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Effects of rapamycin on DC-SIGN expression and biological functions in DC

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1. ABSTRACT

Rapamycin, a macrolide antibiotic, has potent immunosuppressive properties as an antirejection therapy in organ transplantation. Studies show that dendritic cells (DC) are important targets for rapamycin, which can inhibit DC maturation and DC-induced allogeneic T cell proliferation. In this study, we investigated the effects of rapamycin on the expressions of DC-SIGN and transcription factor PU.1 and the function of DC. Treatment with rapamycin significantly reduced the expression of DC-SIGN in a dose-dependent manner associated with suppression of PU.1 gene expression and the ability of DC to migrate and stimulate T cell proliferation. The expression of DC-SIGN was significantly suppressed using PU.1 siRNA. Intriguingly, rapamycin treatment largely decreased the expressions of PU.1 and DC-SIGN in THP-1 cells. In addition, treatment with rapamycin down-regulated the promoter activity of DC-SIGN. In conclusion, rapamycin inhibits DC-SIGN expression and suppresses the ability of DC to migrate and stimulate T cell proliferation through the PU.1 gene transcription pathway.

2. INTRODUCTION

The macrolide antibiotic rapamycin (Rapa), produced by *Streptomyces hygroscopicus*, is a very promising immunosuppressant. Rapa was originally identified as a low-toxic antifungal agent, and was later found to have potent immunosuppressive activity in the prevention of organ rejection (1). After binding to the intracellular immunophilin FK506-binding protein 12 (FKBP12), the resulting complex (Rapa-FKBP12) inhibits the activity of the mammalian target of Rapa (mTOR), which is involved in several signal transduction pathways. Two different mTOR complexes have been identified, mTORC1 and mTORC2. mTORC1 is highly sensitive to Rapa and regulates protein synthesis, transcription, cell survival and autophagy, while mTORC2 is relatively insensitive to Rapa and affects the activity of multiple cellular functions by prolonging Rapa exposure (2). Previously, T cells were considered as the principal therapeutic targets of Rapa (3). However, recent studies have shown that dendritic cells (DC) are important targets for Rapa, which can inhibit DC maturation and DC-induced

allogeneic T cell proliferation (4). DC are critical initiators and participators of the immune response by modulating cell-surface molecules, especially DC-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), a major member of the C-type lectin superfamily. Functionally, DC-SIGN is a pattern recognition receptor (PRR) that recognizes pathogen associated molecular patterns (PAMPs) present on the cell surface of microorganisms and which mediates antigen capture and internalization (5, 6). Upon antigen capture, DC become activated and present antigens to naïve T cells, initiating adaptive immune responses. In addition, DC-SIGN plays an important role in DC migration and adhesion by recognizing intracellular adhesion molecule (ICAM)-2, 3 and Mac-1 (7, 8). DC-SIGN also participates in the immune escape of pathogens and tumors (9). Therefore, as an innate immune molecule, DC-SIGN plays a key role in DC-mediated positive and negative immune regulation in various infectious and inflammatory diseases.

It is well established that Rapa can affect the capacity of DC to uptake antigen and suppress DC maturation and antigen-presenting functions (10,11). Moreover, Rapa can further inhibit early DC differentiation by blocking the potent endogenous DC growth factor-fms-like tyrosine 3 kinase ligand (Flt3L) (12). However, the mechanisms that mediate Rapa actions on DC biological functions and DC-SIGN expression remain unclear. Thus, we used immature DC and DC-like cells to investigate whether Rapa could affect DC-SIGN expression, DC migration and DC-mediated T cell activation, and investigate the mechanism of transcription factor PU.1 on the DC-SIGN expression.

3. MATERIALS AND METHODS

3.1. Materials and reagents

Roswell Park Memorial Institute 1640 (RPMI 1640), Dulbecco's modified Eagle's medium (DMEM), L-glutamine and fetal bovine serum were purchased from Gibco BRL (Crewe, Cheshire, UK). Lymphocyte separation medium was purchased from Shanghai Huajing High-tech Co., Ltd. (Shanghai, China). Anti-human CD14 immunomagnetic beads were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). Recombinant human granulocyte/macrophage colony-stimulating (rhGM-CSF), recombinant human interleukin-4 (rhIL-4), stromal cell-derived factor-1 (SDF-1) were purchased from R&D Systems (Minneapolis, MN, USA). PE-conjugated mouse anti-human DC-SIGN monoclonal antibody (mAb) was purchased from BioLegend (San Diego, CA, USA). Rapa was purchased from Sigma (St Louis, MO, USA). Rabbit anti-human PU.1 antibody and rabbit anti-human DC-SIGN monoclonal antibody were purchased from Abcam (Cambridge, UK). Mouse anti-human GAPDH antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Horseradish peroxidase (HRP)-conjugated anti-mouse IgG was purchased from Cell Signaling Technology (Danvers, MA, USA). The reverse transcription-polymerase chain reaction (RT-PCR) kit was purchased from Takara (Mountain View, CA, USA). DharmaFECTTM1 siRNA Transfection Reagent (T-2004-01) and 5 × siRNA Buffer were purchased from Dharmacon (Lafayette, CO, USA). Lipofectamine™ 2000 was purchased from Life Technologies (Invitrogen, CA, USA). The firefly and Renilla Dual-Glo™ Luciferase Assay System was purchased from Promega (Madison, WI, USA). The electrochemiluminescent (ECL) reagent was purchased from Amersham Biosciences (Amersham, UK). The 5-(and-6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) was obtained from Molecular Probes (Eugene, OR) and 4,5 dimethyl-2-yl 2,5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma Chemical Co.

