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Title: miR-146a enhances regulatory T cells differentiation and function in allergic rhinitis by targeting STAT5b

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ABSTRACT

Background: MicroRNA (miR)-146a, as an important immune regulatory factor with an anti-inflammatory effect, plays a crucial role in regulatory T cells (Tregs) differentiation and function in allergic rhinitis (AR). The present study aimed to investigate the regulatory mechanism employed by miR-146a to control Tregs differentiation and function in AR.

Methods: Expression of miR-146a and STAT5b in peripheral blood mononuclear cells (PBMCs) and nasal mucosa from patients with AR was detected by qPCR and western blotting. Tregs were quantified by flow cytometry in miR-146a knockdown or STAT5b knockdown PBMCs. FOXP3, IL-10, and TGF- β levels were detected by western blotting or ELISA in miR-146a knockdown or STAT5b overexpressing PBMCs, as well as in STAT5b knockdown PBMCs overexpressing miR-146a. The effect of miR-146a on STAT5b was observed by luciferase assay and knockdown experiments.

Results: Levels of miR146a and STAT5b in the nasal mucosa or PBMCs were significantly lower in the AR group than in the control group. There were significantly fewer Tregs in miR-146a knockdown or STAT5b knockdown PBMCs compared to control PBMCs. Expression of FOXP3, IL-10, and TGF- β was decreased in the miR-146a knockdown group but increased in the STAT5b overexpression group. In contrast, miR-146a overexpression increased the levels of these factors, but knockdown of STAT5b significantly inhibited this effect. Luciferase assay and knockdown experiments showed that miR-146a bound directly to STAT5b.

Conclusions: miR-146a enhances Tregs differentiation and function in AR by positively targeting STAT5b.

Keywords: Allergic rhinitis, miR-146a, Treg cells, STAT5b

INTRODUCTION

Allergic rhinitis (AR) is a type I allergy, which is mediated by specific IgEs and is characterized by pruritus, sneezing, rhinorrhea, and nasal congestion.¹ Regulatory T cells (Tregs) represent a specific target of forkhead box P3 (FOXP3)² and play a central role in negative immune regulation. The percentage of Tregs was significantly decreased in patients with AR, which contributes to the development of AR by directly inhibiting the activation of allergen-specific T helper (Th2) cells, mast cells, basophils, and eosinophils or by suppressing the expression of anti-inflammatory mediators, such as transforming growth factor- β (TGF- β) and interleukin (IL)-10.^{3,4}

MicroRNA (miR)-146a has been shown to possess anti-inflammatory properties.⁵⁻⁷ The level of miR-146a and the ratio of Tregs were found to be significantly higher in peripheral blood mononuclear cells (PBMCs) from AR patients after subcutaneous or sublingual immunotherapy, highlighting the use of miR-146a as a marker for the treatment and prognosis of AR.⁸ Another study showed that miR-146a could induce the differentiation of naïve CD4⁺ T cells into Tregs by augmenting the production of dendritic TGF- β , and blocking polarization of Th2 cells in an AR mouse model.⁹ Although miR-146a has been proven to play a crucial role in Treg differentiation and function, the underlying mechanism remains poorly understood.

Signal transducer and activator of transcription 5B (STAT5b) is a critical regulator of FOXP3, which plays a vital role in the differentiation, function, and homeostasis of Tregs.¹⁰ However, its expression and function in AR are not entirely clear. The proportion of CD4⁺ CD25⁺ Foxp3⁺ cells in the spleen of Stat5b knockdown mice was found to be significantly reduced.¹¹ miR-146a could also selectively regulate Treg-mediated suppression of the Th1 response by targeting STAT1 in mouse bone marrow cells.¹² While STAT1 and STAT5 both belong to the STAT family,¹³ it is presently unknown whether miR-146a has a regulatory effect on STAT5b. In this study, we investigated the mechanism employed by miR-146a to regulate Tregs differentiation and function in AR, and particularly the relationship between miR-146a and STAT5b.

MATERIALS AND METHODS

Patients

Thirty-two patients with AR and 28 control subjects were enrolled in the study. Nasal mucosa tissue and peripheral blood were obtained from patients undergoing septoplasty because of nasal septum deviation. All patients were tested for the serum allergen-specific IgE of common inhalant allergens and the diagnosis of AR was made according to the Guidelines for the diagnosis and treatment of AR.¹⁴ Patients who had received antiallergic drugs, as well as nasal or systemic corticosteroid therapy for up to 4 weeks before surgery were excluded. The study protocol was approved by the Ethics Committee of Yantai Yuhuangding Hospital at Qingdao University. Samples were collected after obtaining written informed consent from patients.

