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Research paper

Interactions between canonical Wnt signaling pathway and MAPK pathway regulate differentiation, maturation and function of dendritic cells

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ABSTRACT

Antigen-presenting dendritic cells interpret environmental signals to orchestrate local and systemic immune responses. In this study, the roles of Wnt proteins and their signaling pathway members in the maturation and function of monocyte-derived DCs were investigated. The present study showed higher expression of β-catenin, as well as pGSK-3β in DCs than those in monocytes. Wnt3a, Wnt5a and inhibition of GSK-3β promoted differentiation of DCs, but inhibited maturation of DCs. GSK-3β induced DCs maturation with unconventional phenotypes. Together with β-catenin silence, these treatment lead to reduced secretion of cytokines and chemokines except for IL-10 in comparison with LPS treatment, and significantly promoted proliferation of T cells. Wnt3a and inhibition of GSK-3β increased expression of MAPK signalings (p-ERK, p-p38, p-JNK). However, inhibition of MAPK signalings in turn differently regulated Wnt signaling proteins expression. These data suggest that Wnt pathway regulates DCs differentiation, maturation and function with interaction of MAPK signaling pathways.

1. Introduction

Dendritic cells (DCs) are a heterogeneous group of cells that function in immunosurveillance, antigen-presentation and tolerance [1]. DCs are specialized sentinel cells at the interface of innate and adaptive immunity without direct engagement in effective activities such as pathogen killing. DCs induce tolerance or anergy by stimulating harmless self-Ag or environmental Ag [2,3]. However, DCs are activated and induce an Ag-specific adaptive immune reaction in the presence of exogenous or endogenous danger signals, resulting in a memory response and sensitization. DCs have an optimal antigen-presenting capacity to stimulate resting T cells in the primary immune response. During the process of DCs maturation, immature DCs (iDCs) are unable to internalize antigens and become mature, then presenting antigens to naive T cells. Specific subsets of either murine or human DCs cultured with different stimuli respond with great plasticity in terms of both gene expression and cytokine secretion. Therefore, differentiation and maturation status of DCs may determine the nature of DCs and subsequent immune response [4].

Wnts are secreted cysteine-rich glycosylated and lipid modified proteins [5]. There are 19 Wnt genes identified in human [6] with molecular sizes ranging from 39 kDa to 46 kDa [7]. Wnt signaling is divided into canonical and non-canonical pathways. Canonical pathway is considered to be Wnt/β-catenin pathway, while non-canonical pathways are composed of Wnt-planar cell polarity pathway (Wnt-PCP pathway) and Wnt-$Ca2^{+}$ pathway [8]. Wnt/β-catenin pathway begins with Frizzled (Fz) receptor and the low-density co-receptor lipoprotein receptor-related protein 5/6 (LRP5/6) binding with Wnt ligand. Then, Wnt-LRP-Fz complex activates scaffolding protein Dishevelled (Dvl), which in turn induces disassembly of complex formed by axin, adenomatous polyposis coli (APC), and the serine/threonine kinase glycogen synthase kinase-3β (GSK-3β) [9]. The canonical Wnt/β-catenin pathway is critical for self-renewing in the process of development through regulating cell proliferation and terminal differentiation, as demonstrated in the gut [10,11], hair [12], regulation of hematopoietic stem cells [13], and homeostasis of bone [14,15]. Wnt3a is a representative Wnt protein, inducing accumulation of β-catenin, and activating the canonical Wnt signaling pathway

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[16]. Wnt5a, one of the most extensively studied ligand in the Wnt family, has function of activating non-canonical pathway. It performs significantly during the development of various organs and has postnatal cellular functions [17,18]. Previous studies showed that canonical and non-canonical Wnts directly stimulated murine DCs production of anti-inflammatory cytokines [4]. Wnt3a triggered canonical β-catenin signaling and preferentially induced DCs TGF-β and VEGF production, whereas Wnt5a induced IL-10 through alternative pathways. DKK-3, a Wnt signaling inhibitor, treated DCs up-regulated expression of CD86 and CD40 [19]. In the presence of DKK-3, DCs decreased IL-10, IL-4 production, and shifted naïve CD4 T cells towards Th1 cells through up-regulation of T-bet and down-regulation of GATA-3. Therefore, Wnt proteins and their signaling pathway members may play an important role in DCs maturation and function.

And thus, in this study, the roles of Wnt proteins and their signaling pathway members in the maturation, differentiation and function of monocyte-derived DCs, and cross talk among MAPK pathways, were investigated.

2. Materials and methods

2.1. Antibodies and reagents

The following anti-human antibodies and reagents were used for flow cytometry and cell stimulation. Human CD14 and CD3 microbeads, human FITC-CD14 antibody, were from Miltenyi Biotec (Bergisch Gladbach, Germany). Recombinant human Wnt3a (rWnt3a), rWnt5a, SB216763 were from R&D Systems (Minneapolis, MN, USA). Human APC-CD1a, PE-cy7-CD40, PE-CD83, FITC-HLA-DR, APC-cy7-CD40, Percp-cy5-CD86 antibodies, and isotype controls were from BD Biosciences (San Jose, CA, USA). U0126, SP600125(SP) and SB203580(SB) were from Merck (Darmstadt, German).

