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RESEARCH PAPER

miR-106b-5p induces immune imbalance of Treg/Th17 in immune thrombocytopenic purpura through NR4A3/Foxp3 pathway

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ABSTRACT

Background: Immune imbalance of regulatory T cells (Treg)/T helper 17 cells (Th17) contributes to the development of immune thrombocytopenic purpura (ITP). The dysregulation of miRNAs is important in the development of ITP. However, the role of miR-106b-5p in Treg/Th17 imbalance remains unknown in ITP.

Materials and methods: Peripheral blood was collected from patients with ITP and healthy controls, and CD4 + T cells were further isolated. miR-106b-5p, nuclear receptor subfamily 4 group A member 3 (NR4A3), forkhead box protein 3 (Foxp3), IL-17A, and TGF-β expressions were detected by qRT-PCR, western blot, or ELISA. The effect of miR-106b-5p on NR4A3 was detected by dual-luciferase reporter gene assay.

Results: Compared with healthy controls, miR-106b-5p was elevated in peripheral blood of patients with ITP, and NR4A3 expression was decreased. sh-NR4A3 significantly decreased Foxp3 and TGF-β expressions, indicating that NR4A3 may regulate Treg differentiation via Foxp3. Additionally, NR4A3 was identified to be a target of miR-106b-5p, and miR-106b-5p was able to negatively modulate NR4A3 expression. Moreover, we found miR-106b-5p induced immune imbalance of Treg/Th17 through NR4A3. *In vivo* experiments revealed that silencing miR-106b-5p promoted Treg differentiation and increased the number of platelets, suggesting the relief of ITP.

Conclusion: miR-106b-5p regulated immune imbalance of Treg/Th17 in ITP through the NR4A3/Foxp3 pathway.

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KEYWORDS
mir-106b-5p; NR4A3; immune imbalance; immune thrombocytopenic purpura

Introduction

Immune thrombocytopenic purpura (ITP) is an autoimmune disease due to the abnormal T cell response, which is characterized by antiplatelet autoantibodies destroying platelets [1]. The incidence of ITP was 29 per 1 million person-years, especially in children and in those >60 years of age [2]. Currently, more advanced treatments have been made to control ITP. For example, caplacizumab, an immunoglobulin fragment of the bivalent variable domain, decreases the incidence of ITP-related death and the recurrence of ITP [3]. Preemptive rituximab reduces long-term recurrence in ITP [4]. However, the recurrence rate of ITP remains unsatisfied.

Abnormal function of T lymphocytes can cause the occurrence of ITP [5]. CD4+ CD25+ Foxp3+ regulatory T cells (Treg) play an important role in maintaining peripheral immune tolerance [6]. Researchers have found that the number of Treg in peripheral blood of patients with ITP is remarkably reduced than healthy controls [7]. Th17, a subset of T helper (Th) cells, play an important role in inflammation and autoimmune diseases [8]. The number of Th17 in peripheral blood of patients with ITP is remarkably increased than healthy controls [9]. Emerging pieces of evidence indicate that Treg/Th17 imbalance contributes to the development of ITP [10,11]. However, the underlying mechanism that regulating Treg/Th17 imbalance is still not fully understood.

MicroRNAs (miRNAs) are a class of small non-coding RNAs that regulate gene expression at the post-transcriptional level [12]. More and more researches have shown that dysregulation of miRNAs is involved in the development of autoimmune diseases [13,14]. miR-106b-5p is widely expressed in cancer cells and can act as

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an oncogene or tumor suppressor gene in many cancers [15,16]. Recently, researchers have found that miR-106b-5p in peripheral blood mononuclear cells of patients with ITP is remarkably upregulated than healthy controls using microarray technology [17], indicating that miR-106b-5p may exert function in ITP. However, the regulation mechanism of miR-106b-5p in ITP is still not clear.

In this study, we determine the regulation role of miR-106b-5p in the immune imbalance of Treg/Th17, and whether miR-106b-5p induces Treg/Th17 imbalance through NR4A3/Foxp3 pathway, which may provide potential targets for the treatment of ITP.

Materials and methods

Patients

A total of 20 patients with ITP and 20 healthy controls (NC) were recruited in this study. There were 12 male patients and 8 female patients, aged from 2.5 to 12 years old. Patients with ITP were diagnosed according to the ITP diagnosis criteria of The Subspecialty Group of Hematology, the Society of Pediatrics, Chinese Medical Association. There were also 12 male healthy controls and 8 female healthy controls, aged from 2.4 to 12.5 years old. There was no significant difference in age and sex between patients with ITP and healthy controls. All patients signed informed consents. The experiment was approved by the Ethics Committee of Children's Hospital of Soochow University.

CD4 + T cells isolation and transfection

Peripheral blood was collected from patients with ITP and healthy controls. Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood using Ficoll 400 reagent (Sigma-Aldrich, Missouri, USA) by density gradient centrifugation. CD4 + T cells were isolated from PBMCs using Dynabeads Untouched Human CD4 T Cells Kit (Invitrogen, CA, USA) according to the manufacturer's instructions. The bead-bound cells were depleted, and the remaining negatively isolated human CD4 + T-cells were analyzed in a flow cytometer.

CD4 + T cells were incubated in RPMI 1640 medium (Gibco, NY, USA) for 72 h. Then, CD4 + T cells were seeded in 6-well culture plates for 24 h with RPMI 1640 medium which does not contain penicillin-streptomycin. Lentiviral vector pLKO.1-sh-NR4A3 (sh-NR4A3), overexpressing sequence miR-106b-5p mimic, silencing sequence miR-106b-5p inhibitor were transfected into CD4 + T cells. pLKO.1-sh-control (NC) and scramble sequence was set as a negative control.

