Wnt1 Positively Regulates CD36 Expression via TCF4 and PPAR-γ in Macrophages

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Wnt1 • β-catenin • TCF4 • PPAR-γ • CD36

Abstract
Background: Scavenger receptors including CD36 control the phagocytosis of oxidized low-density lipoprotein and play an important role in macrophage physiology, but the underlying molecular mechanism by which CD36 is regulated in macrophages or during macrophage differentiation from monocytes remains to be determined. Methods: Here, we investigated the relationship between Wnt1 and CD36 during macrophage differentiation. CD36 was suppressed following knockdown of Wnt1 by siRNA, while it was increased by ectopic overexpression of Wnt1 in macrophages. Using a β-catenin inhibitor, peroxisome proliferator-activated receptor gamma (PPAR-γ) siRNA, and transcription factor 4 (TCF4) siRNA, we demonstrated that Wnt1 regulates the expression of CD36 through TCF4 and PPAR-γ. Co-immunoprecipitation, chromatin immunoprecipitation, and immunofluorescence experiments showed that β-catenin interacted with PPAR-γ and that PPAR-γ and TCF4 colocalized in the nucleus. Furthermore, Pax3 regulated Wnt1 via binding to the first binding site in the Wnt1 promoter. Results: Our study demonstrated that during macrophage differentiation from monocytes, Wnt1 promotes CD36 expression via activation of PPAR-γ and TCF4. Conclusions: Our findings suggest that Wnt1 plays an important role in macrophage physiology via activation of the canonical Wnt pathway.

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Introduction

CD36 is a membrane glycoprotein and a member of the scavenger receptor B family. It is expressed in platelets, endothelial cells, smooth muscle cells, and immune cells including macrophages and dendritic cells [1-5]. Accumulating evidence indicates that CD36 plays an important role in macrophage physiology. In addition, enforced expression of CD36 in macrophages promotes endocytosis of oxidized low-density lipoprotein (oxLDL) by binding to oxLDL in a lipid raft-dependent pathway [6]. Meanwhile, oxLDL/CD36 interactions suppress locomotion of macrophages, leading to foam cell accumulation in the local lumen [7]. Moreover, CD36 initiates apoptosis of macrophages with overloaded oxLDL via caspase-3 activation. Furthermore, through stimulation of c-Jun N-terminal kinase 2 and mitogen-activated protein kinase kinase 4, CD36 promotes the phosphorylation of several transcription factors including c-Jun and activating transcription factor 2, which are necessary for macrophage foam cell formation [8]. These data clearly indicate that deregulation of the CD36 signaling pathway contributes to the formation of atherosclerosis [9], but the underlying molecular mechanism by which CD36 is regulated in macrophages or during macrophage differentiation from monocytes remains to be determined.

The Wnt1 signaling pathway participates in multiple biological processes including cell adhesion, migration, and anti-apoptosis [10-12]. Wnt1, a key component of the canonical Wnt pathway, binds cell membrane receptor frizzled-1 (FZD1) and activates lymphoid enhancer-binding factors and transcription factors (TCFs) through β-catenin [13]. In addition, Wnt1 promotes mammary tumorigenesis by inducing matrix metalloproteinase (MMP)-2, MMP-3, and MMP-9 [14]. Furthermore, Wnt1 promotes cell survival through NF-κB activation [15]. Recently, it has been reported that during the differentiation of dendritic cells from monocytes, Wnt1 was decreased by miR-34a [16]. In addition, previous studies have shown that transcription factor peroxisome proliferator-activated receptor gamma (PPAR-γ), which is induced by the mitogen-activated protein kinase pathway, promotes CD36 transcription [17, 18]. However, the alteration of Wnt1 during macrophage differentiation from monocytes and whether Wnt1 influences the physiological functions of macrophages remain unknown.

In this study, we investigated the regulation of CD36 by the Wnt1 pathway and the upstream regulatory pathways of Wnt signaling during macrophage differentiation by co-immunoprecipitation, chromatin immunoprecipitation (ChIP), and immunofluorescence experiments.