3.2. Cell culture, transfection and induction

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood (40 ml) of healthy donors by density gradient centrifugation over Lymphoprep. $CD14^+$ monocytes were isolated from PBMCs using CD14 immunomagnetic beads. The isolated cells were then cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS) supplemented with rhGM-CSF (500 U/ml) and rhIL-4 (1000 U/ml) . After 5 days, immature DC was harvested and transferred to 12-well cell culture plates for subsequent experiments such as transfection and medicine intervention. Immature DCs were transfected by PU.1 siRNA. The PU.1 siRNA sequences were: sense 5'-GGATGACAGATCCCTACCCGT-3', antisense 5'-AGAGAAACAGGTC GACGGGG-3' (Genepharma, Shanghai, China). Four groups were produced: 1) control, 2) PU.1 siRNA, 3) Rapa (10 ng/ml), and 4) Rapa (10 ng/ml) + PU.1 siRNA. Immature DC was transferred to 6-well plates at 2×10^5 cells per well. Transient transfection of siRNA was performed using Lipofectamine™ 2000 reagent.

THP-1 and 293T cells were obtained from the Shanghai Institute of Hematology (Shanghai, China). THP-1 cells were seeded in RPMI-1640 supplemented with 10% FBS at 10^5 cells/ml and were then induced to differentiation by treatment with phorbol 12-myristate 13-acetate (PMA) and IL-4. PMA (10 ng/ml) was added in complete medium and the cells were incubated. After 18 h, the complete medium was removed and RPMI 1640 medium supplemented with PMA (10 ng/ml) was added into cell culture for 6 h of cell starvation. After starvation for 4 h, IL-4 was added at a final concentration of 1000 U/ml. Following serum starvation, THP-1 cells were then supplemented with 10% FBS for an additional 48 h. Finally, the cells were harvested for analysis. The 293T cells were maintained in DMEM with 10% Fetal Bovine Serum. Transfections were performed using Lipofectamine™ 2000 reagent, according to the manufacturer's protocol.

3.3. Flow cytometry

Immature DC was harvested and transferred to 6-well cell culture plate at 10^5 cells/well as described in 3.2. After the cell growth density reached 70%, Rapa was added at final concentrations of 0, 0.1, 1 and 10 ng/ml for 24 h. Cells from each group at a density of 5×10^5 were washed twice with PBS and incubated with FITC-conjugated mouse anti-human DC-SIGN monoclonal antibody for 30 min at 4°C. Cells were washed again and detected by flow cytometry (BD FACSAria™ Cell Sorter). The results were analyzed by FlowJo (TreeStar, San Carlos, CA, USA).

3.4. RT-PCR

Primer design and synthesis: human PU.1 mRNA from GenBank was used as a template to design primers using Primer Designer 5.0 (Scientific and Educational Software, Durham, NC, USA). PU.1 primer sequences were as follows: sense 5'-GCGACCATTACTGGGACTTCC-3', antisense 5'-GGGTATCGAGGACGTGCAT-3', and the length of amplified products was 156 base pairs (bp). β-actin as used as internal control reference: sense 5'-GACTACCTCATGAAGATCCT-3', antisense 5'-GCGGATGTCCACG TCACACT-3', and the length of amplified products was 313 bp. Total RNA was extracted using TRIzol. The PCR reaction system was a mixture of total RNA (0.5 μg), 10 × RT buffer (2.5 μl), $MgCl_2$ (25 mmol/L; 2.5 μl), dNTP (10 mmol/L; 2.5 μl), RNase inhibitor (40 U/μl; 0.5 μl), AMVV-Optimized Taq (2.5U), AMV RTase XL (2.5 U), PU.1 primer (20 μmol/L; 0.5 μl), β-actin primer (20 μmol/L; 0.5 μl), in a final volume of 25 μL with RNase free water. Real-time PCR was performed with initial melting at 95°C for 5 min, then 30 cycles of melting at 94°C for 30s, annealing at 55°C for 30s, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products were stained with ethidium bromide (EB) following resolution on a 1.2% agarose gel, and analyzed by a computer image analysis system (UVIphoto MV).

