Effect of BDNF on Differentiation of Circulating Th17 and Treg Cells in SLE Patients and Exploration of Signal Transduction Pathways

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Research Article

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Abstract

Circulating brain-derived neurotrophic factor(BDNF) is mainly derived from lymphocytes. The serum BDNF level in SLE is decreased, and BDNF may be involved in the pathogenesis of systemic lupus erythematosus(SLE). Our aim is to determine whether BDNF affects the differentiation of CD4+T cells into regulatory T(Treg) or T helper 17(Th17). 30 patients were selected. TGF-β and IL-6 were added to induce differentiation of Treg and Th17. After co-cultured with BDNF, the percentages of $CD4+CD25+CD127^{low}$ and CD4+IL-17A+ were detected by Flow cytometry, and the expression of Foxp3mRNA and RORγtmRNA were detected by Rt-PCR. Under the condition of Th17 and Treg polarization, after co-cultured with BDNF and TrkB IgG, the phosphorylation of Akt, mTORC1 and ERK1/2 were detected by western-blot, the percentages of $CD4+CD25+CD127^{low}$ and CD4+IL-17A+ were detected by Flow cytometry. Under the condition of Th17 polarization, with the increase of BDNF concentration(60ng/ml, 120ng/ml, 350ng/ml), the percentages of CD4+IL-17A+ and the expression of RORγtmRNA were decreased($p<0.01$; $p<0.001$). Under the condition of Treg polarization, the percentages of $CD4+CD25+CD127^{low}$ and the expression of Foxp3mRNA were increased($p<0.001$). Regardless of Th17 or Treg polarization, the phosphorylation of Akt, mTORC1 and ERK1/2 in the BDNF group were reduced($p<0.001$), and the phosphorylation of Akt, mTORC1 in the TrkBIgG group were enhanced($p<0.001$). BDNF down-regulates the differentiation of Th17 and promotes the differentiation of Treg in SLE through inhibiting the activation of PI3K-Akt-mTORC1 axis and ERK1/2 pathway. BDNF could play a certain role in maintaining the balance of Treg/Th17 ratio in SLE.

1. Introduction

Initial CD4+T cells differentiate into regulatory T (Treg) and T helper (Th). Treg are characterized by sustained expression of CD25+, transcription factor Foxp3, and low expression of CD127, which secrete cytokine transforming growth factor-β(TGF-β) and play an essential role in maintaining immunologic homeostasis and preventing the occurrence of autoimmune diseases[1]. Th17, a distinct subset of Th cells characterized by expression of the transcription factor RORγt. Particularly, Th17 plays a pivotal role in the initiation and development of autoimmunity, which secretea profile of potent pro-inflammatory cytokines upon certain stimulation, including interleukin 17(IL-17), IL-2[2] etc. Th17 are involved in the pathogenesis of many autoimmune diseases[3]. TGF-β is a cytokine shared during the differentiation of Th17 and Treg. However, the presence of other pro-inflammatory cytokines during the activation of lymphocytes further regulates the differentiation of these cells. Under the coexistence of IL-6 and TGF-β, CD4+T cells differentiate into Th17, while in the absence of IL-6, TGF-β promotes the differentiation of Treg[4,5]. Therefore, the balance between pro-inflammatory cytokines and anti-inflammatory cytokines is a key factor in the differentiation of CD4+T cells[6]. It was reported that under the co-stimulation of TCR and CD28, the activation, survival and proliferation of Th17 is up-regulated through the activation of PI3K-Akt-mTORC1 axis[7-9]. Activation of the MAPK/ERK pathway also promotes the differentiation of Th17(Sup.1)[10].

Systemic lupus erythematosus(SLE) is a heterogeneous chronic inflammatory autoimmune disorder characterized by the loss of immune tolerance to autoantigen, continuous production of pathogenic autoantibodies and deposition of immune complexes in different organs. Increasing evidences suggested that defects in the number or function of Treg in SLE can lead to an increasing activity in Th17[3,11-13]. Th17 participate in the attack on target cells or tissues through the production of excessive pro-inflammatory cytokines, which ultimately leads to the damage of target tissues[14]. Pro-inflammatory cytokines such as IL-6, IL-8, IL-17, and IFN-γ were significantly elevated in the serum and in cerebrospinal fluid of neuropsychiatric lupus erythematosus (NPSLE) patients[15].

Brain-derived neurotrophic factor(BDNF) plays an essential role in promoting the growth, differentiation and survival of neurons[16]. In addition, BDNF has been found in peripheral blood. Lymphocytes and vascular endothelial cells are the main sources of BDNF[17,18]. BDNF can promote the proliferation of T lymphocytes and has an anti-apoptosis effect on T cells[19,20]. However, the relationship between BDNF and T lymphocytes is unclear. We have reported that serum BDNF in SLE patients is significantly decreased. The serum BDNF level is positively related with SLEDAI scores[21,22]. We speculated that BDNF may involve in the pathogenesis of SLE. Our aimis to future determine whether BDNF can affect the differentiation of peripheral Treg and Th17 in SLE, and to explore the signal transduction pathway.

2. Methods

2.1 Subjects

We recruited thirty naïve SLE patients from March to September 2019 who were definitely diagnosed after hospitalization in the Rheumatology Department of the First Affiliated Hospital of China Medical University.15ml EDTA anticoagulant whole blood was collected from the patients after overnight of fasting. Diagnosis of SLE based on the Revised criteria from Systemic Lupus International Collaborating Clinics(SLICC) and American College of Rheumatology[23]. SLE disease activity index(SLEDAI) scoring system was used to evaluate disease activity, and the patients with≥5 scores were selected[24]. The study was approved by the Ethics Committee of the First Affiliated Hospital of China Medical University(No. 2018-214-3) and conducted in accordance with the declaration of Helsinki. Informed consent and signature will be obtained after detailed description and explanation of the study to all subjects.