Induction of Treg and Th17

CD4 + T cells were seeded in 24-well culture plates pre-coated with 5 μg/ml anti-CD3 antibody and 1 μg/ml soluble anti-CD28 antibody in RPMI 1640 medium supplemented with 10% fetal bovine serum (Gibco), 100 U/ml penicillin and 50 μg/ml streptomycin. For inducing Th17 differentiation, the medium was added with 10 ng/ml IL-6, 10 ng/ml IL-1β, 10 ng/ml TGF-β1, 10 ng/ml IL-23, 10 μg/mL anti-IL-4 and 10 μg/mL anti-IFN-γ. For inducing Treg differentiation, the medium was added with 300 U/ml IL-2, 10 ng/ml TGF-β1, 10 μg/mL anti-IL-4 and 10 μg/mL anti-IFN-γ. The cells were cultured at 37°C for 96 h and collected for further use.

Quantitative real-time RCR (qRT-PCR)

Total RNAs from peripheral blood and CD4 + T cells under different treatments were isolated using TRIzol reagent (Beyotime Biotechnology, Shanghai, China), and inversely transcribed into cDNA using Transcriptor High Fidelity cDNA Synthesis Kit (Roche, Basel, Switzerland). qRT-PCR was conducted on QuantStudio 5 Real-Time PCR System using SYBR Green qPCR Mix (Beyotime Biotechnology) for the detection of miR-106b-5p, NR4A3 mRNA, Foxp3 mRNA, IL-17A mRNA, and TGF-β mRNA expression. The relative expression was calculated with the $2^{-\Delta\Delta Ct}$ method, with U6 or GAPDH as internal references.

ELISA

IL-17A and TGF-β concentrations of serum from patients with ITP and healthy controls, or CD4 + T

cells with different treatments were measured by ELISA kits (Invitrogen) according to the manufacturer's instructions.

Dual-luciferase reporter gene assay

NR4A3 wild type sequence (NR4A3-WT) or NR4A3 mutant sequence (NR4A3-Mut) was inserted into pTK luciferase reporter (Thermo Scientific, CA, USA) and named as pTK-NR4A3-WT and pTK-NR4A3-Mut. Then, 293 T cells were co-transfected with miR-106b-5p mimic and pTK-NR4A3-WT or pTK-NR4A3-Mut using Lipofectamine 2000 reagent (Invitrogen). The relative luciferase activity was measured with the Dual-Luciferase reporter assay system (Promega, Wisconsin, USA).

Western blot

Total proteins from CD4 + T cells under different treatments were isolated using Radio Immunoprecipitation Assay (RIPA) buffer (Beyotime Biotechnology), and the concentration of protein was measured by BCA Protein Assay Kit (Beyotime Biotechnology). Protein was electrophoresed by 12% SDS-PAGE and transferred to polyvinylidene fluoride membranes (Invitrogen). The membranes were blocked with 5% nonfat dry milk and incubated with primary antibodies anti-Foxp3 (1:1000; Abcam, Cambridge, USA), anti-NR4A3 (1:500; Abcam), anti-β-actin (1:5000; Abcam) overnight at 4°C, then incubated with secondary antibody conjugated to HRP (1:4000; Abcam) for 2 h at 25°C. The bands were visualized using enhanced chemiluminescence (ECL) kit (Beyotime Biotechnology) and protein level was quantified using Image J software.

Flow cytometry analysis of the percentage of Treg and Th17

The spleen was collected from the mice, cut into pieces, and ground into homogenate. The homogenate was passed through a 100 μm cell strainer and centrifuged at 200 × g for 5 min. The cells were re-suspended in RPMI 1640 medium and stained with CD4 monoclonal antibodies (eBioscience, CA, USA) for 30 min. Then, cells were stained with PE-conjugated anti-IL-17 monoclonal antibody (eBioscience), and the percentage of Th17 in CD4 + T cells was measured by a flow cytometer (BD Biosciences). For the detection of the percentage of Treg in CD4 + T cells, PE-conjugated anti-CD4 monoclonal antibody (eBioscience), PE-conjugated anti-CD25 monoclonal antibody (eBioscience) and PE-conjugated anti-Foxp3 monoclonal antibody (eBioscience) were added and incubated for 30 min.

In vivo experiments

BALB/c mice were obtained from the laboratory animal center of Soochow University. ITP mice were induced through intraperitoneal injection of antiplatelet antibody MWReg30 (Abcam) using a micro osmotic pump. The micro osmotic pump was filled with sterile saline containing 100 μl MWReg30 (82.5 μg/ml) and bovine serum albumin (1.5 mg/ml) and inserted into the peritoneal cavity of mice. The MWReg30 was released at a rate of 0.5 μl/h for 168 h. Mice in the treatment group were injected with adenovirus vector carrying miR-106b-5p inhibitor by caudal vein four days after the establishment of the ITP model. The mice were divided into the blank control group (n = 5), ITP group (n = 5), and treatment group (n = 5). All mice were sacrificed 168 h later. The spleen and bone marrow were collected from each mouse for HE staining. Orbital blood was collected from each mouse for the detection of TGF-β and IL-17A concentration. Blood was collected from the caudal vein of mice to count the number of platelets. The animal experiment was approved by the Ethics Committee of Children's Hospital of Soochow University.

Statistical analysis

SPSS18.0 software was used to analyze all the data. Data were expressed as mean±SD. The difference between the two groups was tested by Student's *t*-test, and the difference among groups was tested by the one-way ANOVA test. P value<0.05 was regarded as statistical significance.

Results

miR-106b-5p was elevated in peripheral blood of patients with ITP

As shown in Figure 1(a), miR-106b-5p expression was significantly elevated in peripheral blood of patients with ITP (ITP group) than healthy controls (NC group). NR4A3 mRNA level was significantly downregulated in the ITP group than the NC group (Figure 1(b)). The correlation analysis between miR-106b-5p and NR4A3 showed that the NR4A3 mRNA level was negatively correlated with miR-106b-5p expression (Figure 1(c)). In addition, there was no significant change in IL-17A concentration between NC group and ITP group (Figure 1(d)), whereas TGF-β (Treg cell marker molecule) concentration was significantly downregulated in ITP group (Figure 1(e)), indicating that the percentage of Treg cells was decreased, thereby inducing immune imbalance of Treg/Th17.