3.5.Western blot

Cell lysates were clarified by centrifugation at 16,000 × *g* for 10 min at 4°C. Protein in the supernatant was extracted, followed by the addition of 5 × SDS protein loading buffer and boiled at 95°C for 5 min. Markers and samples were electrophoresed by denaturing 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene difluoride (PVDF) membrane. The location of proteins of interest were detected by primary antibody, including rabbit anti-human DC-SIGN polyclonal antibody (dilution 1:500), rabbit anti-human PU.1 monoclonal antibody (1:5000), mouse anti-human GAPDH monoclonal antibody (1:10,000). HRP conjugated-secondary antibody bound to the primary antibody was visualized using enhanced chemiluminescence (ECL) reagent.

3.6. Cell migration assay

Endothelial cells were isolated using collagenase digestion methods from human newborn umbilical cord tissue under sterile conditions. Human umbilical vein endothelial cells were seeded in the upper chamber of 8 μg/ml fibronectin-coated 24-well transwells. When cells reached confluence, DC labeled with the cell-permeable fluorescent dye 5-(6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) were added to upper chambers. In lower chambers, SDF-1 was added at a final concentration of 10 ng/ml. The cells were then incubated at 37°C for approximately 24 h. Finally, DC in the lower chamber were collected for analysis by flow cytometry.

3.7. Mixed lymphocyte reaction

Immature DC (2×10^4 cells/ml) and human $CD4^+$ T cells from normal peripheral blood were plated in 96-well plates at a ratio of 1:10. Cells were incubated at 37°C in a 5% CO_2 incubator and each group was set up in triplicate. After 5 days, 5 mg/ml methylthiazolyldiphenyl-tetrazolium bromide (MTT) solution (20 μl per well) was added to each well and incubated for 4 h. Then the supernatant was removed and 150 μl dimethyl sulfoxide (DMSO) was added. The plates were vibrated for 10 min using an orbital shaker and the absorbance was measured in a microplate reader at 570 nm.

3.8. Dual-luciferase reporter assay

DC-SIGN 5'UTR including the Ets-1 target (23) was cloned into a pGL-3 Basic vector and named pDC-SIGN. Plasmids pDC-SIGN/PU.1 and PcDNA3.1/PU.1 containing PU.1 mRNA were synthesized by Sangon Biotech Company (Shanghai, China). A pRL-SV40 vector with Renilla luciferase and pDC-SIGN was used to transfect 293T cells over-expressing PU.1 or negative control 293T cells, respectively. Rapa (10 ng/ml) was added to 293 T cells after transfection for 24 h. Cells were then incubated at 37°C for another 24 h before harvesting for luciferase assay.

3.9. Statistical analysis

The database was analyzed statistically using Social Sciences (SPSS) 15.0 software package. Enumeration data was analyzed using the chi-squared test and Fisher's exact test. Measurement data were represented as mean ± SD and evaluated by one-way analysis of variance (ANOVA) and t test, as appropriate. P values < 0.05 were considered statistically significant.

4. RESULTS

4.1. Identification of DC phenotype

$CD14^+$ cells were isolated from human PBMCs and cultured in the presence of rhGM-CSF and rhIL-4. On day 5, cells attached to the wall and extended pseudopodia. Then cells were collected and the expression of DC-SIGN (CD209), MHC class II molecules (HLA-DR), CD1a, CD11c, CD54, CD83, CD80 and CD86 were determined by flow cytometry (Figure 1). The percentage of DC-SIGN, MHC class II molecules, CD1a, CD11c, CD54 were highly expressed on the cell surface, while CD83, CD80 and CD86 had a low expression, indicating $CD14^+$ cells had become immature DC.

4.2. Inhibitory effects of Rapa on DC-SIGN expression in human DC

Flow cytometry showed that Rapa has a dose-dependent inhibitory effect on DC-SIGN expression. After 24 h, there was trend for a reduction in DC-SIGN expression by 0.1 ng/ml Rapa compared with the untreated group, although this was not significant ($P > 0.05$). DC-SIGN expression was significantly reduced in the presence of 1 ng/ml and 10 ng/ml Rapa ($P < 0.01$; Figure 2A). The results of flow cytometry were consistent with western blotting analysis (Figure 2B).