Samples

The nasal tissue homogenate was obtained after grinding with liquid nitrogen in a high-pressure homogenizer. Whole blood was collected in EDTA-coated vacutainer tubes. PBMCs were isolated from the blood using Ficoll-Hypaque gradient centrifugation and washed two times with phosphate-buffered saline.

Cell culture and gene expression interference

Human PBMCs from AR patients or control subjects were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C with 5% CO₂. To stimulate lymphocyte proliferation, PBMCs were treated with anti-CD3 and anti-CD28 antibodies. Briefly, 2×10^6 PBMCs were plated in a 6-well plate, pretreated with 5 µg/mL anti-CD3 antibody for 3 h at 37 °C, washed three times with Dulbecco's phosphate-buffered saline, and incubated with 2 µg/mL anti-CD28 antibody for 72 h.

Lenti-GFP-has-miR-146a virus, lenti-GFP-STAT5b virus, lenti-III-GFP-virus, lenti-Off-has-miR-146a-5p virus, and lenti-III-mir-off control virus were purchased from Shanghai GeneChem. To obtain miR-146a knockdown cells, miR-146a overexpressing cells, or STAT5b overexpressing cells, PBMCs were stimulated with anti-CD3/CD28 for 72 h before

lentivirus infection, and the expression of green GFP in PBMCs was observed. The cells were then incubated with 3 $\mu\text{g/mL}$ puromycin for 72 h; the surviving cells stably expressed GFP.

To obtain STAT5b knockdown PBMCs, we applied siRNAs (General Biol) targeting STAT5b (siSTAT5b). Plasmids harboring either siSTAT5b or control siRNA (siNC) were transfected into PBMCs at 60% confluence in 24-well plates. Briefly, 3 μL Lipofectamine[®] RNAiMAX Reagent (Invitrogen) and 1 μL siRNA (10 μM) were each diluted in 50 μL Opti-MEM[®] medium. The diluted siRNA was mixed with diluted Lipofectamine[®] RNAiMAX Reagent and allowed to complex for 5 min at room temperature. The resulting siRNA-lipid complex was co-incubated with PBMCs for 24–48 h at 37 °C.

Dual-luciferase reporter assay

Human embryonic kidney 293 T (HEK-293 T) cells (ATCC, CRL-3216) were inoculated in a 6-well culture plate, and incubated until 70–80% confluence. Combinations of pRL-TK empty + pGL3 + pcDNA3.1-empty plasmid, pRL-TK empty + pGL3 + pcDNA3.1-STAT5b plasmid, pRL-TK-miRNA-146a mimics + pGL3 + pcDNA3.1-empty plasmid, and pRL-TK-miRNA-146a mimics + pGL3 + pcDNA3.1-STAT5b plasmid were dissolved in Opti-MEM[®] serum-free medium (Gibco). Cells were transfected with the plasmid combinations using Lipofectamine 2000 (Invitrogen), incubated for 48 h, and assayed with the Dual-Glo[®] luciferase detection kit (Promega) according to the manufacturer's instructions.

Quantitative real-time PCR (qPCR)

We extracted total RNA from the nasal tissue homogenate and PBMCs using the RNT411 extraction reagent (Mabio) according to the manufacturer's instruction. RNA was quantified on a NanoDrop 2000 (Thermo Fisher Scientific) at 260 nm and cDNA was synthesized from 2 μg of total RNA using the Total-Transcriptome cDNA Synthesis Kit (Applied Biological Materials). miR cDNA synthesis was performed using the miRNA All-in-One[™] cDNA Synthesis Kit (Applied Biological Materials). Gene expression was quantified on a StepOnePlus[™] Real-Time PCR System (Applied Biosystems) and detected with the KAPA SYBR FAST kit (Kapa Biosystems). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a housekeeping

gene and U6 was used to normalize miR-146a expression. The following primers were used: GAPDH (forward primer, 5'-GGG CTC TCT GCT CCT CCC TGT-3'; reverse primer, 5'-ACG GCC AAA TCC GTT CAC ACC-3'), STAT5b (forward primer, 5'-GAG AAC ATT AAG GCC ACC A-3'; reverse primer, 5'-CGG ACC AAC CTC TGT TCA TT-3'), U6 (forward primer, 5'-GGA ACG ATA CAG AGA AGA TTA GC-3'; reverse primer, 5'-GGA ACG ATA CAG AGA AGA TTA GC-3'), and miR-146a (forward primer, 5'-AAT GCG GCC GCT CAA GAG ATC CAC CCA CAT C-3'; reverse primer, 5'-AAT GCG GCC GCT CAA GAG ATC CAC CCA CAT C-3'). Relative gene expression was calculated using the $2^{-\Delta\Delta C_t}$ method.