Exclusion criteria: (1) combination of other autoimmune diseases; (2) acute or chronic infection, including hepatitis virus or human immunodeficiency virus infection; (3) tumors; (4) endocrine and metabolic diseases; (5) end-stage hepatic and renal insufficiency; (6) drug abuse; (7) have taken anti-anxiety and depression drugs.

2.2 Preparation of peripheral blood mononuclear cells

EDTA anticoagulant venous whole blood was layered on a Ficoll-Paque density gradient (GE, USA) and centrifuged at room temperature according to the manufacturer's recommended protocol. The peripheral blood mononuclear cell (PBMC) layer was collected, washed with 1×PBS twice and then resuspended for 107/ml density cells. Cell viability was greater than 95%, as determined by trypan blue exclusion assay under the optical microscope.

2.3 Isolation of CD4+T lymphocytes

PBMCs were resuspended in RPMI-1640 medium(HyClone, Logan, UT, USA) containing 10% heat-inactivated fetal calf serum, 40 umol/l L-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin at room temperature. CD4+T cells were purified by positive selection with immunomagnetic beads using human CD4+T-cell isolation kit according to the manufacturer's instructions(130-045-101, Miltenyi Biotec, Germany). Starting with 1-2×10^7PBMCs, 50-100×10^6 CD4+T cells were typically isolated. The purity of CD4+T cells was more than 90%(Sup.2).

2.4 Cell culture

24-well plate was coated with 1×PBS and anti-CD3(2ug/ml) for 4-6h. CD4+T lymphocytes were mixed with anti-CD28(2ug/ml). TGF-β(2.5ng/ml) was added to induce differentiation of Treg cells, and TGF-β(2.5ng/ml)+IL-6(25ng/ml) were added to induce differentiation of Th17 cells. Then these cells were treated with different concentrations of human recombinant BDNF(60ng/ml, 120ng/ml, 350ng/ml) respectively, and cultured at 37℃ in a 5% CO2 incubator for 72 hours.

2.5 Cell surface/intracellular staining and flow cytometry analysis

PBMCs were resuspended in PBS containing 1% bovine serum albumin. For the staining of surface antigens, cells were incubated with monoclonal antibodies or their isotype control in the dark for 30 min on ice. Intracellular staining of Foxp3 and IL-17A was performed for fixation and permeabilization according to the manufacturer's instructions. The antibodies used for the surface or intracellular marker analysis include FITC-conjugated CD4 antibody(11-0049-42, eBioscience, USA), PE-conjugated CD25 antibody(12-0259-42, eBioscience, USA), APC-conjugated CD127(351315, Biolegend, USA)and isotype for CD127(Mouse IgG1 kappa isotype control, 17-4714-82, eBioscience, USA), PE-conjugated IL-17A(12-7179-42, eBioscience, USA). FACSortk flow cytometry(FACSCalibur, BD Biosciences) was used to evaluate the expression of cell surface and intracellular markers in all samples, and FlowJo v10 software(Tree Star, Ashland, USA) was used for data analysis.

2.6 Quantitative PCR

The cells were cultured and harvested at the indicated times. Trizol Reagent(RP1001, BioTeke, Beijing) was used to isolate total RNA from the cultured cells above according to the manufacturer's instructions. The RNA samples were reverse transcribed using a PrimeScript RT reagent Kit(PR6502, BioTeke, Beijing) to obtain the corresponding cDNA, and finally performed real-time fluorescence quantitative analysis. Quantitative detection of gene transcripts was performed using the following primers: Foxp3, Forward: 5'TGACCAAGGCTTCATCTGTG3', Reverse: 5'GAGGAACTCTGGGAATGTGC3', 179bp; RORγt, Forward: 5'CTGTAACGCGGCCTACTCCT3', Reverse: 5'GGCTGTCCCTCTGCTTCTTG3', 161bp; β-actin, Forward: 5'GGCACCCAGCACAATGAA3', Reverse: 5'TAGAAGCATTTGCGGTGG3', 168bp. Reaction conditions were as follows: 94℃ for 5 min, followed by 40 cycles of 94℃ for 10s, of 60℃ for 20s and of 72℃ for 30s, then 72℃ for 150s, 40℃ for 90s, 25℃ for 60s. TP800 real-time fluorescent quantitative PCR instrument(TaKaRa, Japan) was used for amplification, and the results were quantitatively analyzed by $2^{-\Delta\Delta CT}$.

2.7 Western blot

The cultured cells were rinsed with phosphate-buffered saline(PBS) and lysed in 1% Triton lysis buffer. Total proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically transferred to nitrocellulose membranes. The membranes were blocked with 5% skimmed milk in TBSTbuffer at room temperature for 1h and incubated overnight at 4℃ with the primary antibodies. After the appropriate secondary antibodies were added for 45 min, theproteins were detected using an enhanced chemiluminescence reagent(SuperSignal Western Pico Chemiluminescent Substrate; Pierce, USA) and the optical density of the target strip was analyzed by Gel-Pro-Analyzer.

2.8 Statistical analysis

All the data were presented as a mean ± standard deviation. We used the t-test for statistical analysis for parametric data and the Mann-Whitney U test for non-parametric data. One-way analysis of variance(ANOVA) was used when there were more than two groups. We performed statistical analyses with SPSS17.0 software and GraphPad Prism 5.0(GraphPad Software, USA) and considered a P value less than 0.05 as significance.