4.3. Inhibitory effects of Rapa on DC migration and DC-mediated T cell activation

Previous studies demonstrated Rapa inhibits the

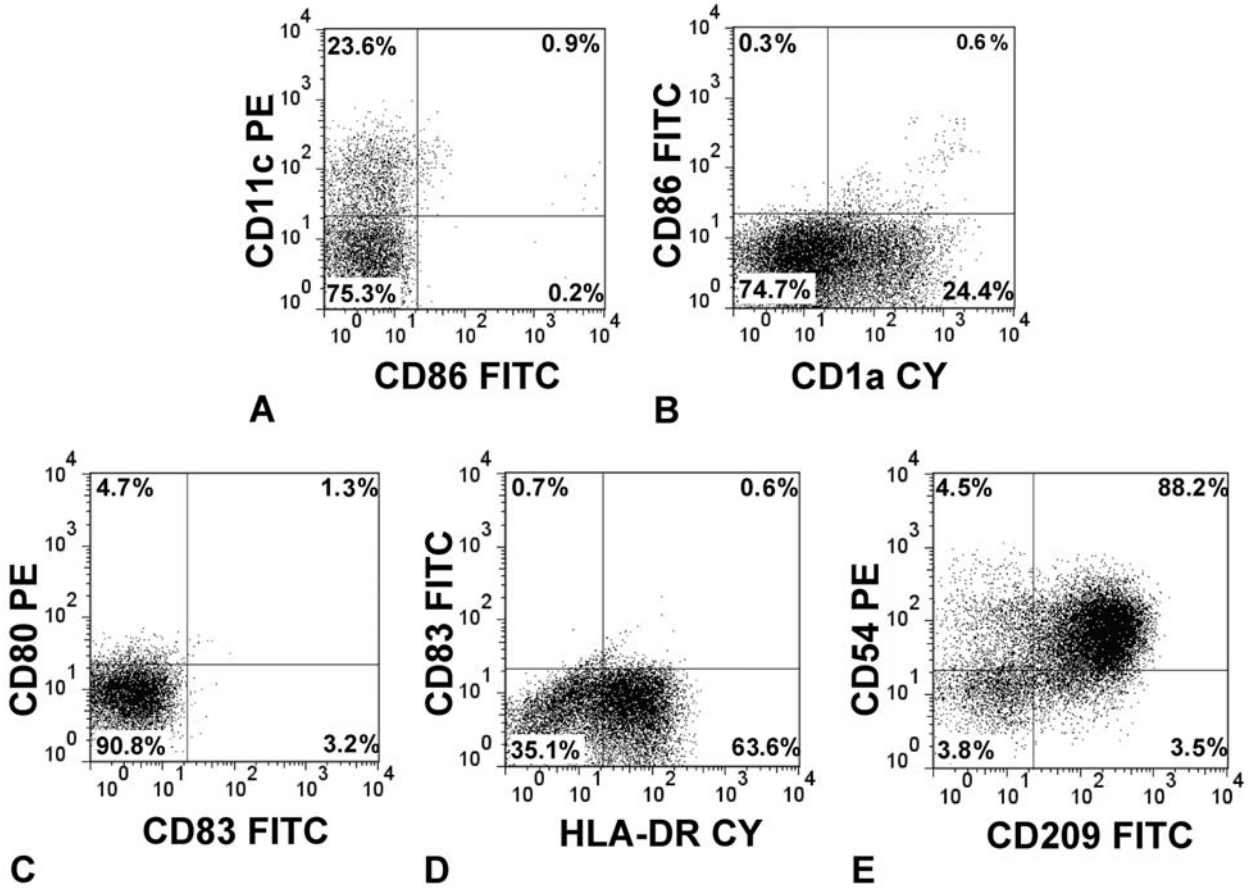

Figure 1. Phenotype analysis of immature DC after 5 days culture. (A-E) Expression of DC-SIGN (CD209), MHC class II molecules (HLA-DR), CD1a, CD11c, CD54, CD83, CD80 and CD86 were assessed by flow cytometry.

expression of DC-SIGN, involving multiple biological processes such as PRR and cell adhesion molecules. In addition, Rapa exerts divergent functions depending on different DC subsets used (13). In this study, we demonstrated the effects of Rapa on PBMC-derived DC. We investigated the migration capability of DC by transendothelial migration assay and the ability of DC to induce T cell proliferation using the MTT test. After 24 h interference with Rapa, DC migration and DC-mediated T cell activation were significantly down-regulated ($P < 0.01$). Thus, Rapa down-regulated DC-SIGN expression in a dose-dependent manner, with a peak value of DC-SIGN expression at a dose of 10 ng/ml (Figure 3).

4.4. Inhibitory effects of Rapa on PU.1 gene expression in human DC

This study demonstrated that Rapa could affect DC-SIGN expression and inhibit DC biological functions. However, the underlying mechanisms of these effects are not fully understood. Reports have shown that the DC-SIGN promoter region contains transcription factor PU.1-binding sites, which promotes DC-SIGN expression (14). In this study, we investigated the effects of Rapa on PU.1 expression and activity. RT-PCR results showed that Rapa had a dose-dependent inhibitory effect on PU.1 mRNA expression. After 24 h interference with Rapa doses of 0.1, 1 and 10 ng/ml, low, moderate and significant inhibitory effects on PU.1 mRNA expression, respectively were observed compared with the untreated group (Figure 4A).