Western blotting

Total proteins were extracted from tissue homogenate and cultured PBMCs using a protein extraction reagent (Solarbio). Protein concentration was measured using the BCA assay (Solarbio) and 20 μ g was separated by denaturing sodium dodecyl sulfate polyacrylamide gel electrophoresis. The proteins were then transferred onto a PVDF membrane, which was blocked with 5% skimmed milk/Tris-buffer saline for 2 h at room temperature and incubated overnight at 4 °C with primary antibodies against STAT5b (1:1000, Abcam), phosphorylated (p)-STAT5b (1:1000, Abcam), and FOXP3 (1:1000, Abcam). The membranes were then washed three times with Tris-buffered saline-Tween 20 (TBST), incubated with goat anti-mouse IgG and goat anti-rabbit IgG (conjugated with peroxidase) for 1 h, washed again with TBST, and finally visualized with an electrochemiluminescence system.

Flow cytometry

Cultured cells were incubated on ice with anti-human CD4-Percp-Cy5.5, anti-human CD25-PE-Cy7, and anti-human CD45RA-APC-Cy7 antibodies (BD Biosciences) for 30 min in the dark. For FOXP3 detection, cells were permeabilized with permeabilization/fixation buffer (Thermo Fisher Scientific), and then stained with anti-human-FOXP3-APC antibody (Thermo Fisher Scientific). Stained samples were assayed on a FACSCalibur flow cytometer (BD Biosciences) and results were analyzed by FlowJo software (Tree Star Inc.). Unstained and

Fluorescence Minus One (FMO) control samples were also measured to help set the gates during data analysis.

Enzyme-linked immunosorbent assay (ELISA)

Anti-CD3/CD28-stimulated and freshly infected PBMCs were used to measure IL-10 and TGF- β levels in the cell culture supernatant by means of an ELISA kit (Elabscience) following the manufacturer's instructions.

Statistical analysis

The Shapiro–Wilk test was used to evaluate the normality of quantitative variables. Normally distributed variables were represented as the mean $\pm$ standard deviation. Comparisons between two groups were performed using Student's paired or unpaired *t*-test. All statistical analyses were performed in SPSS 21.0 (SPSS Inc.). $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Effect of miR-146a on the differentiation and function of Tregs in AR

First, we verified the level of miR-146a by qPCR and found that the expression of miR-146a in PBMCs ($P < 0.05$) and nasal mucosa ($P < 0.001$) was significantly lower in the AR group than in the control group (Figure 1A, B). To investigate whether miR-146a deficiency altered Tregs differentiation and function, we infected PBMCs of control subjects with lenti-Off-has-miR-146a-5p virus to generate miR-146a knockdown cells. In all cases, infection efficiency surpassed 80% and expression of miR-146a was significantly decreased ($P < 0.05$) in the miR-146a knockdown group (Figure 1C). The proportion of differentiated Tregs was determined via flow cytometry by gating the $CD4^+CD25^+CD45RA^-FOXP3^+$ subpopulation of Tregs. Compared with the control group, the proportion of Tregs was significantly lower ($P < 0.001$) in miR-146a knockdown PMBCs (Figure 1D). The expression of FOXP3 ($P < 0.001$), IL-10 ($P < 0.01$), and TGF- β ($P < 0.01$) was also lower in the miR-146a knockdown group than in the control group (Figure 1E, F).

Effect of STAT5b on the differentiation and function of Tregs in AR

The expression of STAT5b in PBMCs ($P < 0.05$) and nasal mucosa ($P < 0.01$) was significantly lower in the AR group than in the control group (Figure 2A, B). PBMCs of control subjects were transfected with control and STAT5b siRNA, revealing a significantly reduced proportion of Tregs in STAT5b knockdown PMBCs compared to the control group ($P < 0.001$) (Figure 2C). PBMCs of AR patients overexpressing STAT5b were generated following infection with lenti-GFP-STAT5b virus at efficiencies above 90% (Figure 2D). Western blot results revealed that STAT5b ($P < 0.01$) and p-STAT5b ($P < 0.05$) were significantly more expressed in the STAT5b overexpressing lentivirus infection group (Figure 2E). The expression of FOXP3 ($P < 0.05$), IL-10 ($P < 0.05$), and TGF- β ($P < 0.05$) was similarly higher in STAT5b overexpressing PBMCs or cell culture supernatant (Figure 2F, G).