Materials and Methods

Reagents

Wnt1 recombinant protein was from Millipore(Billerica, MA, USA). VAX939 and sp216763 were purchased from Sigma-Aldrich(Shanghai, China). oxLDL was from Yiyuan (Guangzhou, China). GM-CSF was obtained from PeproTech(Rocky Hill, NJ, USA).

Cell culture and transfection

Human primary macrophages were induced from monocytes, which were separated from human peripheral blood with human lymphocyte separation medium (Sigma). Monocytes were cultured in flasks with RPMI1640 (Gibco) supplemented with 10% fetal calf serum (Gibco) and 100 ng/mL granulocyte-macrophage colony-stimulating factor (GM-CSF). Culture medium was changed every other day. After 7 days, 1 × 10⁶ cells were seeded in six-well plates with 2 mL of culture medium. Inhibitors were added 30 min before transfection, when needed.

The siRNA transfections were performed in 12-well plates. Cells were cultured in serum-free medium for 30 min before transfection. A total of 2 µL of siRNA (50 nM, Baiao, China) and 3.3 µL of RNAIMAX reagent (Invitrogen, USA) were mixed, incubated for 15 min, and added to each well; the plates were incubated at 37 °C for 24 h. Plasmid transfection was performed as follows: 750 ng of plasmid (Origene, USA) and 10 µL of cDNA transfection reagent (Origene, USA) were mixed, incubated for 10 min, and added to each well; the plates were incubated at 37 °C for 24 h.
Western blotting

Cells were lysed on ice with RIPA lysis buffer (Cell Signaling Technology). The total protein concentration was determined with a bicinchoninic acid assay kit (Bio-Rad). Proteins were resolved on a denaturing 10% SDS-PAGE gel and subsequently transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% dried milk in tris-buffered saline and Tween 20 (10 mM Tris, pH 7.5; 100 mM NaCl; and 0.1% Tween-20), incubated with primary antibodies, and then incubated with horseradish peroxidase-conjugated secondary antibodies. The signals were visualized by enhanced chemiluminescence detection (Pierce, Thermo Fischer Scientific, Bonn, Germany). The following primary antibodies were used: anti-human β-catenin monoclonal antibody (1:1000; Epitomics, USA), anti-human Wnt1 monoclonal antibody (1:1000; Millipore, USA), anti-human PPAR-γ monoclonal antibody (1:1000; Cell Signaling Technology, USA), anti-human GAPDH monoclonal antibody (1:2000 Nuoyang, Hangzhou, China), anti-human TCF4 monoclonal antibody (1:500 Sango,Shanghai, China), rabbit IgG antibody (1:2000; Cell Signaling Technology, USA), and anti-CD36 antibody (1:1000; abcam, USA).

Isolation of mRNA and RT-PCR

Total mRNA was isolated from cultured cells using the RNA simple total RNA kit (Tiangen, China) according to the manufacturer’s instructions. Total cDNA was synthesized using a primescriptRT reagent kit (Takara, Japan). RT-PCR consisted of 40 cycles of 95°C for 5 s, 60°C for 30s and 95°C for 30s. 18s rRNA served as an endogenous control. Primers were: CD36 forward (GGCTGTGACCGGAACTGTG) and reverse: (AGGTCTCCAACTGGCATTAGAA); 18s rRNA forward (CCGCACTTGATACGGTTCCT) and reverse (CCAGGCTGATCTATCCCACTG).

Oil red O staining

Cells were treated with oxLDL (40 µg/mL) for 24 h, washed twice with phosphate-buffered saline (PBS), and then stained with oil red O working solution (Jiancheng, Nanjing, China) for 10 min. After washing with PBS, 1 mL of compound stain dye for nuclear and extracellular matrix staining was added to the cells. All experiments were performed at room temperature.

Co-immunoprecipitation

Cell lysates were precleared by incubation with 20 µL of protein A/G agarose (Santa Cruz, USA) for 1 h and incubated with bait antibody at 4 °C with rotation overnight. After centrifugation, the supernatant was incubated with 80 µL of protein A/G agarose at 4 °C with rotation for 4 h. The agarose beads were washed three times with cold PBS, followed by elution with 40 µL of protein lysis buffer (Beyotime, Shanghai, China). Then, 10 µL of 5× loading buffer (Beyotime, Shanghai, China) was added, and the mixture was boiled for 5 min and subjected to western blotting.