4.5. Effects of Rapa on DC-SIGN expression in human DC through PU.1 gene silencing

To confirm the intervention function of Rapa on DC-SIGN expression, siRNA against PU.1 was used. RT-PCR showed that DC-SIGN expression was decreased after transfection of PU.1-siRNA for 24 and 48 h, indicating that siRNA could effectively down-regulate PU.1 gene expression (Figure 4B). Compared with the control group, the Rapa (10 ng/ml) + PU.1 siRNA group caused a reduction in DC-SIGN expression, which was significantly greater than in the PU.1 siRNA and Rapa (10 ng/ml) groups (Figure 4C). This suggested that Rapa could down-regulate the expression of DC-SIGN and may involve PU.1.

4.6. Rapa inhibits DC-SIGN and PU.1 expression in THP-1 cells and suppresses DC-SIGN Promoter activity

To further clarify the inhibitory effects of Rapa on DC-SIGN expression, the human leukemia monocytic cell

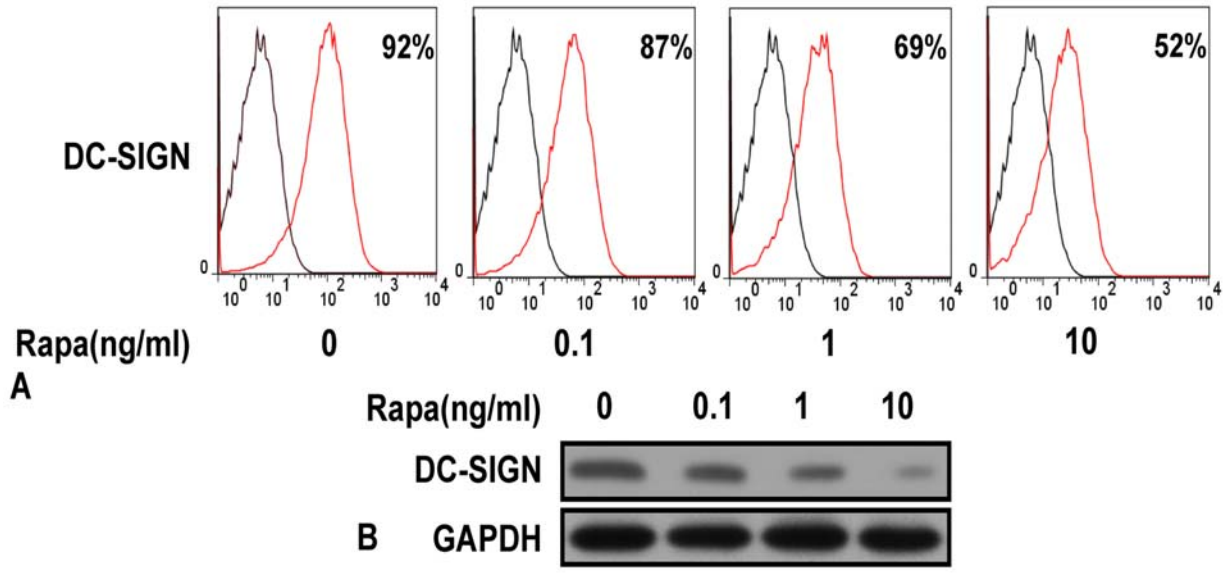

Figure 2. Effects of Rapa on DC-SIGN. Immature DC were cultured at a density of 2×10^6 cells per well in 6-well plates and treated with different concentrations of Rapa as indicated. (A) DC-SIGN expression cells were measured by surface staining and flow cytometry. (B) Western blot was performed to detect DC-SIGN expression. Data are representative of more than three independent experiments.