miR-146a positively regulates the expression of STAT5b

Given the important role of miR-146a and STAT5b in AR, we constructed a double luciferase reporter gene to clarify the regulatory relationship between miR-146a and STAT5b in HEK-293 T cells. Accordingly, miR-146a overexpression significantly up-regulated luciferase activity of the STAT5b 3'UTR (Figure 3A). In contrast, miR-146a knockdown significantly decreased gene and protein expression of STAT5b ($P < 0.01$), as well as the level of p-STAT5b ($P < 0.05$) in human PBMCs (Figure 3B, C).

miR-146a regulates Tregs differentiation and function by targeting STAT5b

Finally, we constructed STAT5b knockdown PBMCs from control subjects using siRNA and infected them with lenti-GFP-has-miR-146a virus to induce miR-146a overexpression. The levels of STAT5b ($P < 0.001$), FOXP3 ($P < 0.01$), IL-10 ($P < 0.01$), and TGF- β ($P < 0.01$) were significantly decreased in STAT5b knockdown PBMCs overexpressing miR-146a compared with the miR-146a overexpression group alone (Figure 4). This finding indicated that STAT5b knockdown could inhibit the positive effect of miR-146a on Tregs differentiation and function.

DISCUSSION

The mechanism underlying AR involves the relative increase in Th2 cells, which can lead to an imbalance in the Th1/Th2 ratio.^{1,15} Tregs can inhibit the polarization of Th2 cells by secreting TGF- β ;^{3,16} however, Treg-mediated inhibition can be reversed after blocking the IL-10 receptor *in vivo*.^{17,18} miR-146a is highly expressed in Tregs,¹⁹ and participates in their development and function. However, the mechanism by which miR-146a regulates the progression of AR inflammation via Tregs remains unclear. In this study, we found that expression of miR-146a and STAT5b was decreased in nasal mucosa tissues and PBMCs from AR patients. Furthermore, we report that miR-146a positively targets STAT5b, thus additionally increasing the ratio of Tregs, as well as the production of IL-10 and TGF- β in PBMCs. This finding points to a new pathway through which miR-146a regulates Tregs differentiation and function in AR.

Previous studies reported a significantly reduced level of miR-146a in nasal epithelial cells from AR patients and an AR mouse model.⁹ Our present results confirmed the low expression of miR-146a in PBMCs and nasal mucosa tissues in patients with AR. We stimulated the differentiation of CD4⁺ T cells by adding anti-CD3/CD28 antibodies to PBMCs from AR patients and inhibited the expression of miR-146a through lentiviral transduction. The resulting drop in the proportion of Tregs, as well as the decreased expression of IL-10 and TGF- β secreted by Tregs, confirmed the role of miR-146a in the differentiation and function of Tregs. The immune tolerance mediated by Tregs depends on miRNAs. Conditional elimination of Dicer in mice has been shown to block the inhibitory function of Tregs due to impairment of miRNAs.²⁰ Previous studies suggested that ectopic overexpression of miR-146a resulted in a slight up-regulation of FOXP3 in Jurkat T lymphoblasts.²¹ Here, we also found that miR-146a could positively regulate the expression of the FOXP3 transcription factor. FOXP3 acts as a Treg lineage specification factor and thus identifies Tregs independently of CD25 expression. It is closely related to secretion of TGF- β and IL-10 by Tregs, further suppressing specific immune responses mediated by Th1 and Th2.^{22,23} Our results indicate that miR-146a could regulate the development and function of Tregs and thus affect the immune response of patients with AR.

We report that miR-146a up-regulated target gene expression by binding to the STAT5b 3'UTR as indicated by a dual luciferase reporter gene assay. Lentiviral transduction inhibited the expression of miR-146a, revealing that the latter had a positive regulatory effect on STAT5b and p-STAT5b in PBMCs. Previously, miR-146a was found to reduce the expression of specific target protein-coding genes by promoting mRNA degradation or inhibiting translation after transcription.²⁴ However, some studies have reported that miRNAs can play a regulatory role in a non-traditional way.²⁵⁻²⁸ For example, the β -actin (Actb) gene results in two alternative transcripts that terminate with tandem poly(A) sites; the longer transcript contains a conserved mmu-miR-34a/34b-5p target site, and miR-34 combines with Actb 3'UTR to up-regulate target gene expression.²⁹ miR-369 guides the association of AGO2 and fragile X mental retardation associated protein 1 (FXR1) with Au-rich elements (Ares) in the 3'UTR of tumor necrosis factor alpha (TNF- α) mRNA to trigger translation.²⁷ miR-Let-7 was reported to exert a similar miRNA-mediated up-regulation of target transcripts at the 3'UTR of high mobility group A2 (HMGA2).²⁷