NR4A3 regulated Treg differentiation

The previous report has shown that NR4A3 activated the promoter of Foxp3 to drive Treg differentiation [18]. To further investigate whether NR4A3 could regulate Foxp3 expression in ITP, CD4 + T cells were isolated from PBMC of healthy controls and patients with ITP. As shown in Figure 2(a), sh-NR4A3 decreased NR4A3 and Foxp3 expressions while having no significant effect on miR-106b-5p expression. In addition, in response to the sh-NR4A3 transfection, the mRNA level and concentration of TGF-β was decreased (Figure 2(b,d)) while the mRNA level and concentration of IL-17A didn't change obviously(Figure 2 (b,c)), indicating that NR4A3 may regulate the differentiation of Treg through Foxp3.

miR-106b-5p induced immune imbalance of Treg/Th17

According to the bioinformatics software, there were binding sites between NR4A3 and miR-106b-5p. To confirm their binding, NR4A3-WT (or NR4A3-Mut) and miR-106b mimic were co-transfected into 293 T cells. The results showed that the relative luciferase activity of NR4A3-WT was significantly decreased in the miR-106b mimic group, whereas the relative luciferase activity of NR4A3-Mut did not significantly change after the

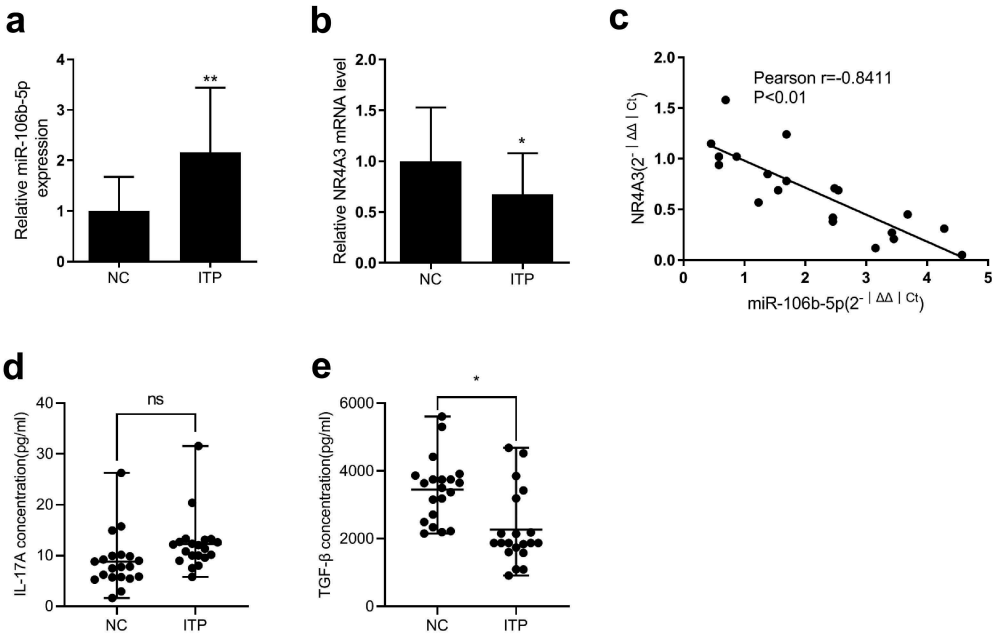

Figure 1. miR-106b-5p was elevated in the peripheral blood of patients with ITP. Peripheral blood samples were collected from healthy controls (n = 20) and patients with ITP (n = 20). (a). miR-106b-5p expression was detected in the healthy control group (NC) and ITP group using qRT-PCR. (b). NR4A3 mRNA level was detected using qRT-PCR. (c). The correlation analysis between miR-106b-5p and NR4A3. (d). IL-17A concentration in the NC group and ITP group was detected using ELISA. (e). TGF-β concentration was detected using ELISA. *p < 0.05, **p < 0.01 vs NC.