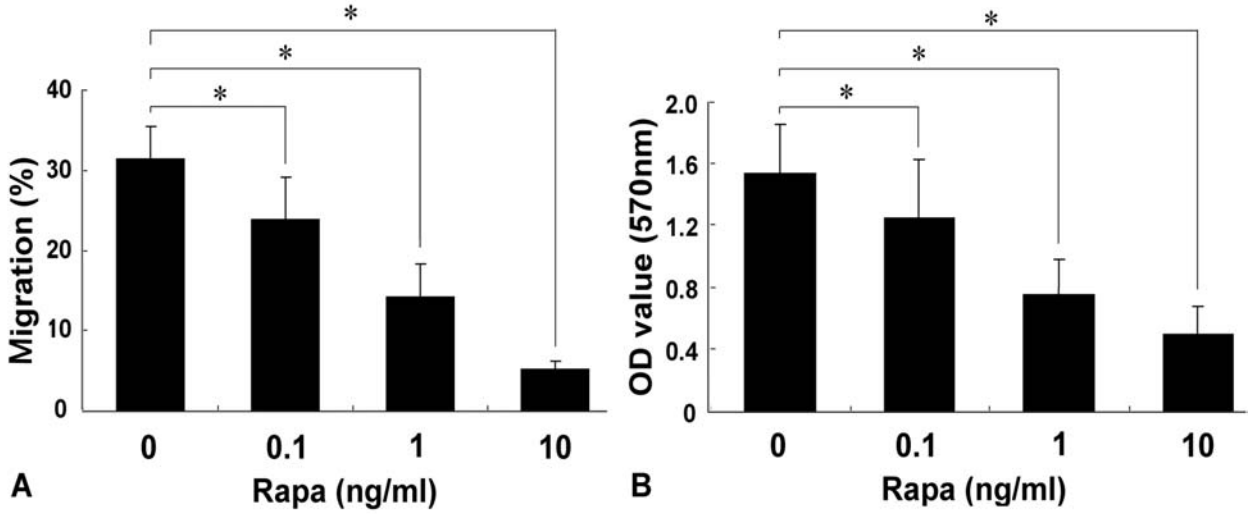

Figure 3. Inhibitory effects of Rapa on DC biological function. (A) Human umbilical vein endothelial cells were isolated from freshly obtained umbilical cords and were seeded on transwell plates. DC were labeled with CFSE and added to the upper chambers. SDF-1 (10 ng/ml) was added into the lower chambers. After 24 h, transmigrated DC in the lower compartment were collected and analyzed by flow cytometry. The percentage of transmigration was calculated by dividing the number of DC in the lower chambers and multiplying by 100. (B) Immature DC, co-cultured with freshly isolated $CD4^+$ T cells from healthy donors at a ratio of 1:10, were treated with various concentrations of Rapa. On day 5, MTT assay was performed in triplicate to measure T cell proliferation. Data represents mean ± SD of three separate experiments. *$P < 0.01$ compared with the untreated group.

line THP-1 was used. THP-1 can be induced to differentiate into DC-like cells by treatment with PMA and IL-4. Western blotting showed that Rapa inhibited the expression of PU.1 and DC-SIGN in THP-1 cells (Figure 5A), similar to the results observed for DC.

Promoter activity of DC-SIGN was low in pDC-SIGN transfected 293T cells (control), but was significantly increased when co-transfected with PU.1 in 293 T cells (PU.1 group) ($P < 0.01$). However, DC-SIGN promoter activity was significantly down-regulated by

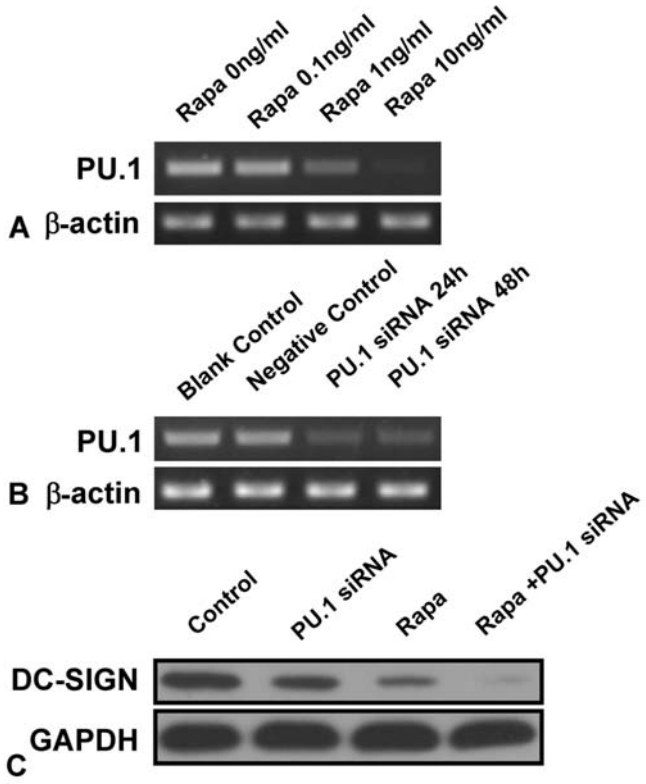

Figure 4. Effects of Rapa on DC-SIGN expression in DC with PU.1 gene silencing. (A) Immature DC were treated with different concentrations of Rapa for 24 h as indicated. PU.1 mRNA expression was determined by RT-PCR. (B) mRNA expression was determined by RT-PCR after transfection of PU.1-siRNA for 24 h and 48 h, respectively. (C) Immature DC were treated with PU.1 siRNA for 48h and/or added Rapa (10 ng/ml) for 24 h. Four groups were produced: 1) Control, 2) PU.1 siRNA, 3) Rapa (10 ng/ml), and 4) Rapa (10 ng/ml) + PU.1 siRNA. Western blotting was performed to detect DC-SIGN expression. Data are representative of more than three independent experiments.