Our analysis of STAT5b in AR found low expression of STAT5b in the PBMCs and nasal mucosa of AR patients. The JAK-STAT pathway triggers the downstream release of type I/II cytokines, such as ILs, interferons, and multiple growth factors.³⁰ STAT5b sends a downstream signal to many cytokines, such as IL-2, IL-4, and IL-5, which is essential for the growth of immune cells and the immune response, especially T cells and natural killer cells.^{31,32} Our study shows that the expression of IL-10 and TGF- β was increased in STAT5b overexpressing PBMCs. The activation of Stat5a/b, which is one of the earliest IL-2 family-mediated signaling pathways, can rapidly deliver signals from the membrane to the nucleus.³³ The absence of STAT5b leads to the abnormal function of Tregs, as well as immune deficiency and development of various lethal abnormalities in mice.^{11,34,35} In addition, we found that FOXP3 expression was increased in STAT5b overexpressing cells. Notably, STAT5b might bind to the FOXP3 gene through its own transcriptional activation regions, in turn regulating expression of FOXP3, IL-10, and TGF- β . Indeed, mice completely deficient in Stat5 possess substantially fewer Foxp3⁺ cells.¹¹ As with

miR-146a, STAT5b appears to exert a positive regulatory effect on Tregs differentiation and function in AR.

Overall, both miR-146a and STAT5b promoted the synthesis of FOXP3 and IL-10 during Tregs differentiation, as well as TGF- β secretion by Tregs. We speculated that miR-146a regulated the differentiation and function of Tregs through STAT5b. To verify this hypothesis, we constructed PBMCs stably overexpressing miR-146a through lentivirus infection and further knocked down STAT5b by siRNA. We found that miR-146a had a positive regulatory effect on STAT5b, FOXP3, IL-10, and TGF- β expression. Importantly, STAT5b knockdown could reverse the effect of miR-146a. Previous studies have shown that the proportion of Tregs in peripheral blood is lower in patients with AR than in healthy individuals;³⁶ hence, we speculate that lower STAT5b expression is one of the reasons for fewer Treg cells in such patients, with miR-146a playing an active role in the regulatory process. In addition, Tregs act by expressing immunosuppressive cytokines, such as IL-10 and TGF- β , whose impaired production has been reported in AR patients.³⁷ These results provide further proof that miR-146a controls the immunosuppressive function of Tregs by regulating STAT5b.

In summary, miR-146a can enhance the differentiation and function of Tregs by targeting STAT5b. Specifically, miR-146a up-regulates the expression of STAT5b in AR through an unconventional regulatory mechanism. In the next step, we will continue to explore new regulatory targets of miR-146 on Tregs differentiation and function in AR, and further verify them through in vivo experiments. Our results show that STAT5b serves as a regulatory target for miR-146a, playing an important role in the development of AR and providing an intervention strategy for the treatment of AR.

AUTHOR CONTRIBUTIONS

Xicheng Song, Luo Zhang, and Yu Zhang contributed to the design and conception of the study. Yu Zhang, Yujuan Yang, Jing Guo, Limei Cui, Liping Yang, Yumei Li, Yakui Mou, and Chuanliang Jia performed the experiments and analyzed the data. Yu Zhang, Yujuan Yang, and Jing Guo drafted the paper. Xicheng Song and Luo Zhang contributed to the interpretation of the data. All authors contributed to the main text and read and approved the final version of the manuscript.

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FIGURE LEGENDS

Figure 1. Effect of miR-146a on the differentiation and function of Tregs in AR

(A) Expression of miR-146a in PBMCs from controls (n = 8) and AR patients (n = 8) as determined by qPCR. (B) Expression of miR-146a in nasal mucosa from controls (n = 13) and AR patients (n = 16) as determined by qPCR. (C) miR-146a control and knockdown lentivirus were used to infect PBMCs of control subjects, marked as Lenti-GFP and Lenti-simiR-146a, respectively. Infection efficiency is shown by GFP fluorescence (green). Relative gene expression of miR-146a as detected by qPCR (n = 6). Scale bar: 400 μ m. (D) Percentage of CD4⁺CD25⁺CD45RA⁻FOXP3⁺Tregs undergoing differentiation in Lenti-simiR-146a and Lenti-GFP groups as determined by flow cytometry (n = 8). (E) Expression of FOXP3 in Lenti-simiR-146a and Lenti-GFP groups as determined by western blotting (n = 6). (F) Relative levels of IL-10 and TGF- β in Lenti-simiR-146a and Lenti-GFP groups as measured by ELISA in cell supernatant (n = 6). ***P < 0.001, **P < 0.01, *P < 0.05. PBMCs: peripheral blood mononuclear cells; AR: allergic rhinitis.