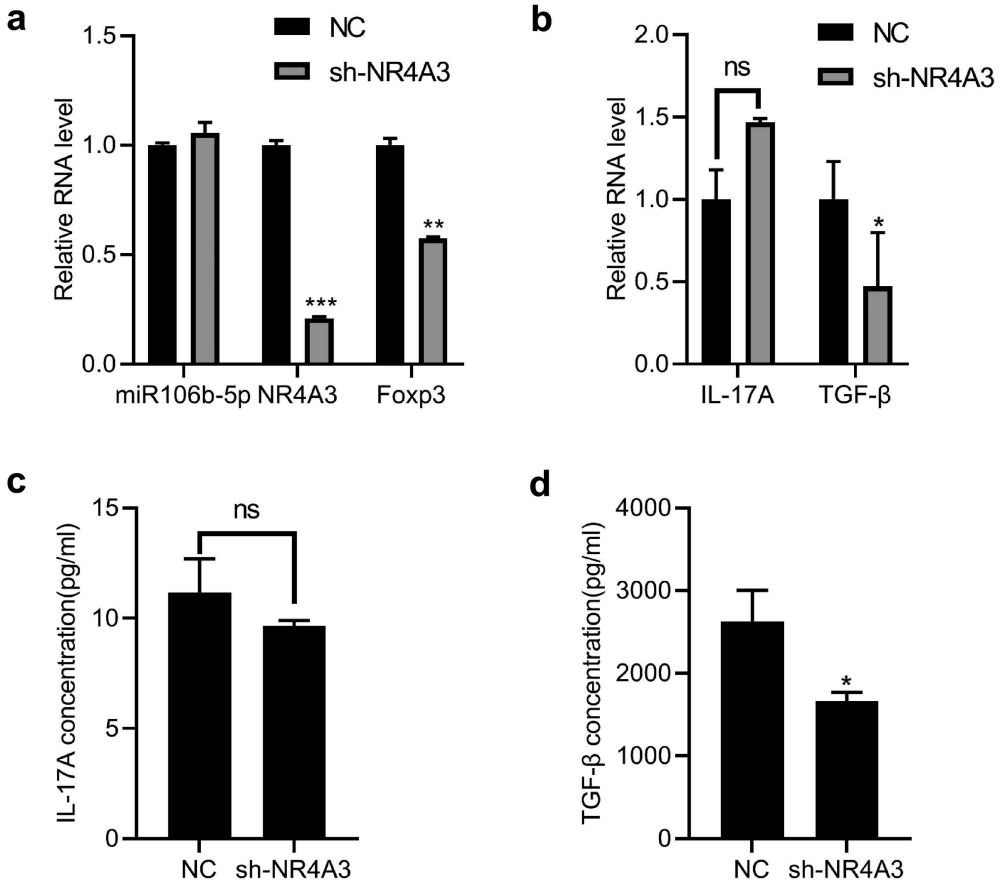

Figure 2. NR4A3 promoted Foxp3 and TGF-β expression. CD4 + T cells were isolated from PBMC in the NC group and ITP group. Then, CD4 + T cells were transfected with lentiviral vector pLKO.1-sh-NR4A3 or pLKO.1-sh-control (NC). (a). miR-106b-5p, NR4A3 and Foxp3 expressions were detected in the NC group and sh-NR4A3 group using qRT-PCR. (b). IL-17A and TGF-β mRNA levels were detected using qRT-PCR. (c). IL-17A concentration was detected using ELISA. (d). TGF-β concentration was detected using ELISA. *p < 0.05, **p < 0.01 vs NC.

treatment of miR-106b mimic (Figure 3(a)). Then, CD4 + T cells from healthy controls were transfected with miR-106b-5p mimic. As shown in Figure 3(b,c), miR-106b-5p mimic significantly increased miR-106b-5p expression in CD4 + T cells and significantly decreased mRNA and protein levels of NR4A3 and Foxp3. In addition, miR-106b-5p mimic significantly decreased the mRNA level and concentration of TGF-β (Figure 2(e,g) while having no effect on the mRNA level and concentration of IL-17A (Figure 2(d,f). Therefore, we speculated that miR-106b-5p inducing the immune imbalance of Treg/Th17 via reducing the Treg differentiation. To confirm our speculation, miR-106b-5p transfection was performed to silence miR-106b-5p in CD4 + T cells. The results of Figure 3(h–m) proved that the interference of miR-106b-5p could promote CD4 + T cells polarized toward a Treg phenotype.

miR-106b-5p regulated immune imbalance of Treg/Th17 through NR4A3

To determine whether miR-106b-5p regulated the percentage of Treg through NR4A3, CD4 + T cells from healthy controls were transfected with miR-106b-5p mimic or NR4A3 + miR-106b-5p mimic. We found that miR-106b-5p mimic significantly decreased TGF-β concentration, and NR4A3&miR-106b-5p-overexpress reversed the inhibition effect of miR-106b-5p mimic on TGF-β concentration (Figure 4(a)). However, miR-106b-5p mimic or NR4A3&miR-106b-5p-overexpress did not significantly change IL-17A concentration (Figure 4(b)). These findings indicated that miR-106b-5p regulated the percentage of Treg through NR4A3. We further detected TGF-β, IL-17A, Foxp3, and NR4A3 expressions in CD4 + T