3. Results

3.1 Clinical characteristics of the subjects

The mean age of individuals was 29.7 ± 11.6 years old(mean ± SD), age range(18 to 48 years old), and the average course of disease was 3.1 months. All patients were naïve SLE who without the treatment of hormones or immunosuppressive agents. The SLEDAI scores of all patients were ≥ 5 points. Lung involvement included interstitial pneumonia, pleural effusion and pleural disease.Among SLE patients

with neuropsychiatric symptoms, two was epilepsy, one was stroke and one was acute confusional state(Table 1).

Table 1
Clinical features and manifestations of SLE patients

characteristics	SLE(n = 30)
Age, mean(S.D.), years	29.7(11.6)
Male/Female, n	3/27
Naïve patients, n	30
Disease duration, median, months	3.1
Clinical manifestations, n (%)	
Malar rash	12(40)
Photosensitivity	9(30)
Oral ulcer	11(36.7)
Arthritis/Arthralgia	8(26.7)
Autoimmune hemolytic anemia	12(40)
Leukopenia	19(63.3)
Lymphopenia	28(93.3)
Thrombocytopenia	16(53.3)
LN	19(63.3)
NPSLE	4(13.3)
Lung involvement	6(20)
SLEDAI	All ≥ 5, 15 ± 6
LN: lupus nephritis; NPSLE: neuropsychiatric lupus erythematosus; SLEDAI: SLE disease activity index.	

3.2 Effect of BDNF on proliferation and differentiation of Th17 in CD4 + T lineage

After co-cultured for 72 hours, the proportion of CD4 + IL-17A + and the expression of RORγtmRNA in the TGF-β + IL-6 group were increased significantly. Figure 1A enumerated the flow diagram that the proportion of CD4 + IL-17A+. The result showed that under the condition of Th17 polarization, the

proportion of CD4 + IL-17A + and the expression of RORγtmRNA gradually decreased after the addition of different concentration BDNF(60ng/ml, 120ng/ml, and 350ng/ml)($p < 0.01$,$p < 0.001$ respectively. Figure 1B and Fig. 1C). Our result revealed that BDNF inhibited the proliferation and differentiation of Th17 by down-regulating the expression of RORγtmRNA in CD4 + T lineage, and the effect was augmented with the increase of BDNF concentration.

3.3 Effect of BDNF on proliferation and differentiation of Treg in CD4 + T lineage

The proportion of CD4 + CD25 + $CD127^{low}$ in the TGF-β group was increased significantly. Figure 2A enumerated the flow diagram that the proportion of CD4 + CD25 + $CD127^{low}$. The result showed that under the condition of Treg polarization, the proportion of CD4 + CD25 + $CD127^{low}$ was gradually increased after the addition of 120ng/ml and 350ng/ml BDNF($p < 0.001$,Fig. 2B), but the difference was not significant($p = 0.181$) in low-dose BDNF group(60ng/ml). The expression of Foxp3mRNA was gradually increased after addition of BDNF(60ng/ml, 120ng/ml, and 350ng/ml)($p < 0.001$, Fig. 2C). Our result revealed that BDNF promoted the proliferation and differentiation of Treg by up-regulating the expression of Foxp3mRNA in CD4 + T lineage, and the effect was augmented with the increase of BDNF concentration.

3.4 Study of the signal transduction pathway mediated by BDNF during the development of Th17 and Treg

3.4.1 BDNF inhibits the proliferation and differentiation of Th17 through PI3K-Akt-mTORC1 axis and ERK1/2 pathway

Under the condition of Th17 polarization, the phosphorylation of Akt, mTORC1 and ERK1/2 was detected by western-blot. The results were showed in Fig. 3A and B. TGF-β + IL-6 group was the positive control, and the phosphorylation of Akt, mTORC1 and ERK1/2 was significantly enhanced. Compared with the positive control, the phosphorylation of Akt, mTORC1 and ERK1/2 in the TGF-β + BDNF group was reduced($p < 0.001$), while there was no significant difference in the phosphorylation of Akt in the TrkBIgG pretreatment group. Although the phosphorylation of mTORC1 in the TrkBIgG group was decreased compared with the positive control, the phosphorylation was also significantly enhanced when compared with the TGF-β + IL-6 + BDNF group($p < 0.001$). Our result demonstrated that BDNF down-regulated the development ofTh17 by inhibiting the activation ofPI3K-Akt-mTORC1 axis and ERK1/2 pathway. Meanwhile, the proportion of Th17 was consistent with the activation of PI3K-Akt-mTORC1 axis(Fig. 3C).

3.4.2 BDNF upregulates the proliferation and differentiation of Treg through PI3K-Akt-mTORC1 axis and ERK1/2 pathway

Under the condition of Treg polarization, the results were showed in Fig. 4A and B. The phosphorylation of Akt, mTORC1 and ERK1/2 was significantly reduced in the TGF-β group. Compared with the TGF-β group, the phosphorylation of Akt and mTORC1in the TGF-β + BDNF group was reduced($p < 0.001$,respectively), although the phosphorylation of ERK1/2 was also reduced in the TGF-β + BDNF group, but the difference was not significant ($p = 0.086$), while there was no significant difference in the phosphorylation of Akt and mTORC1 in the TrkBIgG pretreated group. Our result demonstrated that BDNF up-regulated the proliferation and differentiation of Treg by inhibiting the activation ofPI3K-Akt-mTORC1 axis, probably also including the inhibition of the ERK1/2 pathway. Figure 4C showed the proportion of Treg.

4. Discussion

For the first time we studied the effect of BDNF on the proliferation and differentiation of peripheral blood CD4+T cells into Th17 and Treg in SLE patients. The results showed that BDNF inhibited the development of Th17 and up-regulated the development of Treg. In addition, we also explored the signal transduction pathway, which indicated that BDNF affect the development of Th17 and Treg through inhibiting the activation of PI3K-Akt-mTORC1 axis and ERK1/2 pathway.