ChIP assays

ChIP assays were performed with a kit from Cell Signaling Technology according to the manufacturer’s instructions. In brief, 5 × 10⁶ macrophages were fixed with 1% formaldehyde for 10 min at 37 °C. Next, chromatin was sheared with 5 µL of nuclease at 37 °C for 20 min, yielding DNA fragments of 200–800 bp. Following preclearing with 20 µL of protein A/G agarose at 4 °C for 1 h, the samples were incubated with 2 µg of bait antibody or control IgG antibody (Epitomics, Cell Signaling Technology) at 4 °C with rotation overnight. Complexes including input were incubated in 5 M NaCl at 65 °C for 6 h to reverse crosslinks, resuspended in proteinase K solution at 45 °C for 1 h, and then purified using a DNA purification kit (Beyotime). The purified DNA was subjected to polymerase chain reaction (PCR) analyses. The primers used for PCR detection were as follows:

- CD36 promoter binding sites for PPAR-γ: Forward, 5'-GGGCTTATGGTGGTTCAT-3'; Reverse, 5'-AGATGAAAGCTGATGTGCAA-3'.
- CD36 promoter binding sites for TCF4, site-1: Forward, 5'-GGTCCACATGGAAGAAGTTG-3'; Reverse, 5'-GCAGCAAACCTCAATTAGC-3'. Site-2: Forward, 5'-GGTCCACATGGAAGAAGTTG-3'; Reverse, 5'-AGATGAAAGCTGATGTGCAA-3'.
- Wnt1 promoter binding sites for Pax3, site-1: Forward, 5'-GGGTCCATGCTTGAAATT-3'; Reverse, 5'-GGGTCCATGCTTGAAATT-3'. Site-2: Forward, 5'-GGGTCCATGCTTGAAATT-3'; Reverse, 5'-GGGTCCATGCTTGAAATT-3'. Site-3: Forward, 5'-GGGTCCATGCTTGAAATT-3'; Reverse, 5'-GGGTCCATGCTTGAAATT-3'.
Confocal microscopy imaging

Macrophages were seeded on glass in the bottom of a cell culture dish (NEST, USA). After treatment, the cells were fixed and permeabilized at 4 °C for 10 min. After incubation with primary antibody (1:100) at 37 °C for 2 h, the cells were washed with PBS and stained with goat-anti-rabbit FITC IgG or goat-anti-mouse rhodamine IgG (Nuoyang, China) (1:200) at 37 °C for 1 h, followed by DAPI staining (Sigma, USA). The cells were examined using a Zeiss Confocal Microscope Imaging System (Carl Zeiss, Jena, Germany).

Enzyme-linked immunosorbent assay (ELISA)

Macrophages were cultured in 6-well plates with GM-CSF (100 ng/mL). The secretory protein Wnt1 in the cell culture supernatant was measured using an ELISA kit from Antigenix America (New York, USA), according to the manufacturer’s instructions.

Statistical analysis

Pairs of data were evaluated using the Student’s t-test. P < 0.05 was considered statistically significant. Each experiment was performed at least three times.