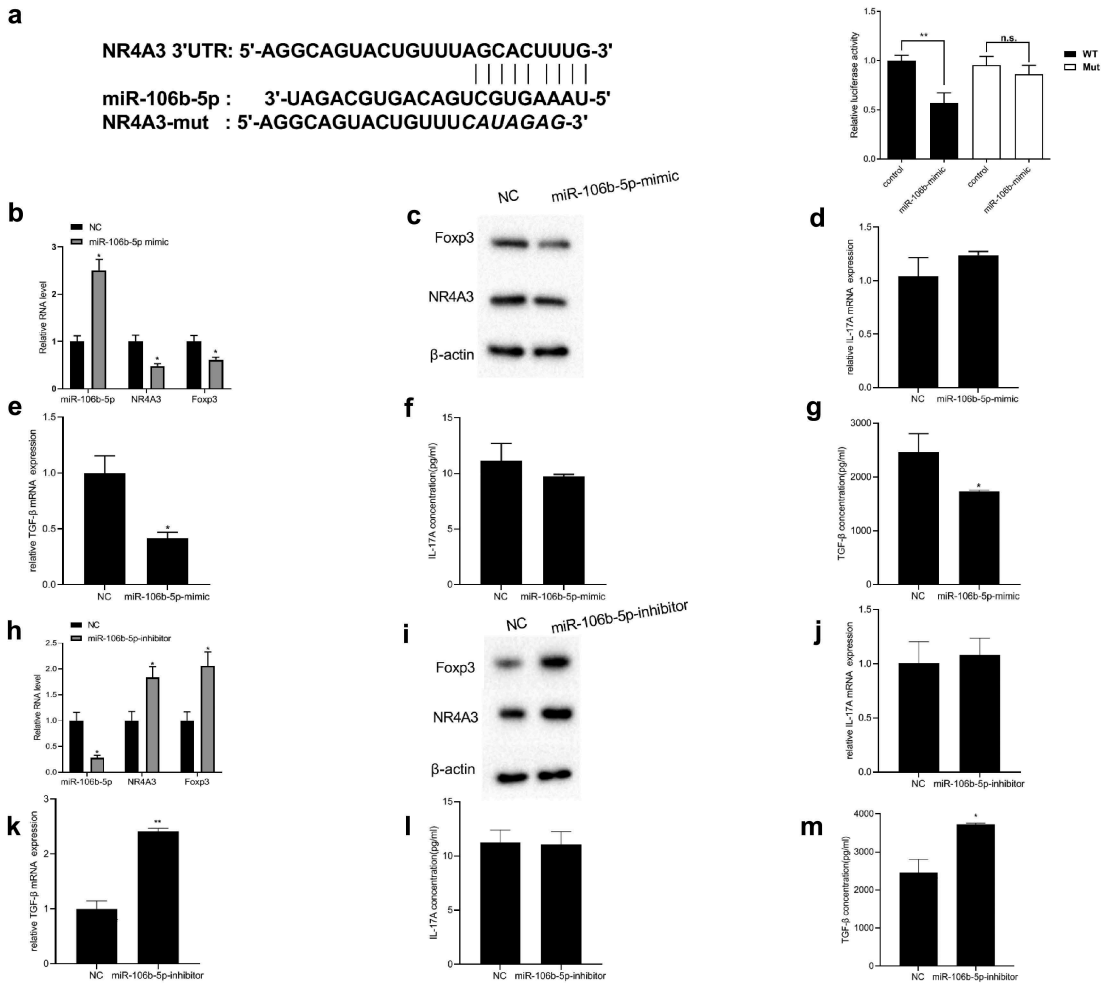

Figure 3. NR4A3 is a target of miR-106b. (a). The binding sites between NR4A3 and miR-106b-5p. Luciferase reporter gene assay was used to confirm the binding of NR4A3 and miR-106b-5p. **p < 0.01 vs control. CD4 + T cells from healthy controls were transfected with miR-106b-5p mimic. NC was a negative control. (b). miR-106b-5p,NR4A3, and Foxp3 expressions were detected in the NC group and miR-106b-5p mimic group using qRT-PCR. (c). Protein levels of NR4A3 and Foxp3 were detected in the NC group and miR-106b-5p mimic group using western blot. (d,e). IL-17A and TGF-β mRNA levels were detected using qRT-PCR. (f,g). IL-17A and TGF-β concentrations were detected using ELISA. CD4 + T cells from patients with ITP were transfected with miR-106b-5p inhibitor. NC was a negative control. (h). miR-106b-5p,NR4A3, and Foxp3 expressions were detected in the NC group and miR-106b-5p inhibitor group using qRT-PCR. (i). Protein levels of NR4A3 and Foxp3 were detected using western blot. (j,k). IL-17A and TGF-β mRNA levels were detected using qRT-PCR. (l,m). IL-17A and TGF-β concentrations were detected using ELISA. *p < 0.05, **p < 0.01 vs NC.

cells. Results showed that miR-106b-5p mimic significantly decreased TGF-β mRNA level, Foxp3 mRNA and protein levels, and NR4A3 mRNA and protein levels. On the contrary, in cells transfected with NR4A3+ miR-106b-5p mimic, the NR4A3 overexpression abolished the effect of miR-106b-5p mimic on cells, increased the expression levels of TGF-β, Foxp3, and NR4A3 (Figure 4(c,d)). The above data indicated that the promoting effect of miR-106b-5p on the imbalance of Treg/Th17 depended on its negative regulation of NR4A3.

Silencing miR-106b-5p promoted Treg differentiation and increased the number of platelets

We established the ITP mice model to observe the effect of adenovirus vector carrying miR-106b-5p inhibitor on the relief of ITP *in vivo*. Firstly, the miR-106b-5p expression was obviously decreased in CD4 + T cells of the miR-106b-5p inhibitor group, indicating the infection of miR-106b-5p inhibitor was worked (Figure 5(b)). The miR-106b-5p interference