Figure 2. Effect of STAT5b on the differentiation and function of Tregs in AR

(A) STAT5b mRNA expression in PBMCs obtained from control subjects (n = 8) and AR patients (n = 8), as determined by qPCR. (B) STAT5b protein expression in nasal mucosa obtained from control subjects (n = 6) and AR patients (n = 8) as determined by western blotting. (C) Percentage of CD4⁺CD25⁺CD45RA⁻FOXP3⁺Tregs undergoing differentiation in PBMCs obtained from control subjects and transfected with siNC and siSTAT5b, as determined by flow cytometry (n = 6). NC: negative control. (D) STAT5b overexpression and control lentivirus were transduced into PBMCs obtained from AR patients, and marked as Lenti-STAT5b and Lenti-GFP, respectively. Infection efficiency is shown by GFP fluorescence (green). Relative gene expression of STAT5b as determined by qPCR (n = 8). Scale bar: 400 μ m. (E) Expression of STAT5b and p-STAT5b in Lenti-GFP and Lenti-STAT5b cells as determined by western blotting (n = 6). (F) Expression of FOXP3 in Lenti-GFP and Lenti-STAT5b cells as determined by western blotting (n = 6). (G)

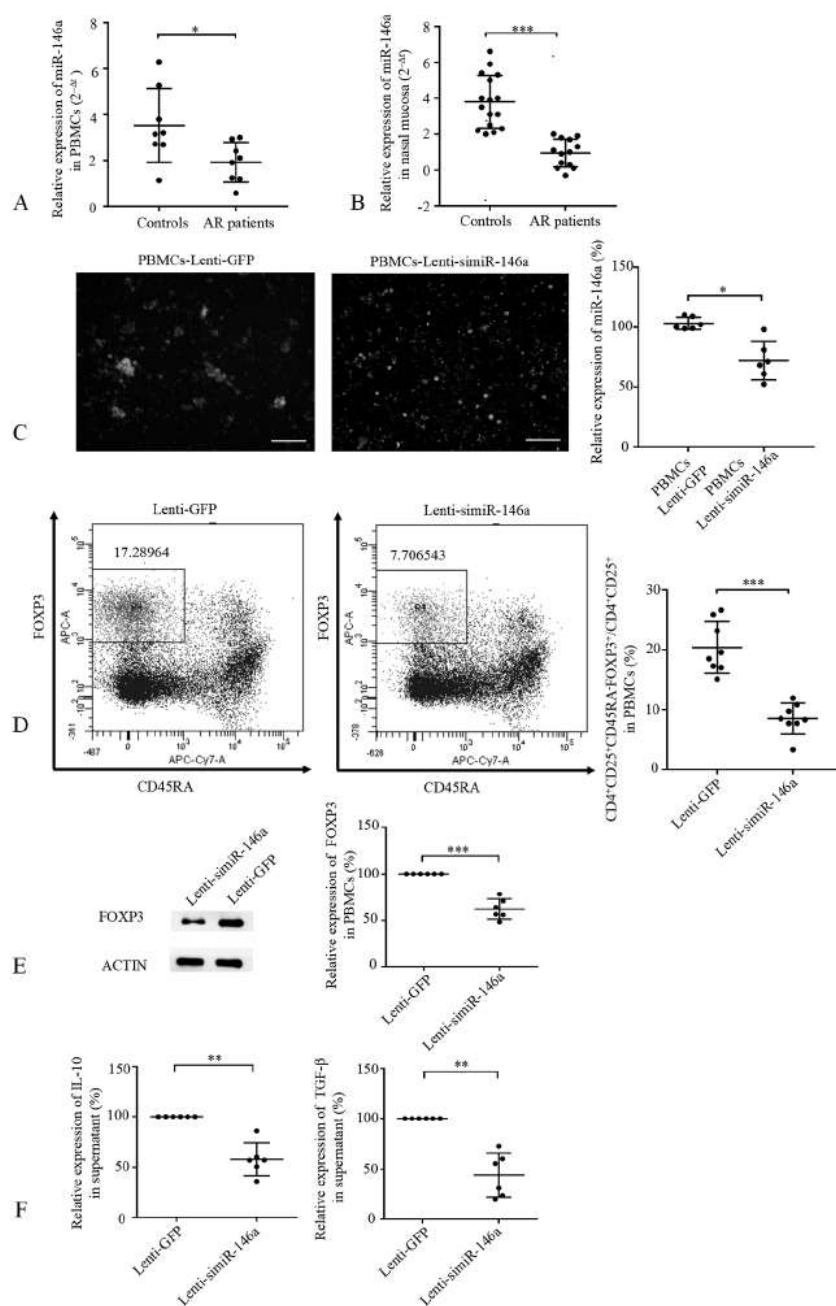
Relative expression of IL-10 and TGF- β in Lenti-GFP and Lenti-STAT5b cells as detected by ELISA (n = 6). ***P < 0.001, **P < 0.01, *P < 0.05.