Results

Wnt1 positively regulates CD36 expression in macrophages

At first, we validated GM-CSF stimulated-monocytes differentiated into M1 macrophages through detecting CD11b+ iNOS and Arg-1. As shown in Fig. 1A, GM-CSF stimulated-monocytes had a high expression of CD11b+(79.3%±5.6%) which is the main marker of macrophages different from dendritic cells. After GM-CSF treatment for seven days, cells showed high expression of iNOS and low expression of Arg-1, which means GM-CSF treatment made monocytes differentiated into M1 macrophages (Fig. 1B). To investigate whether Wnt1 regulates CD36, we knocked down Wnt1 by siRNA and overexpressed Wnt1 with the plasmid pCMV-Wnt1 in human primary macrophages. Western blots and RT-PCRs showed that downregulation of Wnt1 by siRNA led to a decrease of CD36. In contrast, ectopic overexpression of Wnt1 resulted in an increase of CD36 (Fig. 2A). Wnt1 is a secreted protein and activates Wnt1 signaling by binding to the cell surface receptor FZD1. Next, we treated macrophages with different concentrations of Wnt1 recombinant protein and determined the protein and mRNA levels of CD36 by immunoblotting and RT-PCR. We found that CD36 was upregulated by Wnt1 recombinant protein in a concentration-dependent manner (Fig. 2B). These results clearly demonstrated that CD36 is positively regulated by the Wnt1 pathway.

Fig. 1. M1 macrophages identification. A. the red peak means CD11b+ cells (79.3%±5.6%) which is the main marker of macrophages different from dendritic cells. B. monocytes were treated with GM-CSF (100 ng/mL) for seven days, then iNOS and Arg-1 were detected using RT-PCR. contrast to monocytes, cells with GM-CSF treatment show high expression of iNOS and low expression of Arg-1, which means GM-CSF treatment made monocytes differentiated into M1 macrophages. The data presented here are mean±SD of three independent experiments.*P<0.05.
Wnt1 increases uptake of oxLDL in macrophages

It has been well documented that CD36 regulates macrophage physiology, including oxLDL uptake. The positive regulation of CD36 by Wnt1 suggests that Wnt1 may affect oxLDL uptake of macrophages, which is mediated by CD36. To this end, we performed oil red O staining. As shown in Figure 2C, overexpression of Wnt1 by the plasmid pCMV-Wnt1 promoted oxLDL uptake of oxLDL by macrophages, which was reversed by CD36-specific siRNA.

Wnt1 interacts with CD36 in macrophages at the cell surface

Since Wnt1 functions through binding to a cell surface receptor (FZD1, FRP5/6) [19] and CD36 is a protein that mainly floats in a cell’s surface membrane, we examined whether there is an association between Wnt1 and CD36 in the cell surface. We performed co-immunoprecipitation of macrophage lysates with an anti-Wnt1 antibody. The results showed...
that Wnt1 interacted with CD36. Moreover, treatment of macrophages with GM-CSF led to an enhanced association of Wnt1 with CD36 (Fig. 3A). Consistently, confocal microscopy imaging demonstrated that Wnt1 and CD36 had weak colocalization in the macrophage cell surface, and the interaction was impressively enhanced in the presence of GM-CSF (Fig. 3B).

**Wnt1 upregulates CD36 through PPAR-γ and TCF4**

Previous studies have demonstrated that Wnt1 activates the canonical Wnt1 pathway via binding to a receptor complex including LRP5, LRP6, and FZD1. We wished to determine whether Wnt1 regulates CD36 through the activation of canonical Wnt1 signaling. To test this hypothesis, we first transfected macrophages with pCMV-Wnt1 together with siRNAs of the control, LRP5, LRP6, and FZD1 and determined CD36 expression by immunoblotting and RT-PCR. In comparison to the negative control siRNA, siRNA of LRP5, LRP6, or FZD1 apparently reversed the promoting effect of pCMV-Wnt1 on CD36 expression (Fig. 4A). Since β-catenin is a key protein through which the canonical Wnt pathway functions, we further...
tested the CD36 protein and mRNA expression levels following ectopic overexpression of Wnt1 in the presence or absence of the β-catenin inhibitor VAX939. The stimulation of CD36 by Wnt1 was dramatically abolished by VAX939 (Fig. 4B). In contrast, the β-catenin agonist
SP216763 significantly increased the CD36 expression level (Fig. 4E). It has been reported that PPAR-γ is involved in CD36 transcription through the PKC–PPAR-γ pathway [20]. In agreement, overexpression of Wnt1 resulted in an increase of PPAR-γ expression (Fig. 4C). To test whether PPAR-γ plays a role in the upregulation of CD36 by Wnt1, we cotransfected macrophages with PPAR-γ siRNA and pCMV-Wnt1 and found that CD36 was decreased with PPAR-γ siRNA, in contrast to the control siRNA in the presence of Wnt1 overexpression (Fig. 4D). Moreover, ectopic overexpression of PPAR-γ with the plasmid pCMV-PPAR-γ increased CD36 expression (Fig. 4E). TCF4 is the downstream transcription factor that is activated in the canonical Wnt pathway. We tested whether TCF4 participates in CD36 upregulation by Wnt1. We performed co-immunoprecipitation of macrophage lysates with an anti-β-catenin antibody and PPAR-γ antibody (Fig. 5A). ChIP assays were performed with pCMV-Wnt1 transfection using antibodies against PPAR-γ and β-catenin. The chromatin collected before immunoprecipitation served as an input control (p < 0.05). The data presented here are representative or mean±SD of three independent experiments.*P<0.05.