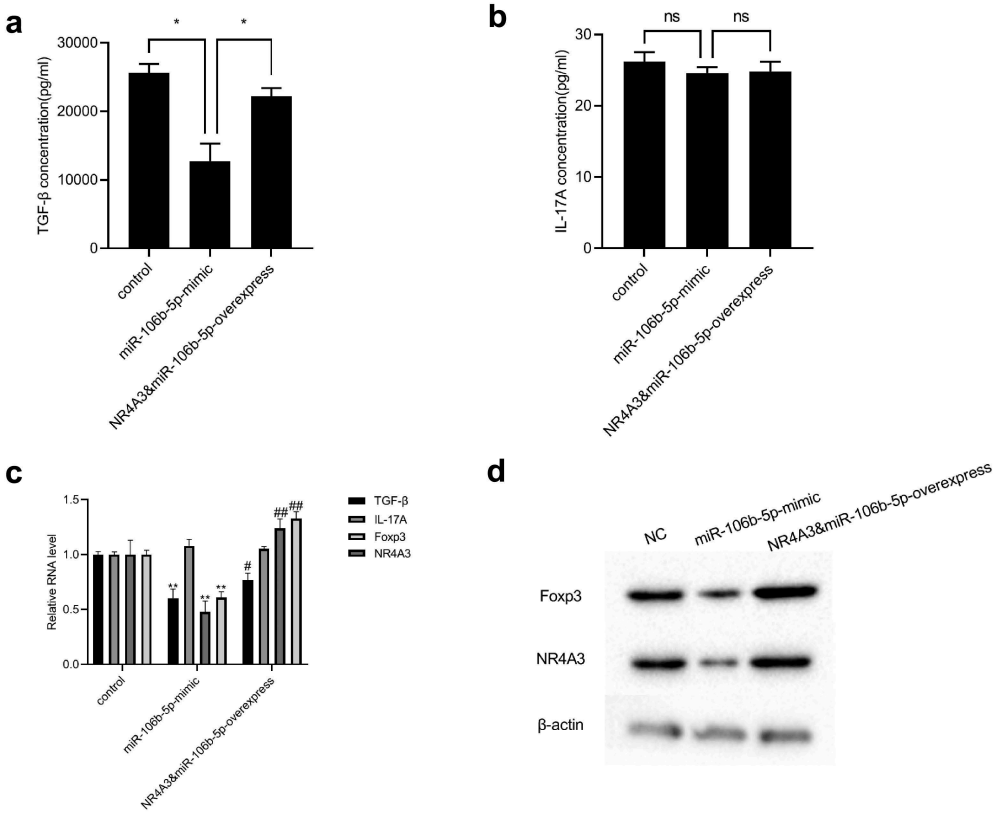

Figure 4. miR-106b-5p regulated CD4 + T cell differentiation through NR4A3. CD4 + T cells from healthy controls were induced and differentiate into Treg and Th17 for 96 h. CD4 + T cells were transfected with miR-106b-5p mimic or NR4A3&miR-106b-5p-overexpress 72 h before the end of induced differentiation. (a,b). TGF-β and IL-17A concentrations were detected in control, miR-106b-5p mimic and NR4A3&miR-106b-5p-overexpress groups using ELISA. *$p < 0.05$ vs control or miR-106b-5p mimic. (c). TGF-β, IL-17A, Foxp3, and NR4A3 expressions were detected using qRT-PCR. **$p < 0.01$ vs control. #$p < 0.05$, ##$p < 0.01$ vs miR-106b-5p mimic. (d). Protein levels of Foxp3 and NR4A3 were detected using western blot.

effectively decreased the number of megacaryocyte in the spleen and bone marrow tissues (Figure 5(a)) of mice and increased the number of platelets (Figure 5(f)). In addition, in miR-106b-5p inhibitor-treated ITP mice, the TGF-β level in orbital blood and CD4 + T cells was significantly decreased while the IL-17A level didn't change significantly (Figure 5(c,d)). To further obverse the effect of the miR-106b-5p inhibitor on Treg differentiation, the percentage of Th17 and Treg was detected by flow cytometry. As shown in Figure 5(g), the percentage of Treg in the spleen was significantly inhibited in the ITP group than the control group and promoted in the treatment group. On the contrary, there was no significant change in the percentage of Th17 among the three groups. These findings indicated that silencing miR-106b-5p promoted Treg differentiation and increased the number of platelets.

Discussion

Treg/Th17 imbalance is closely related to many autoimmune diseases, such as ITP, rheumatoid arthritis, and systemic lupus erythematosus [19,20]. Treg plays an immunosuppressive function to induce T cell tolerance in autoimmune disease, which is characterized by expressing Foxp3 [21,22]. The deficiency of Treg has been observed in patients with ITP, which causes the loss of immune tolerance [23]. Th17 plays an important role in driving inflammation, which is characterized by producing IL-17A [24,25]. The number of Th17 and the concentration of IL-17 are elevated in patients with ITP [9,11], which leads to pro-inflammatory effects and tissue damage [26]. Increasing pieces of evidence have shown that natural Treg/Th17 decreases in patients with ITP, and Treg/Th17 imbalance functions as a contributor to the development of ITP