Recently the theory of neural-immune network has been proposed[25,26]. There were several reports on serum BDNF level in NPSLE patients, suggesting that BDNF was associated with the disease activity and brain parenchymal injury of NPSLE[27]. We also reported BDNF level was significantly decreased in serum of SLE[21]. However, the relationship between BDNF and T cell subpopulation is ambiguous.

Aberrant immune response of T lymphocytes plays a crucial role in the pathogenesis of SLE(Sup.3)[28]. Increasing quantity of T helper cells attributes to excessive secretion of inflammatory cytokines. It was reported that there existed an imbalance of cytokines in the serum of SLE[14,29], with elevated cytokines such as IL-4, IL-6, IL-17A. Such imbalance is not limited to SLE flares, but is the hallmark of the disease, since also patients with quiescent disease display a TH17/Treg ratio favoring Th17. Amount of evidences demonstrated that Th17 cells and IL-17A play important roles in the pathogenesis of SLE[28,30]. Differentiation of Th17 and Treg is interrelated and restricted. RORγt and Foxp3 are the key transcription factors for the differentiation of Th17 and Treg[4-6,31]. TGF-β can induce the expression of RORγt and Foxp3 simultaneously, but the interaction between Foxp3 and RORγt inhibits the action of RORγt[5]. Only in the presence of IL-6 the inhibition of Foxp3 on RORγt can be removed and the development of Th17 be promoted[5,32]. Otherwise, Foxp3 promotes the development of Treg[33,34]. As we known, serum IL-6 level in SLE is increased[15], and IL-6 activates STAT3, thereby down-regulating the expression of Foxp3, resulting in the proportion of Th17 cells increases in SLE[13].

Our result showed that the proportion of CD4+IL-17A+(Th17) decreased significantly with the increasing BDNF concentration, while the proportion of CD4+CD25+CD127low(Treg) increased. In addition, we found that the expression of RORγtmRNA showed a decreasing intendancy, while the expression of Foxp3mRNA was increased. We proved that BDNF inhibited the differentiation of Th17 by down-regulating the

expression of RORγtmRNA, and promoted the differentiation of Treg by up-regulating the expression of Foxp3mRNA. Previously, we have reported that serum BDNF level is lower in active stage of SLE[21]. We speculated that decreased BDNF level in serum of SLE probably weakened its inhibition on the proliferation of Th17, and the deveolpment of Th17 increased, participating in the pathogenesis of SLE. However, BDNF level gradually increased during the convalescence of the disease, restored its inhibition on Th17 and finally up-regulated the deveolpment of Treg. From the results above we speculate that BDNF appears to have a vital role in maintaining the balance of Treg/Th17 ratio.

Previous studies confirmed that the activity of PI3K/Akt pathway was enhanced in murine lupus[35]. The inhibitor of PI3K could improve the symptoms of glomerulonephritis in MRL/Faslpr SLE mice and reduced the mortality[36]. MAPK/ERK was involved in the pathogenesis of SLE[37] and MAPK inhibitor had been shown to reduce autoimmune responses[38]. The differentiation and function of Th17 are controlled by a variety of intracellular signaling pathways and complex transcription factor networks[39]. It was reported that the PI3K-Akt-mTORC1 axis had positive regulating effect on the differentiation of Th17[8,9,40]. In the CD4+T lineage, both PI3K and mTORC1 inhibitors can increase the differentiation of Treg[40]. Kurebayashi Y[39] also reported that the PI3K-Akt-mTORC1-S6K1 axis has positive regulating effect on the differentiation of Th17 by inhibiting the expression of Gfi1 and promoting nuclear translocation of RORγt[8]. As the downstream of mTORC1, S6K1 induces the expression of transcription factors EGR1 and EGR2[41], and EGR1 and EGR2 directly bind to the Gfi1 promoter to inhibit the expression of Gfi1 and accelerate the differentiation of Th17[8]. S6K2 is the nuclear counterpart of S6K1, which has the role of nuclear localization signal. S6K2 can transport RORγt to the nucleus after binding to RORγt by a back-loading manner. The expression of S6K2 is dependent partly on mTORC1 after TCR stimulation. Therefore, the PI3K-Akt-mTORC1-S6K2 pathway also up-regulates the differentiation of Th17 through the nuclear translocation of RORγt[8]. Otherwise, Th17 differentiation is also positively regulated by HIF-1, a transcription factor induced by hypoxia. Recent studies have shown that STAT3-induced HIF-1 binding to Foxp3 leads to the degradation of Foxp3 proteome[42], which removes its inhibition on RORγt. Reports have shown that both hypoxia and HIF-1 have positive and negative regulatory effects on the differentiation of Th7 and Treg respectively[43,44].

In addition to RORγt, STAT3 is also an important transcription factor for the differentiation of Th17[45], and IL-6 is the necessary factor for the activation of STAT3[46]. Ren[47] reviewed the mechanism that mTORC1 up-regulated the expression of IL-17 by STAT3, HIF-1, S6K1 and S6K2. In addition, as the downstream pathway of IL-6 and TGF-β, MAPK/ERK is also involved in differentiation of Th17 and the development of autoimmune diseases. Studies have shown that blocking the activation of ERK pathway can alleviate autoimmune response mediated by Th17 in EAE mouse model. Liu[10] reported the role of ERK in the development of Th17 and Treg. They demonstrated that blocking the activation of the IL-6-induced ERK pathway under the condition of Th17 polarization could down-regulate the expression of RORγt, inhibiting the differentiation of Th17, and up-regulate the differentiation of Treg. In vitro, they also demonstrated that T cells treated with ERK inhibitor produced more TGF-β, reduced differentiation of Th17, and reduced intestinal inflammatory response in colitis[10].