Translocation of β-catenin stabilizes PPAR-γ, which binds to the CD36 promoter

Both β-catenin and PPAR-γ are transcription factors functioning in the nucleus. Several studies have shown that β-catenin and PPAR-γ are functionally linked [21-23]. Our results suggest that the association of β-catenin with PPAR-γ might be enhanced in response to Wnt1. To this end, we performed co-immunoprecipitation of macrophage lysates with an anti-β-
catenin antibody. The results showed that PPAR-γ interacted with β-catenin under normal growth conditions. Interestingly, overexpression of Wnt1 led to an enhanced association of PPAR-γ with β-catenin (Fig. 5A). To test whether Wnt1 overexpression promotes the recruitment of β-catenin and PPAR-γ to the CD36 promoter, we performed ChIP assays with β-catenin- and PPAR-γ-specific antibodies and found that Wnt1 overexpression significantly increased the binding of β-catenin and PPAR-γ to the DR-1 motif sequence [20] of the CD36 promoter (Fig. 5B). In addition, confocal microscopy imaging demonstrated that there was no detectable colocalization of PPAR-γ and β-catenin in the nucleus of unstimulated macrophages. Wnt1 overexpression both increased the protein levels and enhanced the association of β-catenin and PPAR-γ (Fig. 6). Similarly, treatment with SP216763, a β-catenin agonist, led to increased protein levels and enhanced association of β-catenin and PPAR-γ (Fig. 6).

PPAR-γ and TCF4 promote CD36 transcription in a cooperative manner

Our results showed that TCF4 is involved in CD36 transcription by Wnt1; therefore, we predicted the binding sites for TCF4 on the CD36 promoter by bioinformatics analysis. There are two binding sites for TCF4 on the CD36 promoter (Fig. 7A). Moreover, our ChIP analyses with the anti-TCF4 antibody showed that Wnt1 overexpression promoted the recruitment of TCF4 to the second binding site of the CD36 promoter (Fig. 7B). Further co-immunoprecipitation with an anti-PPAR-γ antibody demonstrated that there was a detectable association between TCF4 and PPAR-γ and that Wnt1 overexpression enhanced this association (Fig. 7C). Confocal microscopy analyses showed that TCF4 and PPAR-γ had no detectable association in unstimulated macrophages, but Wnt1 overexpression apparently enhanced TCF4 and PPAR-γ colocalization in the nucleus (Fig. 7D).

GM-CSF induces Pax3, which in turn promotes Wnt1 transcription via binding to the Wnt1 promoter in macrophages

To explore the upstream regulatory mechanism of Wnt1 in macrophages, we first measured Pax3, a transcription factor that is involved in Wnt1 transcription in neural crest development and CD36 expression during macrophage differentiation [24]. As shown in Figure 8A, Wnt1, CD36, and Pax3 were all increased by GM-CSF in a concentration-dependent manner.

Since Wnt1 can be secreted by cells and is present in the cell supernatant, we detected the Wnt1 content in the cell supernatant with GM-CSF treatment and found that Wnt1 was
increased during macrophage differentiation by GM-CSF (Fig. 8B). To test whether Pax3 regulates Wnt1 in macrophages, we determined the Wnt1 content following knockdown of Pax3 by Pax3 siRNA and overexpression of Pax3 with the plasmid pCMV-Pax3 in macrophages. Knockdown of Pax3 by siRNA led to a decrease of Wnt1, while ectopic overexpression of Pax3 resulted in an increase of Wnt1 (Fig. 8C).