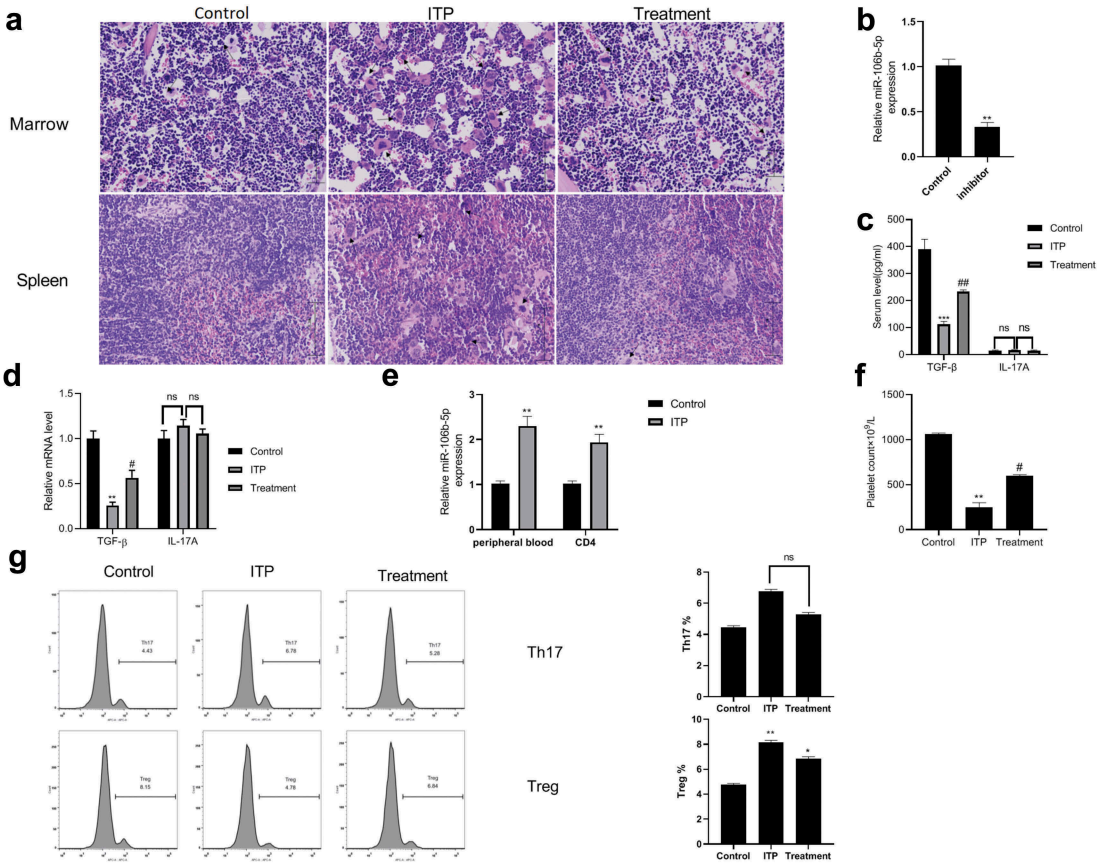

Figure 5. Silencing miR-106b-5p promoted Treg differentiation and increased the number of platelets. The mice were divided into a control group (n = 5), ITP group (n = 5), and treatment group (n = 5). (a). HE staining of spleen and bone marrow in the three groups. (b). The miR-106b-5p silencing efficacy was measured by qRT-PCR. (c). TGF-β and IL-17A concentrations were detected in orbital blood using ELISA. (d). TGF-β and IL-17A levels were detected in CD4 + T cells using qRT-PCR. (e). The expression levels of miR-106b-5p were measured in peripheral blood and CD4 + T cells by qRT-PCR. (f). Blood was collected from the caudal vein of mice, and the number of platelets was counted by an automatic blood cell counter. (g). The percentage of Treg and Th17 were detected in spleen using flow cytometry. **p < 0.01, ***p < 0.001 vs control. #p < 0.05, ##p < 0.01 vs ITP.

[10,11]. This study herein identified a new regulatory pathway concerning Treg/Th17 imbalance, involving miR-106b-5p, NR4A3 and Foxp3. Briefly, we clarified that high expression of miR-106b-5p negatively regulated Foxp3 expression via targeting NR4A3, thus reducing Treg differentiation and inducing Treg/Th17 imbalance. With this study performed, the significance of miR-106b-5p and NR4A3 in the pathogenesis of ITP was emphasized, helping us to have a more comprehensive understanding of the pathogenesis of ITP and providing novel perspectives for the clinical treatment of ITP.

NR4A3, as a member of the NR4A family, is an orphan nuclear receptor that plays an important role in the regulation of cancer cell proliferation, apoptosis, differentiation, and metabolism [27,28]. Studies have reported that NR4A3 induced the expression of transcription factor Foxp3 and promoted Treg differentiation [18,29]. However, whether NR4A3 can regulate Foxp3 expression and Treg differentiation in ITP are not clear. In this study, we found NR4A3 mRNA level in peripheral blood was remarkably decreased in patients with ITP, and TGF-β secretion was also decreased. Silencing NR4A3 reduced Foxp3 mRNA level in CD4 + T cells, decreased TGF-β mRNA level, and inhibited TGF-β secretion. These results indicated that NR4A3 can positively regulate Foxp3 expression and Treg differentiation in ITP.

Dysregulation of miRNAs is important in the development of autoimmune diseases, such as ITP and rheumatoid arthritis [30]. However, the role of specific miRNA in ITP is still largely unknown. In this study, we aimed to identify specific abnormally

expressed miRNA in ITP and tried to provide potential targets for the treatment of ITP. We found that miR-106b-5p was elevated in peripheral blood of patients with ITP compared with healthy controls, indicating miR-106b-5p may play a critical role in ITP.

Numerous studies have shown that miRNAs are involved in various physiological processes via negatively regulating gene regulation through binding to the 3′-untranslated region (UTR) of their target genes [13,31]. In line with this, previous studies showed that miR-106b-5p took part in the regulation of g cell growth, proliferation, apoptosis and cell cycle, and so on through this mechanism. For example, Gu at al. reported that miR-106b-5p promoted the viability and invasion of hepatocellular carcinoma cells via targeting RUNX3 [32]. Wei at al. found that in non-small cell lung cancer, miR-106b-5p promoted cell proliferation and inhibited cell apoptosis by regulating BTG3 [33]. In the present study, according to the prediction of bioinformatics software, we found there were binding sites between miR-106b-5p and NR4A3 3′-UTR. The dual-luciferase assay showed that miR-106b-5p mimic inhibited the luciferase activity of NR4A3 WT. Further loss- and gain-of-function experiments determined that miR-106b-5p negatively regulated Foxp3 and TGF-β expression through regulation of NR4A3.

In conclusion, the current study elucidated that miR-106b-5p could negatively regulate NR4A3 and Foxp3, thus acting as a promoter of Treg/Th17 imbalance. Our study highlighted the role of miR-106b-5p in ITP pathogenesis and emphasized its regulatory relationship with NR4A3 and Foxp3. Although there are many aspects to be improved in the experiment, we still hope this study can provide a new perspective for the clinical treatment of ITP.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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References