2.2. In vitro differentiation of DCs

Peripheral blood from healthy donors was supplied by Chengdu Blood Centre. Ten donors were enrolled in experiment and monocytes were from different donors. Study protocol was approved by West China Hospital Ethics Committee. Heparinized blood (120 ml) was collected from donor and peripheral blood mononuclear cells (PBMCs) were isolated by standard Ficoll-Hypaque density gradient centrifugation. Monocytes were magnetically selected using anti-human CD14 microbeads from PBMCs [20]. The purity of monocytes was estimated by flow cytometry analysis on the basis of CD14 expression, and the purity was higher than 95%. Monocytes were cultured for 6 days (2×10^6/ml) according to our previous study [21]. Briefly, cells were re-suspended in RPMI 1640 supplemented with GM-CSF (1000 U/ml), IL-4 (500 U/ml), 100 U/ml penicillin and 100 μg/ml streptomycin. On days 3 and 5, half of the original medium was removed and replaced by fresh medium containing GM-CSF and IL-4, with or without rWnt3a, rWnt5a or SB216763.

2.3. RNA interference

Small interfering RNAs targeting β-catenin, the unrelated sequence positive control (siRNA mimics) were purchased from Guangzhou Ribobio Corporation. The following primers were used for siRNA: sense 5′-CAGUUGUGGUUAAGCUCUUdTdT-3′ and antisense 3′-dTdTGUCAACACCAAUUCGAGAA-5′ (see Supplementary information). iDCs (2×10^7/ml) were transfected using Lipofectamine 2000 reagents according to the manufacturer's instructions. Knockdown efficiency was determined by RT-PCR. .

2.4. DCs treatment

On day 5, immature DCs (iDCs) were stimulated for 24 h with 100 ng/ml LPS (Sigma, Saint Louis, Missouri, USA). In some experiments, iDCs were treated with 100 ng/ml TNF-α (Sigma, Saint Louis, Missouri, USA), β-catenin siRNA, 10nM SB216763, 100 ng/ml rWnt3a, 500 ng/ml rWnt5a for 24 h, respectively.

2.5. Mixed lymphocyte reaction

Human $CD3^+$ T cells were separated from PBMC by using anti-CD3 conjugated microbeads. Harvested DCs were resuspended in medium containing 0.08 mg/ml mitomycin C to inhibit cell proliferation. Cells were incubated at 37 °C in the dark for 20 minutes, and then washed three times in Hanks' balanced salt solution. DCs were incubated in flat-bottom 96-well tissue culture plates (corning, stone, UK) with allogeneic T cells labeled previously with 4 μM CFSE at ratio of 1:10. T cell proliferation was assessed by flow cytometry after 5 days coculture.

2.6. Flow cytometry analysis

For phenotypes measurement, cells were washed after treatment and conjugated with FITC-CD14, APC-CD1a, PE-cy7-CD40, PE-CD80, FITC-HLA-DR, APC-cy7-CD83, Percp-cy5-CD86 antibodies or isotype controls at 4 °C for 20 min. For T cell proliferation, CSFE positive cells were detected. Analysis was conducted with FACSCalibur flow cytometry (BD Biosciences). Data analysis was performed by using software Cell Quest (BD Biosciences).

2.7. Western blotting

Different groups of DCs (2×10^6 cells) were lysed with cell lysis buffer, and protein concentration was detected using an Enhanced BCA Protein Assay Kit (Bioteke, China). Samples were stored at −80 °C. Cell lysates were electrophoresed on SDS-PAGE and blotted onto PVDF membranes (Millipore Corp, Billerica, MA, USA). After blockade of nonspecific protein binding, the blots were probed overnight with primary antibodies (GAPDH, phosphorylated β-catenin, β-catenin, GSK-3β, phosphorylated GSK-3β, phosphorylated IκBα, p65, phosphorylated p38, p38, phosphorylated ERK1/2, ERK1/2, phosphorylated JNK1/2, JNK1/2) diluted in Primary Antibody Dilution Buffer (1:1000, Cell Signaling, Beverly, MA, USA). The blots were then probed with secondary antibodies correspondingly, conjugated with horseradish peroxidase (1:2000). Antibody binding was detected with the enhanced chemiluminescence kit (Millipore Corp, Billerica, MA, USA). Densitometer scans of the blots were performed using Quantity One (BioRad, Munich, Germany). Results were showed as relative quantity.

2.8. Cytokines and chemokines detection

The levels of cytokines and chemokines, including IFN-γ, IL-10, IL-17, IL-2, IL-23, IL-8, TNF-α, IL-1β, MIP-1α, MIG, MCP-1, RANTES, in the DC culture supernatants stimulated with various conditions were assessed by cytometric beads array according to the manufacturer's instructions (eBioscience, California, USA).

2.9. Statistical analysis

Data were presented as mean ± standard deviation (SD). Comparisons of group means were assessed by Student's *t*-test or one-way analysis of variance with a post hoc Dunnett's test. P value < 0.05 was considered as statistically significant. All