Interleukin (IL)-4 prevents the differentiation of monocytes to macrophages. Our results suggest that IL-4 prevents Wnt1 and Pax3 expression. Indeed, IL-4 stimulation suppressed the expression of both Wnt1 and Pax3 in macrophages (Fig. 8D).

To assess the role of Pax3 during macrophage differentiation, we treated macrophages with GM-CSF following transfection with Pax3 siRNA. In contrast to control siRNA, Pax3 siRNA prevented the stimulation of Wnt1 by GM-CSF (Fig. 8E). These results demonstrated that Pax3 is involved in the GM-CSF-mediated Wnt1 regulatory process.

Finally, we performed bioinformatic analyses to predict the binding sites for Pax3 on the Wnt1 promoter. There are three Pax3 binding sites present in the 5’-proximal promoter of Wnt1 (Fig. 9A). To validate this prediction, we performed co-immunoprecipitation of macrophage lysates with an anti-Pax3 antibody and found that GM-CSF treatment resulted
in enhanced recruitment of Pax3 to the first binding site of the Wnt1 promoter (Fig. 9B). Furthermore, we mutated the first binding site from CTGC to GAGCG of the Wnt1 promoter by site-directed mutagenesis and inserted it upstream of the luciferase reporter pGL4.20 vector (Promega) to obtain plasmids pGL4.20-WNT1-wt and pGL4.20-WNT1-mt. Luciferase reporter assays showed that, in contrast to the wild-type promoter, the mutated promoter reduced the luciferase activity with GM-CSF, which could not be restored by pCMV-Pax3 transfection (Fig. 9C).
Wang et al.: Wnt1 Regulates CD36 Expression

**Fig. 9.** Pax3 promotes Wnt1 expression via binding to the Wnt1 promoter. A. Bioinformatics prediction of Pax3 binding sites on the Wnt1 promoter. B. ChIP assays showed that GM-CSF treatment strengthened Pax3 binding to the first binding site. C. Mutation of the first binding site decreased the normalized luciferase activity induced by GM-CSF treatment or pCMV-Pax3 transfection. The data presented here are representative or mean±SD of three independent experiments.*P<0.05.

**Discussion**

In this study, we showed that Wnt1 positively regulated CD36 expression through cooperation of PPAR-γ and TCF4 and enhanced the phagocytic function of macrophages. We further demonstrated that Wnt1 upregulated CD36 by promoting the recruitment of β-catenin and PPAR-γ to the CD36 promoter. Moreover, we found that GM-CSF induced Pax3, which in turn promoted Wnt1 transcription via binding to the Wnt1 promoter in macrophages. Thus, our study has provided new insights into the regulation of CD36 during macrophage differentiation from monocytes.

Desheng’s work has suggested that activation of PPAR-γ suppresses β-catenin expression [25]. In addition, Shlomo and Emmelie found that β-catenin stimulates the expression of PPAR-γ in FH535 and human colon cancer cells, respectively [26, 27]. Our results indicated that overexpression of Wnt1 promoted the nuclear translocation of β-catenin, which in turn stabilized PPAR-γ. The differences among these studies may be due to the different cells that were used and the treatment conditions. Although we showed the relationship between β-catenin and PPAR-γ in macrophages with Wnt1 overexpression, the underlying mechanism of how Wnt1 regulates PPAR-γ remains unclear. Moreover, it is possible that other genes in macrophages are regulated by the interaction between β-catenin and PPAR-γ. Meanwhile, to
the best of our knowledge, the regulation of CD36 expression in quiescent macrophages is unknown [28].