We explored the signal transduction pathway that BDNF affected differentiation of CD4+T. Our data showed that the phosphorylation of Akt, mTORC1 and ERK1/2 were increased after the addition of TGF-β+IL-6, and the proportion of CD4+IL-17A+ were increased, indicating that we successfully induced the differentiation of Th17 by activating the PI3K-Akt-mTORC1 axis and the ERK1/2 pathway. While the phosphorylation of Akt, mTORC1, ERK1/2 decreased in the BDNF group, the corresponding CD4+IL-17A+ ratio also declined. In the group pre-treated with TrkBIgG, there was no significant change in the phosphorylation of Akt and mTORC1. From the results above we hypothesized that combination of BDNF and TrkB may directly or indirectly down-regulate the development of Th17. We also proved that the PI3K-Akt-mTORC1 axis and ERK1/2 pathway were indeed affected by BDNF/TrkB complex. Similarly, Under the condition of Treg polarization induced by TGF-β, the phosphorylation of Akt and mTORC1 was also reduced in the BDNF group, nevertheless, the percentages of CD4+CD25+CD127^{low} were increased. In the TrkBIgG group, there was no significant change in the phosphorylation of Akt and mTORC1. The phosphorylation of ERK1/2 was also decreased. Our data demonstrated that BDNF inhibited the development of Th17 regardless of Th17 or Treg polarization, and in turn promoted the development of Treg. Compared with initial CD4+T, under the condition of Th17 polarization, the activation of PI3K-Akt-mTORC1 axis was inhibited more obviously(Fig.3A). The amount of Th17 may be one of the reasons, or there may exist a certain correlation between BDNF and TGF-β or IL-6, or the downstream pathway of TGF-β and IL-6. Whether BDNF has a synergistic effect with TGF-β is indecisive. Although expressions of both Foxp3 and RORγt require TGF-β, the signaling cascade of downstream of TGF-β is different. For example, Smad4 seems to be necessary to induce both Foxp3 and RORγt, nevertheless, however, TGF-β induces Foxp3 expression through Smad2-/Smad3[48]. Smad pathway is necessary for TGF-β-induced Foxp3 expression[49]. It was reported that the ERK pathway negatively regulated the expression of Foxp3 and inhibited the differentiation of Treg, which is also dependent on the cytokine TGF-β[50]. In conclusion, the deep understanding about the molecular mechanism of BDNF affecting the PI3K- Akt - mTORC1 axis and ERK1/2 pathway remains to be further explored.

5. Conclusion

Our results demonstrated that BDNF was involved in the pathogenesis of SLE. BDNF not only promotes the proliferation of T lymphocytes, but also regulates the proliferation and differentiation of Treg and Th17. This discovery gives us a deeper understanding of the effect of BDNF on T lymphocytes, and BDNF may have profound and important meaning for autoimmune diseases.

Declarations

AUTHOR CONTRIBUTIONS

Bailing Tian and Xiaoyu Hou collect the samples and performed the experiments. Bailing Tian and Mengmeng Zhao collected and analyzed the data. Bailing Tian prepared the manuscript. Mengmeng Zhao contributed to the conception of the study. All authors have read and approved the manuscript.

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Availability of data and material:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of interest:

The authors declare that they have no competing interests.

Ethics approval:

The project was approved by the clinical scientific research section of the Ethics Committee of The First Hospital Of China Medical University (No. 2018-214-3).

Consent to participate:

This study was approved by the ethics committee of China Medical University (Shenyang, China). All procedures were following the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki.

Consent for publication:

All patients provided written informed consent.

Code availability:

Not applicable.