Rapa in the PU.1 group ($P < 0.01$), while no significant change was observed in controls (Figure 5B). Thus, DC-SIGN promoter activity was inhibited by Rapa, and the effects were regulated by PU.1.

5. DISCUSSION

Recently, increasing attention has been paid to immune tolerance induced by immunosuppressants, especially Rapa, a macrolide antibiotic and a new potent immunosuppressive agent. It has a stronger immunosuppressive effect, but lower toxicity compared with FK506 (1,15). Clinical studies indicated that Rapa and its derivatives can be effective against cancer, kidney transplant rejection and immune inflammatory diseases (1,15). Furthermore, Rapa prevents DC migration and DC-mediated T cell differentiation by blocking mTOR and thus exerts an immunosuppressive effect. Among various DC subsets, Rapa mediates different biological functions. Rapa affects bone-marrow derived DC, increases pro-inflammatory cytokine production and NF-κB expression, and decreases levels of IL-10 and STAT3, potentially leading to upregulated T cell differentiation. However, Rapa cannot effectively activate the NF-κB signaling pathway in LPS-stimulated monocyte-derived dendritic cell (moDC), and thus fails to stimulate T cell differentiation (13). Therefore, the mechanism of Rapa remains unexplained and is the focus of current research.

Based on the important role of DC in inflammatory-immune diseases, the intervention and regulation of DC-surface molecules has attracted much attention (16-18). DC-SIGN, a member of the C-type lectin receptor (CLR) family, is a DC innate immune molecular containing pattern recognition receptor with adhesion functions. A recent study suggested DC-SIGN might be involved in regulating DC maturation and function by forming a trimolecular receptor complex with C1q and gC1qR (19). As DC-SIGN can bind to the HIV gp120 protein and promote $CD4^+$ T cell infection by DC resulting in immunodeficiency, it has begun to attract increasing attention (20). Studies demonstrated that the glycosyl group in hyperglycemia could competitively bind to DC-SIGN, leading to a decline in immune function, and thus become an important treatment target for diabetic patients with infection and chronic inflammation complications (21). Other studies reported that the carbohydrate structure of intravenous immunoglobulins (IVIG) could provide an

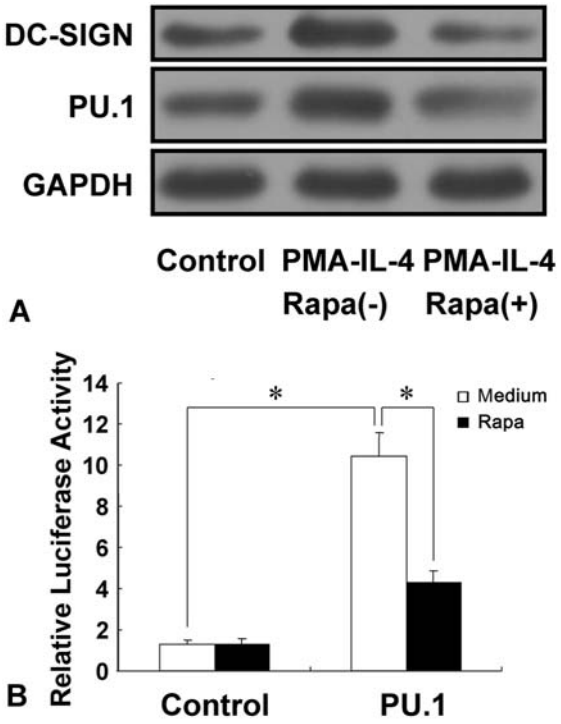

Figure 5. Inhibitory effects of Rapa on PU.1 and DC-SIGN expression. (A) In the presence of PMA and IL-4, DC-SIGN was induced in THP-1 cells. The expression of PU.1 and DC-SIGN protein was determined by western blotting. (B) Dual-luciferase reporter assay was used to detect DC-SIGN promoter activity. 293 T cells transfected with DC-SIGN reporter plasmid and an internal control plasmid were used as control. 293 T cells co-transfected with DC-SIGN reporter plasmid and PU.1 plasmid as the PU.1 group. Data represents mean ± SD of three separate experiments. *P < 0.01, compared with relative control group.

anti-inflammatory mechanism for regulating Th2 cytokine production in a DC-SIGN dependent manner (22). All these studies further suggest that multiple DC-SIGN-induced immunological functions would provide important clues to treat and prevent certain immune disorders and inflammatory diseases.

