differentiation and function of multiple effector T cell subsets, especially IFN-γ or IL-17-producing T cells [16, 21, 22]. Experiments with gene-deficient mice indicate that Th17 cells and IL-17 play a crucial role in the pathogenesis of EAMG [23, 24]. Moreover, previous studies have shown that serum IL-17 concentrations were higher in MG patients compared with controls and correlated with anti-AchR antibody titers [25, 26].

In addition to supporting T cell development and homeostasis, high concentration of IL-7 can lead to increased B cell survival and antibody production in the presence of T cells [27]. Consequently, IL-7 plays a pathogenic role in multiple autoimmune diseases, including rheumatoid arthritis (RA) [28, 29], inflammatory bowel disease (IBD) [30], psoriasis [31], spondylarthritis [32], and type 1 diabetes [33].

The 3′-UTR of the IL-7 transcript contains an evolutionarily conserved miR-181 binding site, and our luciferase reporter assay confirmed IL-7 as a direct target gene of miR-181c. Next we measured IL-7 expression in serum from MG patients and found a significantly increased expression of IL-7 in MG patients compared with healthy controls, with higher serum levels of IL-7 in generalized MG than in ocular MG. We also found that the level of IL-7 in serum presents a positive correlation with the expression level of IL-17 in the whole cohort of patients with MG. The positive correlation between IL-7 and IL-17 may be partly explained by IL-7-induced significant cell proliferation and secretion of IL-17 by PBMCs and CD4(+) T cells [16, 34].

Correlation analysis further revealed that PBMC miR-181c presents an obvious negative correlation with the serum levels of both IL-7 and IL-17 in MG patients. Generally, IL-7 is produced by stromal cells at lymphopoietic sites in the bone marrow, gut, spleen, thymus, and lymph nodes [35]. However, expression of IL-7 was recently found in PBMCs from patients with metabolic syndrome and healthy persons [36]. In the present study, we also detected considerable amount of IL-7 in cultured MG PBMCs, but we only detected small amount of IL-7 in cultured healthy control PBMCs (data not shown). To further explore the mechanisms involved in the cytokine regulation of miR-181c, we genetically manipulated its expression in gain- and loss-of-function experiments to explore whether miR-181c could influence in vitro cytokine production. The result showed that knockdown of miR-181c significantly enhanced production of IL-7 and IL-17 in cultured MG PBMCs, while overexpression of miR-181c resulted in a significant decrease in the expression of these two inflammatory cytokines.

It is worthwhile to point out that there are some limitations in our study. In our present experiment condition, the specific cell types expressing miR-181c and responsible for IL-7 production in the culture system were not investigated. In fact, unlike in normal conditions, dendritic cells and macrophages have the ability to express significantly high levels of IL-7 in pathological conditions [37, 38]. Then, in the inflammatory context of MG patients, whether miR-181c was downregulated in dendritic cells and macrophages, leading to increased IL-7 production, needs to be confirmed by more detailed study in the future. Moreover, previous studies have demonstrated that downregulation of miR-181c in activated human PBMC CD4(+) T lymphocytes could increase the IL-2 expression [9], and IL-2 could induce DC activation [39]. So we speculate that

the miR-181c-downregulated CD4+ T cells from MG patients could produce more IL-2 and thus promote DC activation and secretion of IL-7.

Overall, our results are the first demonstration that miR-181c expression was downregulated and associated with the severity of MG. miR-181c was able to negatively regulate the production of proinflammatory cytokines IL-7 and IL-17. miR-181c might serve as a potential therapeutic target in the treatment of MG and other inflammatory diseases.

Acknowledgments We would like to thank all patients and normal control persons for their collaboration. This work was supported by National Nature Science Foundation of China (81072465), Natural Science Fund of the Educational Committee of Jiangsu Province (10KJD320003), Special foundation of president of the Xuzhou Medical College (2010KJZ01), Key medical talents fund of Jiangsu Province (H201130), Jiangsu Province ordinary university postgraduate research innovation fund (CXLX11_0734).

Conflict of interest The authors have no financial conflict of interest.