[1] Audia S, Mahévas M, Samson M, et al. Pathogenesis of immune thrombocytopenia. Autoimmun Rev. 2017;16:620–632.
[2] Mariotte E, Azoulay E, Galicier L, et al. Epidemiology and pathophysiology of adulthood-onset thrombotic microangiopathy with severe ADAMTS13 deficiency (thrombotic thrombocytopenic purpura): a cross-sectional analysis of the French national registry for thrombotic microangiopathy. Lancet Haematol. 2016;3:e237–245.
[3] Scully M, Cataland SR, Peyvandi F, et al. Caplacizumab treatment for acquired thrombotic thrombocytopenic purpura. N Engl J Med. 2019;380:335–346.
[4] Jestin M, Benhamou Y, Schelpe A-S, et al. Preemptive rituximab prevents long-term relapses in immune-mediated thrombotic thrombocytopenic purpura. Blood. 2018;132:2143.
[5] Song I, Kim J, Kwon K, et al. Expression of CD154 (CD40L) on stimulated T lymphocytes in patients with idopathic thrombocytopenic purpura. Hematology. 2016;21:187–192.
[6] Pabbisetty SK, Rabacal W, Volanakis EJ, et al. Peripheral tolerance can be modified by altering KLF2-regulated Treg migration. Proc Natl Acad Sci U S A. 2016;113:E4662–E4670.
[7] Li F, Ji L, Wang W, et al. Insufficient secretion of IL-10 by Tregs compromised its control on over-activated CD4+ T effector cells in newly diagnosed adult immune thrombocytopenia patients1. Immunol Res. 2015;61:269–280.
[8] Xu Z-S, Zhang H-X, Li -W-W, et al. FAM64A positively regulates STAT3 activity to promote Th17 differentiation and colitis-associated carcinogenesis. Proc Nat Acad Sci. 2019;116:10447.
[9] Qiao J, Li X, Wu Y, et al. An increased expression profile of Th9/IL-9 correlated with Th17/IL-17 in patients with immune thrombocytopenia. Platelets. 2017;28:287–294.
[10] Yu S, Liu C, Li L, et al. Inactivation of Notch signaling reverses the Th17/Treg imbalance in cells from patients with immune thrombocytopenia. Lab Invest. 2014;95:157.
[11] Hu Y, Wang X, Yu S, et al. Neutralizations of IL-17A and IL-21 regulate regulatory T cell/T-helper 17 imbalance via T-helper 17-associated signaling pathway in immune thrombocytopenia. Expert Opin Ther Targets. 2015;19:723–732.
[12] Michlewski G, Cáceres JF. Post-transcriptional control of miRNA biogenesis. RNA. 2019;25:1–16.
[13] Jernås M, Nookaew I, Wadenvik H, et al. MicroRNA regulate immunological pathways in T-cells in immune thrombocytopenia (ITP). Blood. 2013;121:2095–2098.
[14] Qian BH, Ye X, Zhang L, et al. Increased miR-155 expression in peripheral blood mononuclear cells of primary immune thrombocytopenia patients was correlated with serum cytokine profiles. Acta Haematol. 2015;133:257–263.

[15] Nishida N, Nagahara M, Sato T, et al. Microarray analysis of colorectal cancer stromal tissue reveals upregulation of two oncogenic miRNA clusters. Clin Cancer Res. 2012;18:3054.
[16] Dong P, Kaneuchi M, Watari H, et al. MicroRNA-106b modulates epithelial–mesenchymal transition by targeting TWIST1 in invasive endometrial cancer cell lines. Mol Carcinog. 2014;53:349–359.
[17] Qian C, Yan W, Li T, et al. Differential expression of miR-106b-5p and miR-200c-3p in newly diagnosed versus chronic primary immune thrombocytopenia patients based on systematic analysis. Cell Physiol Biochem. 2018;45:301–318.
[18] Sekiya T, Kashiwagi I, Yoshida R, et al. Nr4a receptors are essential for thymic regulatory T cell development and immune homeostasis. Nat Immunol. 2013;14:230.
[19] Xie M, Wang J, Gong W, et al. NF-κB-driven miR-34a impairs Treg/Th17 balance via targeting Foxp3. J Autoimmun. 2019;102:96–113.
[20] Fan M, Li Y, Yao C, et al. Dihydroartemisinin derivative DC32 attenuates collagen-induced arthritis in mice by restoring the Treg/Th17 balance and inhibiting synovitis through down-regulation of IL-6. Int Immunopharmacol. 2018;65:233–243.
[21] Ryba-Stanisławowska M, Sakowska J, Zieliński M, et al. Regulatory T cells: the future of autoimmune disease treatment. Expert Rev Clin Immunol. 2019;15:777–789.
[22] Rai G, Das S, Ansari MA, et al. Phenotypic and functional profile of Th17 and Treg cells in allergic fungal sinusitis. Int Immunopharmacol. 2018;57:55–61.
[23] Bao W, Bussel JB, Heck S, et al. Improved regulatory T-cell activity in patients with chronic immune thrombocytopenia treated with thrombopoietic agents. Blood. 2010;116:4639–4645.
[24] Poissonnier A, Guégan J-P, Nguyen HT, et al. Disrupting the CD95–PLCγ1 interaction prevents Th17-driven inflammation. Nat Chem Biol. 2018;14:1079–1089.
[25] Korn T, Bettelli E, Oukka M, et al. IL-17 and Th17 Cells. Annu Rev Immunol. 2009;27:485–517.
[26] Lee A, Lim HX, Kim MS, et al. Vibrio vulnificus infection induces the maturation and activation of dendritic cells with inflammatory Th17-polarizing ability. Int J Mol Med. 2018;41:531–540.
[27] Hirano T, Nagasaki-Maeoka E, Ishizuka Y, et al. Forced expression of NR4A3 induced the differentiation of human neuroblastoma-derived NB1 cells. Med Oncol. 2019;36:66.
[28] Close AF, Dadheech N, Villela BS, et al. The orphan nuclear receptor Nor1/Nr4a3 is a negative regulator of β-cell mass. J Biol Chem. 2019;294:4889–4897.
[29] Policardo L, Seghieri G, Anichini R, et al. Effect of diabetes on hospitalization for ischemic stroke and related in-hospital mortality: a study in Tuscany, Italy, over years 2004–2011. Diabetes Metab Res Rev. 2015;31:280–286.
[30] Wang Y, Huang R, Song C, et al. Some viral microRNAs were up-regulated in megakaryocytes incubated with immune thrombocytopenia plasma. Eur J Haematol. 2013;90:220–227.
[31] Li JQ, Hu SY, Wang ZY, et al. Long non-coding RNA MEG3 inhibits microRNA-125a-5p expression and induces immune imbalance of Treg/Th17 in immune thrombocytopenic purpura. Biomed Pharmacother. 2016;83:905–911.
[32] Gu H, Gu S, Zhang X, et al. miR-106b-5p promotes aggressive progression of hepatocellular carcinoma via targeting RUNX3. Cancer Med. 2019;8:6756–6767.
[33] Wei K, Pan C, Yao G, et al. MiR-106b-5p promotes proliferation and inhibits apoptosis by regulating BTG3 in non-small cell lung cancer. Cell Physiol Biochem. 2017;44:1545–1558.