In the current study, we demonstrated that Rapa inhibits DC-SIGN expression and correspondingly prevents DC migration and DC-mediated T cell stimulation, using DC from human peripheral blood. Therefore, the down-regulating effects of Rapa on DC functions are correlated with the expression of DC-SIGN. On that basis, we investigated the mechanism of Rapa activity on DC-SIGN expression. The DC-SIGN promoter region contains numerous transcription factor binding sites, such as AP-1, Sp1, Ets-1and NF-κB. Mutation or deletion of these binding sites, especially Ets-1, may decrease the activity of the DC-SIGN promoter region (23, 24). PU.1, a member of the Ets-1 family, has binding sites in the DC-SIGN promoter region and can enhance the transactivation of the DC-SIGN promoter alone or by synergizing with c-Myb/RUNX3 (14). Moreover, studies demonstrated that miR-155 down-regulated DC-SIGN expression by acting on 3'UTR of PU.1 mRNA (25). Therefore, we attempted to observe the effects of Rapa on the expression of PU.1 and DC-SIGN and observed the expression of PU.1 and DC-SIGN was decreased in Rapa-treated DC.

We hypothesized that Rapa exerted inhibitory effects on DC-SIGN expression by down-regulating the transcription factor PU.1. When using siRNA to silence PU.1, DC-SIGN expression decreased, indicating PU.1 could regulate DC-SIGN expression. These results are consistent with a study by Dominguez-Soto et al. (14). Moreover, with the addition of Rapa further decreased DC-SIGN expression suggesting it may down-regulate the expression of DC-SIGN by PU.1. To demonstrate this, THP-1 and 293 T cell lines were used. Western blotting (Figure 5A) and dual-luciferase reporter assay (Figure 5B) demonstrated that Rapa significantly suppressed the expression of DC-SIGN, and that the inhibitory effect of Rapa may be regulated by PU.1.

PU.1 belongs to ETS family members, which regulate the genetic transcription of antigen receptors such as toll-like receptors and the mannose receptor that mediate immune responses against microorganisms. PU.1 is a regulatory factor that affects DC-SIGN biological functions and thus participates in DC maturation and differentiation (26-29). In the present report, we suggest that the inhibitory effects of Rapa on DC-SIGN expression were associated with the PU.1 gene transcription pathway.

The serine/threonine kinase, mTOR, can regulate cell growth, proliferation, differentiation, migration, and survival (13). Inactivation of mTOR inhibits the secretion of C-type lectin receptors and cytokines from immune cells (30, 31). Thus, we further speculated that Rapa inhibited PU.1 gene expression via inactivation of mTOR and thus affected DC-SIGN expression and functions. However, further investigation into the mechanisms of Rapa on DC-SIGN expression, such as whether Rapa acts on mTORC1 or mTORC2 signaling pathways and whether other transcription factors are involved in the inhibitory effects of Rapa, are required.

6. ACKNOWLEDGMENTS

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Abbreviations: DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; Rapa: Rapamycin; DC: dendritic cell; FKBP12: FK506-binding protein 12; mTOR: mammalian target of Rapa; mTORC1: mammalian target of Rapa complex 1; mTORC2: mammalian target of Rapa complex 2; PRR: pattern recognition receptor; PAMPs: pathogen associated molecular patterns; ICAM: intercellular adhesion molecule; Mac-1: macrophage 1 antigen; Flt3L: fms-like tyrosine 3 kinase ligand; RPMI 1640: Roswell Park Memorial Institute 1640; rhGM-CSF: recombinant human granulocyte/macrophage colony-stimulating; rhIL-4: recombinant human interleukin-4; SDF-1: stromal cell-derived factor-1; PE: phycoerythrin; mAb: monoclonal antibody; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; RT-PCR: transcription-polymerase chain reaction; PBMCs: peripheral blood mononuclear cells; FBS: fetal bovine serum; PMA: phorbol-12-myristate-13-acetate; PBS: phosphate buffer saline; SDS-PAGE: sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF: polyvinylidene fluoride; HRP: horseradish peroxidase; ECL: enhanced chemiluminescence; CFSE: carboxyfluorescein succinimidyl ester; MTT: methylthiazolyldiphenyl-tetrazolium bromide; DMSO: dimethyl sulfoxide; IL-4: interleukin-4; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; IL-10: interleukin-10; STAT3: signal transducer and activator of transcription 3; LPS: lipopolysaccharide; moDC: monocyte-derived dendritic cell; CLR: C-type lectin receptor; C1q: complement 1q; gC1qR: receptor for globular head domain of complement component C1q; IVIG: intravenous immunoglobulin; Th2: T-helper cell type 2; AP-1: activator protien-1; Ets-1: E26 transformation specific-1; Sp1: specificity protein 1.

Key Words: Rapamycin, DC-SIGN, dendritic cell, transcription factor PU.1

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