References

1. Vincent A. Unravelling the pathogenesis of myasthenia gravis. Nat Rev Immunol. 2002;2(10):797–804.
2. Meriggioli MN, Sanders DB. Autoimmune myasthenia gravis: emerging clinical and biological heterogeneity. Lancet Neurol. 2009;8(5):475–90.
3. Berrih-Aknin S. Myasthenia gravis: Paradox versus paradigm in autoimmunity. J Autoimmun. 2014;52:1–28.
4. O'Connell RM, Rao DS, Chaudhuri AA, Baltimore D. Physiological and pathological roles for microRNAs in the immune system. Nat Rev Immunol. 2010;10:111–22.
5. Pillai RS, Bhattacharyya SN, Filipowicz W. Repression of protein synthesis by miRNAs: how many mechanisms? Trends Cell Biol. 2007;17:118–26.
6. Xiao C, Srinivasan L, Calado DP, et al. Lymphoproliferative disease and autoimmunity in mice with increased miR-17-92 expression in lymphocytes. Nat Immunol. 2008;9:405–14.
7. Thai TH, Calado DP, Casola S, et al. Regulation of the germinal center response by microRNA-155. Science. 2007;316:604–8.
8. Rebane A, Runnel T, Aab A, et al. MicroRNA-146a alleviates chronic skin inflammation in atopic dermatitis through suppression of innate immune responses in keratinocytes. J Allergy Clin Immunol. 2014;134(4):836–47.
9. Xue Q, Guo ZY, Li W, et al. Human activated CD4(+) T lymphocytes increase IL-2 expression by downregulating microRNA-181c. Mol Immunol. 2011;48(4):592–9.
10. Poussin MA, Goluszko E, David CS, Franco JU, Christadoss P. HLA-DQ6 transgenic mice resistance to experimental autoimmune myasthenia gravis is linked to reduced acetylcholine receptor-specific IFN-γ, IL-2 and IL-10 production. J Autoimmun. 2001;17(3):175–80.
11. Paas-Rozner M, Sela M, Mozes E. The nature of the active suppression of responses associated with experimental autoimmune myasthenia gravis by a dual altered peptide ligand administered by different routes. Proc Natl Acad Sci USA. 2001;98(22):12642–7.
12. Zhang L, Dong LY, Li YJ, Hong Z, Wei WS. The microRNA miR-181c controls microglia-mediated neuronal apoptosis by suppressing tumor necrosis factor. J Neuroinflamm. 2012;6(9):211.
13. Drachman DB. Medical progress: myasthenia gravis. N Engl J Med. 1994;330:1797–810.
14. Osserman KE, Genkins G. Studies in myasthenia gravis: review of a twenty-year experience in over 1200 patients. Mt Sinai J Med. 1971;38:497–537.
15. Fry TJ, Mackall CL. The many faces of IL-7: from lymphopoiesis to peripheral T cell maintenance. J Immunol. 2005;174:6571e6.
16. Bikker A, Moret FM, Kruize AA, Bijlsma JW, Lafeber FP, van Roon JA. IL-7 drives Th1 and Th17 cytokine production in patients with primary SS despite an increase in CD4 T cells lacking the IL-7Rα. Rheumatology (Oxford). 2012;51(6):996–1005.
17. Ma X, Zhou J, Zhong Y, et al. Expression, regulation and function of microRNAs in multiple sclerosis. Int J Med Sci. 2014;11(8):810–8.
18. Haghikia A, Haghikia A, Hellwig K, et al. Regulated microRNAs in the CSF of patients with multiple sclerosis: a case–control study. Neurology. 2012;79(22):2166–70.
19. Abdelsadik A, Trad A. Toll-like receptors on the fork roads between innate and adaptive immunity. Hum Immunol. 2011;72(12):1188–93.
20. Zhang L, Li YJ, Wu XY, Hong Z, Wei WS. MicroRNA-181c negatively regulates the inflammatory response in oxygen–glucose-deprived microglia by targeting Toll-like receptor 4. J Neurochem. 2015;132(6):713–23.
21. Hartgring SA, Bijlsma JW, Lafeber FP, van Roon JA. Interleukin-7 induced immunopathology in arthritis. Ann Rheum Di. 2006;65(Suppl 3):iii69–74.
22. Hartgring SA, Willis CR, Bijlsma JW, Lafeber FP, van Roon JA. Interleukin-7-aggravated joint inflammation and tissue destruction in collagen-induced arthritis is associated with T-cell and B-cell activation. Arthritis Res Ther. 2012;14(3):R137.
23. Wang W, Milani M, Ostlie N, et al. C57BL/6 mice genetically deficient in IL-12/IL-23 and IFN-γ are susceptible to experimental autoimmune myasthenia gravis, suggesting a pathogenic role of non-Th1 cells. J Immunol. 2007;178(11):7072–80.
24. Bai Y, Liu R, Huang D, et al. CCL2 recruitment of IL-6-producing CD11b+ monocytes to the draining lymph nodes during the initiation of Th17-dependent B cell-mediated autoimmunity. Eur J Immunol. 2008;38(7):1877–88.
25. Roche JC, Capablo JL, Larrad L, et al. Increased serum interleukin-17 levels in patients with myasthenia gravis. Muscle Nerve. 2011;44(2):278–80.
26. Zheng S, Dou C, Xin N, et al. Expression of interleukin-22 in myasthenia gravis. Scand J Immunol. 2013;78(1):98–107.
27. Sammicheli S, Ruffin N, Lantto R, Vivar N, Chiodi F, Rethi B. IL-7 modulates B cells survival and activation by inducing BAFF and CD70 expression in T cells. J Autoimmun. 2012;38(4):304–14.
28. Hartgring SA, Willis CR, Alcorn D, et al. Blockade of the interleukin-7 receptor inhibits collagen-induced arthritis and is associated with reduction of T cell activity and proinflammatory mediators. Arthritis Rheum. 2010;62(9):2716–25.
29. Pickens SR, Chamberlain ND, Volin MV, et al. Characterization of interleukin-7 and interleukin-7 receptor in the pathogenesis of rheumatoid arthritis. Arthritis Rheum. 2011;63(10):2884–93.
30. Totsuka T, Kanai T, Nemoto Y, et al. IL-7 Is essential for the development and the persistence of chronic colitis. J Immunol. 2007;178(8):4737–48.
31. Bonifati C, Trento E, Cordiali-Fei P, et al. Increased interleukin-7 concentrations in lesional skin and in the sera of patients with plaque-type psoriasis. Clin Immunol Immunopathol. 1997;83:41–4.
32. Rihl M, Kellner H, Kellner W, et al. Identification of interleukin-7 as a candidate disease mediator in spondylarthritis. Arthritis Rheum. 2008;58(11):3430–5.