Our results implied that upregulation of Wnt1 by cytokines such as GM-CSF through Pax3 plays an important role during macrophage differentiation from peripheral blood-derived monocytes. The study by Benjamin showed that Pax3 interacts with the promoter of Wnt1 via a conserved binding site during neural crest development [24]. In agreement, our bioinformatics analyses found that there were three Pax3 binding sites in the 5′-proximal promoter of Wnt1 in macrophages. Furthermore, our ChIP analyses validated the binding of Pax3 to the first binding site of the Wnt1 promoter. Thus, it is important to further elucidate other transcription factors that are involved in the regulatory mechanism of Wnt1 during macrophage differentiation.

Our observation that Wnt1 controls CD36 expression during macrophage differentiation provided a link between the conserved Wnt pathway and CD36 and disclosed a new role for the Wnt pathway in scavenger receptor expression, introducing new insight on scavenger receptor-related diseases such as atherosclerosis. Moreover, several studies have shown that the transcription factors NRF2 and CBFB also participate in the regulation of CD36 gene expression [20, 29, 30]. In future work, it will be interesting to investigate whether NRF2 and CBFB are involved in the regulation of CD36 by the Wnt1 pathway as well as the relationship among Pax3, NRF2, and CBFB in the regulation of CD36 transcription during macrophage differentiation and macrophage foam cell formation.

In conclusion, our study demonstrated that during the process of macrophage differentiation, Wnt1 promoted CD36 expression via TCF4 and PPAR-γ. To the best of our knowledge, this is the first study to reveal the CD36 regulatory mechanism during macrophage differentiation from monocytes.

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Disclosure Statement

None declared.