Figure 3. miR-146a regulates the expression of STAT5b in human PBMCs

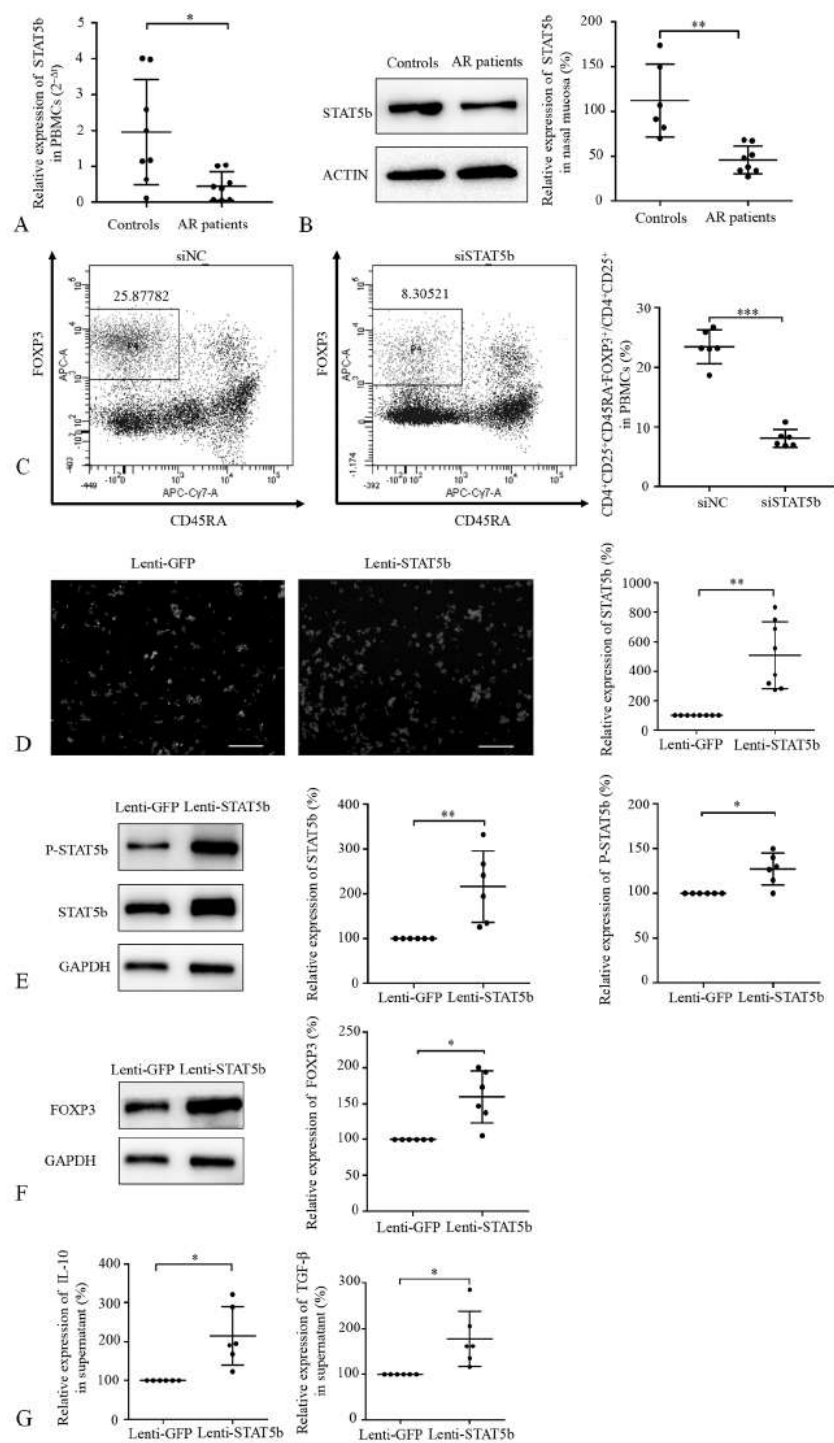
(A) Luciferase activity was measured after transfection with the following sets of plasmids in HEK-293 T cells: pRL-TK empty + pGL3 + pcDNA3.1-empty (empty); pRL-TK-empty + pGL3 + pcDNA3.1-stat5b (STAT5b 3'UTR); pRL-TK-miR-146a imics + pGL3 + pcDNA3.1-empty (miR-146a mimics); and pRL-TK-miR-146a mimics + pGL3 + pcDNA3.1-stat5b (miR-146a mimics STAT5b 3'UTR) (n = 3). (B) Relative gene expression of STAT5b in Lenti-simiR-146a and Lenti-GFP groups as detected by qPCR (n = 6). (C) Protein level of STAT5b and p-STAT5b as determined by western blotting (n = 6). ***P < 0.001, **P < 0.01, *P < 0.05.