33. Hofer J, Hofer S, Zlamy M, et al. Elevated proportions of recent thymic emigrants in children and adolescents with type 1 diabetes. Rejuvenation Res. 2009;12(5):311–20.
34. Yang Y, Xiao X, Li F, Du L, Kijlstra A, Yang P. Increased IL-7 expression in Vogt–Koyanagi–Harada disease. Invest Ophthalmol Vis Sci. 2012;53(2):1012–7.
35. Mackall CL, Fry TJ, Gress RE. Harnessing the biology of IL-7 for therapeutic application. Nat Rev Immunol. 2011;11(5):330–42.
36. Saidijam M, Tootoonchi AS, Goodarzi MT, et al. Expression of interleukins 7 and 8 in peripheral blood mononuclear cells from patients with metabolic syndrome: a preliminary study. Indian J Med Res. 2014;140(2):238–43.
37. Harnaha J, Machen J, Wright M, Lakomy R, Styche A, Trucco M, Makaroun S, Giannoukakis N. Interleukin-7 is a survival factor for CD4+ CD25+ T-cells and is expressed by diabetes-suppressive dendritic cells. Diabetes. 2006;55(1):158–70.
38. Jana M, Mondal S, Jana A, Pahan K. Interleukin-12 (IL-12), but not IL-23, induces the expression of IL-7 in microglia and macrophages: implications for multiple sclerosis. Immunology. 2014;141(4):549–63.
39. Hu X, Cao Y, Meng Y, Hou M. A novel modulation of structural and functional changes of mouse bone marrow derived dendritic cells (BMDCs) by interleukin-2(IL-2). Hum Vaccin Immunother. 2015;11(2):516–21.