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Figures

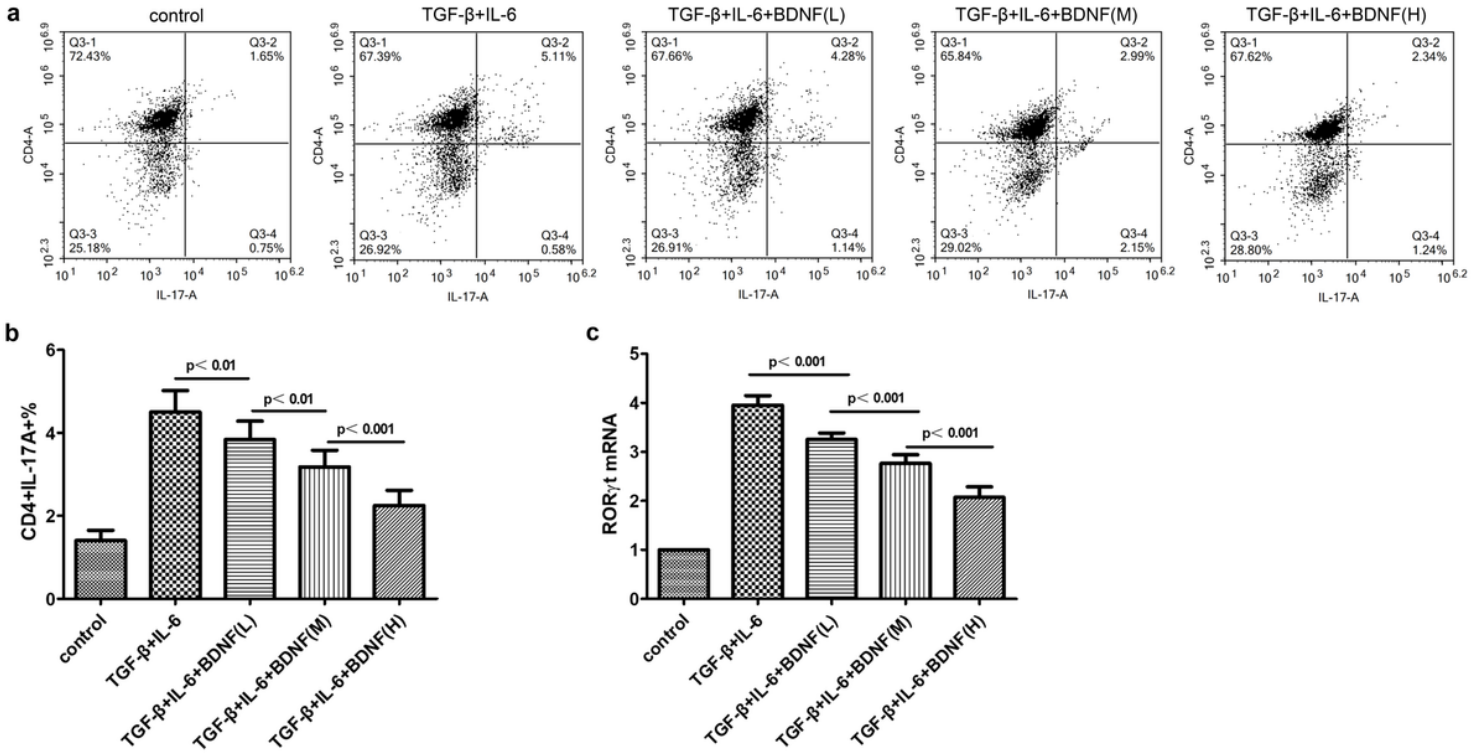

Figure 1

Effect of BDNF on the development of Th17 Under the condition of Th17 polarization: (A)Detection of the proportion of CD4+IL-17A+ cells in each group treated with different concentrations of BDNF by Flow Cytometry.(B)Comparison of the effect of BDNF on Th17 differentiation in each group. The proportion of CD4+IL-17A+ cells increased significantly after induction by TGF-β+IL-6. After the treatment of BDNF with different concentrations, the proportion of CD4+IL-17A+ cells in each group decreased($p < 0.01$). (C)Comparison of the effect of BDNF on RORγtmRNA in each group. After induction by TGF-β+IL-6, the expression level of RORγtmRNA significantly increased. After the treatment of BDNF with different concentrations, the expression level of RORγtmRNA in each group decreased($p < 0.001$).

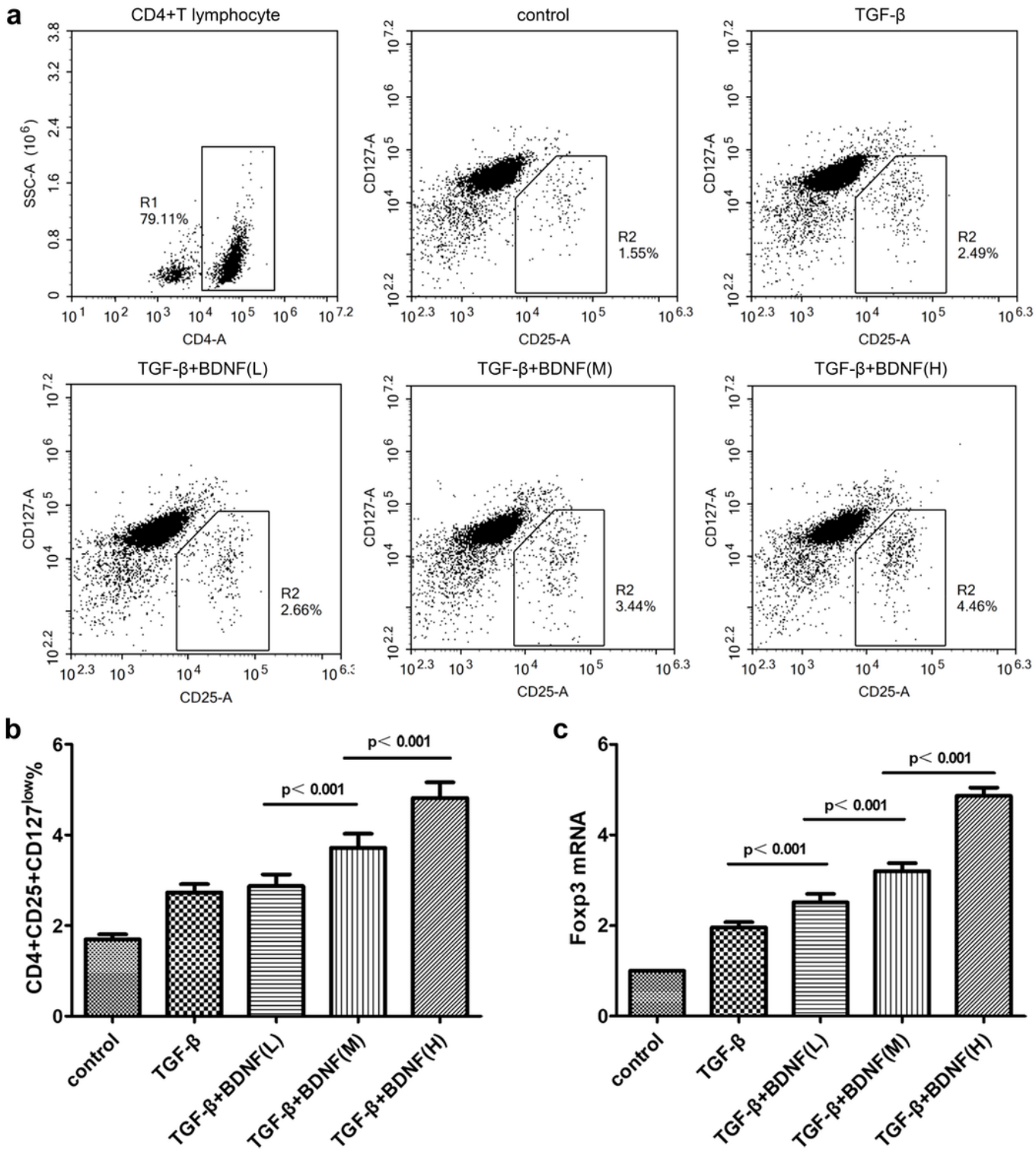

Figure 2

Effect of BDNF on the development of Treg Under the condition of Treg polarization: (A)Detection of the proportion of CD4+CD25+CD127low cells in each group treated with different concentrations of BDNF by Flow Cytometry. (B)Comparison of the effect of BDNF on Treg differentiation in each group. After induction by TGF-β, the proportion of CD4+CD25+CD127low increased significantly. The proportion of CD4+CD25+CD127low cells in the group treated with 120ng/ml and 350ng/ml BDNF increased(p <