Figure 4. miR-146a regulates Tregs differentiation and function by targeting STAT5b

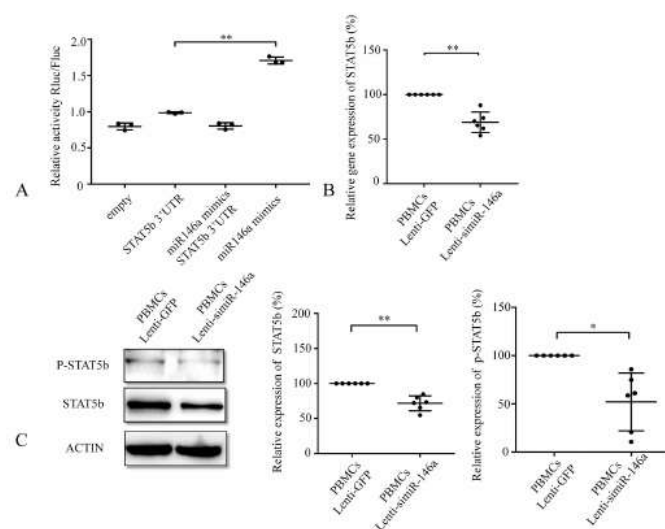
(A) STAT5b siRNA and control siRNA were transfected into PBMCs obtained from control subjects. Then, PBMCs were further infected with miR-146a overexpression and control lentiviruses to generate samples siNC + Lenti-GFP, siSTAT5b + Lenti-GFP, siNC + Lenti-miR-146a, and siSTAT5b + Lenti-GFP + Lenti-miR-146a. Relative gene expression of STAT5b as detected by qPCR (n = 6). (B) Expression of FOXP3 as determined by qPCR (n = 6). (C, D) Relative level of IL-10 and TGF- β in cell culture supernatant as measured by ELISA (n = 6). ***P < 0.001, **P < 0.01, *P < 0.05.



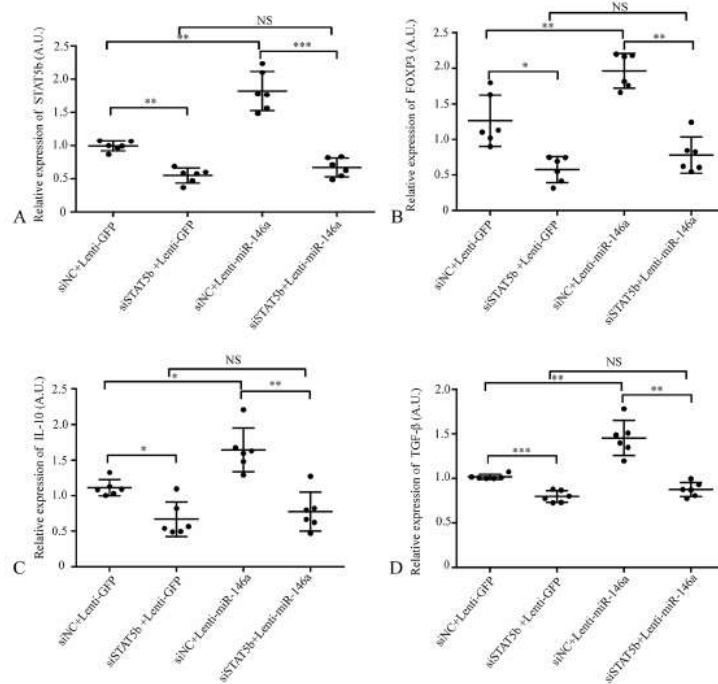
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