References

1 Talle MA, Rao PE, Westberg E, Allegar N, Makowski M, Mittler RS, Goldstein G: Patterns of antigenic expression on human monocytes as defined by monodonal antibodies. Cell Immunol 1983;78:83-99.
2 Asch AS, Barnwell J, Silverstein RL, Nachman RL: Isolation of the thrombospondin membrane receptor. J Clin Invest 1987;79:1054-1061.
3 McGregor JL, Catimel B, Parmentier S, Clezardin P, Dechavanne M, Leung LL: Rapid purification and partial characterization of human platelet glycoprotein IIb. Interaction with thrombospondin and its role in platelet aggregation. J Biol Chem 1989;264:501-506.
4 Swerlick RA, Lee KH, Wick TM, Lawley TJ: Human dermal microvascular endothelial but not human umbilical vein endothelial cells express CD36 in vivo and in vitro. J Immunol 1992;148:78-83.
5 Matsumoto K, Hirano K, Nozaki S, Takamoto A, Nishida M, Nakagawa-Toyama Y, Janabi MY, Ohy T, Yamashita S, Matsuzawa Y: Expression of macrophage (Mphi) scavenger receptor, CD36, in cultured human aortic smooth muscle cells in association with expression of peroxisome proliferator activated receptor-gamma, which regulates gain of Mphi-like phenotype in vitro, and its implication in atherogenesis. Arterioscler Thromb Vasc Biol 2006;20:1027-1032.
6 Zeng Y, Tao N, Chung KN, Heuser JE, Lublin DM: Endocytosis of oxidized low density lipoprotein through scavenger receptor CD36 utilizes a lipid raft pathway that does not require caveolin-1. J Biol Chem 2003;278:45931-45936.
7 Park YM, Drazba JA, Vasanji A, Egelhoff T, Febbraio M, Silverstein RL: Oxidized LDL/CD36 interaction induces loss of cell polarity and inhibits macrophage locomotion. Mol Biol Cell 2012;23:3057-3068.
8 Rahman S0, Lennon DJ, Febbraio M, Podrez EA, Hazen SL, Silverstein RL: A CD36-dependent signaling cascade is necessary for macrophage foam cell formation. Cell Metabolism 2006;4:211-221.
9 Wintergerst ES, Jell J, Rahner C, Asmis R: Apoptosis induced by oxidized low density lipoprotein in human monocyte-derived macrophages involves CD36 and activation of caspase-3. Eur J Biochem 2000;267:6050-6059.
10 Hinck L, Nelson WJ, Papkoff J: Wnt-1 modulates cell-cell adhesion in mammalian cells by stabilizing beta-catenin binding to the cell adhesion protein cadherin. J Cell Biol 1994;124:729-741.
11 Chen S, Guttridge DC, You Z, Zhang Z, Fribley A, Mayo MW, Kitajewski J, Wang CY: Wnt-1 signaling inhibits apoptosis by activating beta-catenin/T cell factor-mediated transcription. J Cell Biol 2001;152:87-96.
12 Schiavone D, Dewilde S, Vallania F, Turkson J, Di Cunto F, Poli V: The RhoU/Wrch1 Rho GTPase gene is a common transcriptional target of both the gp130/STAT3 and Wnt-1 pathways. Biochem J 2009;421:283-292.
13 Logan CY, Nusse R: The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 2004;20:781-810.
14 Blavier L, Lazaryev A, Dorey F, Shackleford GM, De Clerck YA: Matrix metalloproteinases play an active role in Wnt1-induced mammary tumorigenesis. Cancer Res 2006;66:2691-2699.
15 Shang YC, Chong ZZ, Hou J, Maisee K: Wnt1, FoxO3a, and NF-kappaB oversee microglial integrity and activation during oxidant stress. Cell Signal 2010;22:1317-1329.
16 Hashimi ST, Fulcher JA, Chang MH, Gov L, Wang S, Lee B: MicroRNA profiling identifies miR-34a and miR-21 and their target genes JAG1 and WNT1 in the coordinate regulation of dendritic cell differentiation. Blood 2009;114:404-414.
17 Shiffman D, Mikita T, Tai JT, Wade DP, Porter JG, Seilhamer JJ, Somogyi R, Liang S, Lawn RM: Large scale gene expression analysis of cholesterol-loaded macrophages. J Biol Chem 2000;275:37324-37332.
18 Zhao M, Liu Y, Wang X, New L, Han J, Brunk UT: Activation of the p38 MAP kinase pathway is required for foam cell formation from macrophages exposed to oxidized LDL. Apmis 2002;110:458-468.
19 Clevers H, Nusse R: Wnt/beta-catenin signaling and disease. Cell 2012;149:1192-1205.
20 Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM: PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 1998;93:241-252.
21 Easwaran V, Pishvaian M, Salimuddin, Byers S: Cross-regulation of beta-catenin-LEF/TCF and retinoid signaling pathways. Curr Biol 1999;9:1415-1418.
22 Lu D, Cottam HB, Corr M, Carson DA: Repression of beta-catenin function in malignant cells by nonsteroidal antiinflammatory drugs. Proc Natl Acad Sci U S A 2005;102:18567-18571.
23 Ross SE, Hemati N, Longo KA, Bennett CN, Lucas PC, Erickson RL, MacDougald OA: Inhibition of adipogenesis by Wnt signaling. Science 2000;289:950-953.
24 Fenby B, Fotaki V, Mason J: Pax3 regulates Wnt1 expression via a conserved binding site in the 5′ proximal promoter. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms 2008;1779:115-121.
25 Lu D, Carson DA: Repression of beta-catenin signaling by PPAR gamma ligands. Eur J Pharmacol 2010;636:198-202.
26 Handel S, Simon JA: A small-molecule inhibitor of Tcf/beta-catenin signaling down-regulates PPARgamma and PPARdelta activities. Mol Cancer Ther 2008;7:521-529.
27 Jansson EA, Are A, Greicius G, Kuo IC, Kelly D, Arulampalam V, Pettersson S: The Wnt/beta-catenin signaling pathway targets PPARgamma activity in colon cancer cells. Proc Natl Acad Sci U S A 2005;102:1460-1465.
28 Yesner LM, Huh HY, Pearce SP, Silverstein RL: Regulation of monocyte CD36 and thrombospondin-1 expression by soluble mediators. Arterioscler Thromb Vasc Biol 1996;16:1019-1025.
29 Calvo D: Structural and functional characterization of the human CD36 gene promoter. J Biol Chem 1996;271:7781-7787.
30 Ishii T: Role of nrf2 in the regulation of CD36 and stress protein expression in murine macrophages: Activation by oxidatively modified LDL and 4-Hydroxynonenal. Circ Res 2004;94:609-616.