0.001).(C)Comparison of the effect of BDNF on Foxp3mRNA in each group. After the induction by TGF-β, the expression level of Foxp3 mRNA significantly increased. After the treatment of BDNF, the expression level of Foxp3mRNA in each group increased($p < 0.001$).

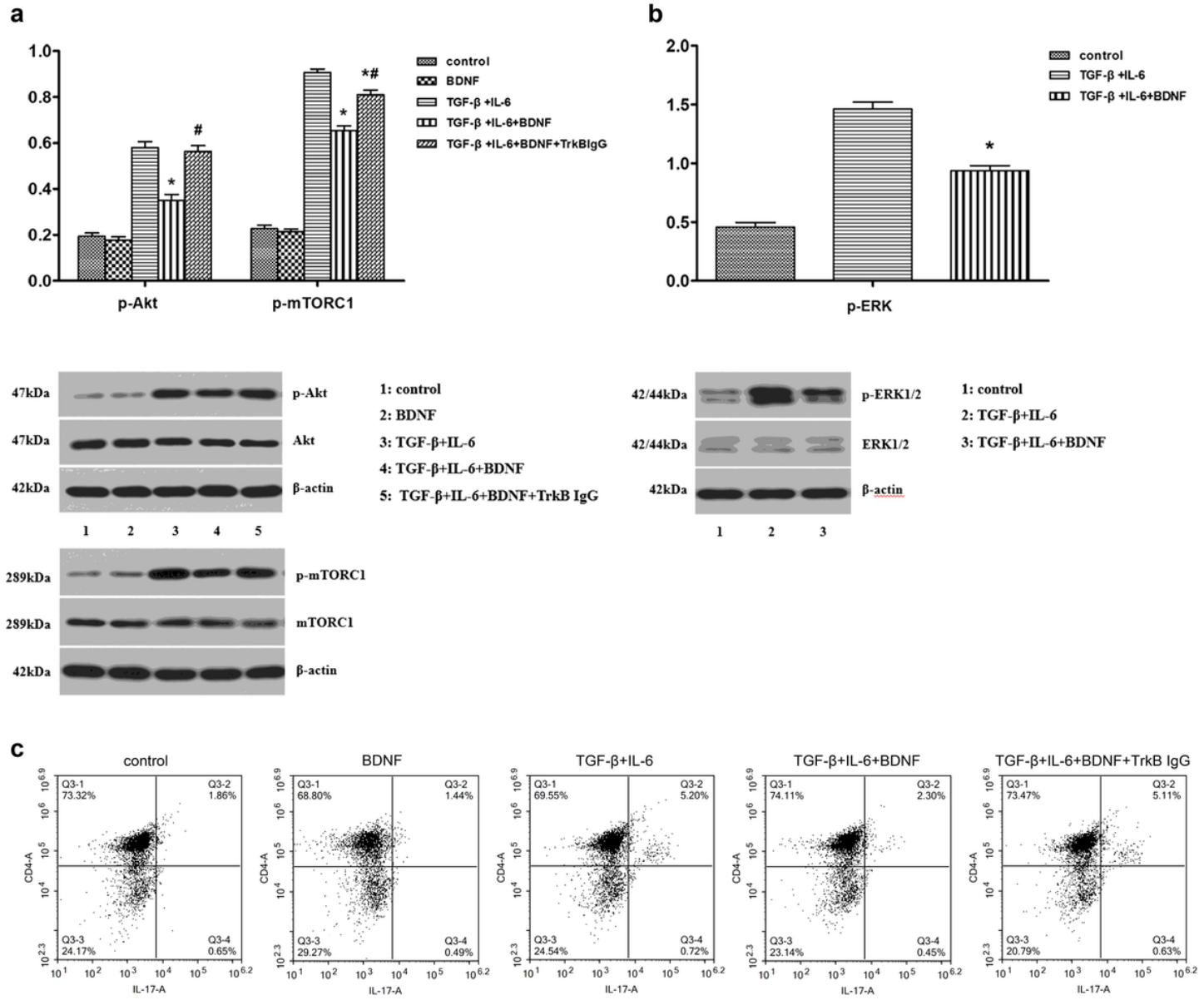

Figure 3

Effects of BDNF on PI3K-Akt-mTORC1 and ERK1/2 pathway under the condition of Th17 cell polarization A.(1)Phosphorylation of Akt and mTORC1 was significantly enhanced after the induction by TGF-β+IL-6. (2)Phosphorylation of Akt and mTORC1 in the BDNF(350ng/ml) group was significantly reduced(compared with the TGF-β+IL-6 group, *p<0.001). (3)Phosphorylation of Akt was significantly enhanced in the TrkBIgG group(compared with the TGF-β+IL-6+BDNF group, #p<0.001, no difference between TGF-β+IL-6 group and TGF-β+IL-6+BDNF+TrkBIgG group); Phosphorylation of mTORC1 was significantly enhanced compared with TGF-β+IL-6+BDNF group(#p < 0.001), and reduced compared with TGF-β+IL-6 group(*p<0.001)(X±SD,N=5). B.(1)Phosphorylation of ERK1/2 was significantly enhanced after the induction by TGF-β+IL-6. (2)Phosphorylation of ERK1/2 in the 350ng/ml BDNF group was

significantly reduced(compared with the TGF-β+IL-6 group, *p < 0.001) (X±SD,N=5). C.Detection of the proportion of CD4+IL-17A+ cells in each group by flow cytometry which was consistent with activation of signal pathway(including control, BDNF, TGF-β+IL-6, TGF-β+IL-6+BDNF, and TGF-β+IL-6+BDNF +TrkB IgG group).

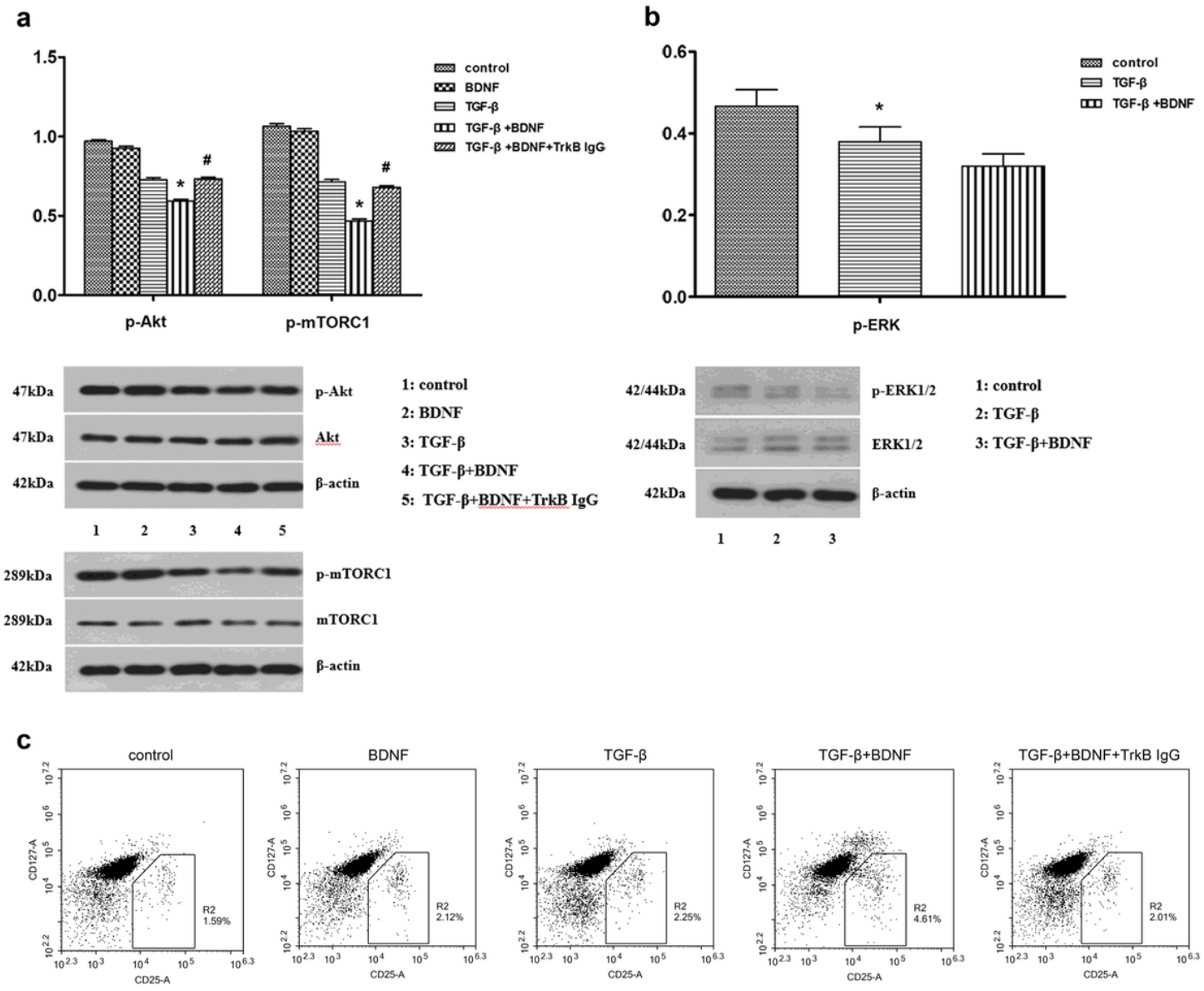

Figure 4

Effects of BDNF on PI3K-Akt-mTORC1 and ERK1/2 pathway under the condition of Treg cell polarization A.(1)Phosphorylation of Akt and mTORC1 reduced significantly after the induction by TGF-β . (2)Phosphorylation of Akt and mTORC1 in the BDNF group(350ng/ml) further reduced(compared with the TGF-β group, *p<0.001). (3)Phosphorylation of Akt and mTORC1 was significantly enhanced in the TrkBIgG group(compared with the TGF-β+BDNF group, #p<0.001, no difference between TGF-β group and the TGF-β+BDNF+TrkBIgG group)(X±SD,N=5). B.(1)Phosphorylation of ERK1/2 was significantly reduced after the induction by TGF-β(compared with the control group, *p=0.025). (2)Phosphorylation of ERK1/2 in the TGF-β +350ng/ml BDNF group was further reduced(compared with the TGF-β group, p=0.086)

(X±SD,N=5). C.Detection of the proportion of CD4+CD25+CD127low cells in each group by flow cytometry(including control, BDNF, TGF-β, TGF-β+BDNF and TGF-β+BDNF +TrkBIgG group).

Supplementary Files

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