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Inhibition of siglec-1 by lentivirus mediated small interfering RNA attenuates atherogenesis in apoE-deficient mice

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ABSTRACT

Background: Siglec-1 is highly expressed on circulating monocytes and plaque macrophages in atherosclerotic patients, but the exact role of Siglec-1 in atherosclerosis has not been elucidated.
Methods: Lentiviral vector containing small interfering RNA targeting Siglec-1 (Lv-shSiglec-1) or control vector (Lv-shNC) were injected intravenously into 6-week old *Apoe*$^{-/-}$ mice. Then onset of atherosclerosis was observed.
Results: Siglec-1 was highly expressed in aortic plaques and it can be down-regulated by Lv-shSiglec-1 injection. The plaque area and serum pro-inflammatory cytokine (IL-1β, IL-6, TNF-α and IL-17A) levels in Lv-shSiglec-1 mice were significantly lower than Lv-shNC mice, whereas IL-10 was higher. Moreover, plaque macrophages accumulation in aortic wall in Lv-shSiglec-1 mice was diminish, partly by decreased secretion of MCP-1/CXCL2 and CCR2/CXCR2 of aortas and monocytes, respectively. Furthermore, silencing of Siglec-1 can attenuate oxLDL uptake by peritoneal macrophages.
Conclusions: Inhibition of Siglec-1 can prevent atherosclerotic lesion formation by suppress monocytes-endothelial cells adhesion and macrophages accumulation.

1. Introduction

Atherosclerosis (AS) is an inflammatory disease, macrophage is particularly important in the initiation of immune responses to plaque antigens, especially lipid antigens. As a major biomarker of macrophage activation, Siglec-1 (sialoadhesin, CD169) is mainly expressed on resident macrophages and can be induced to express on circulating monocytes in inflammatory settings, such as SLE [1] and SS [2]. Siglec-1 is also expressed in a subset of semi-mature, myeloid-like pDCs in human blood. Proportions of Siglec-1-positive/Siglec-1-negative pDCs were higher in SLE than in healthy controls [3]. Ip et al. [4] found that the proteolipid protein (PLP) overexpressing mice displays demyelination and almost all CD11b+ cells from the mice express Siglec-1, but in Siglec-1-deficient PLP mice, upregulation of CD8+ T-cells and CD11b+ macrophages is reduced and pathological alterations are ameliorated. Jiang et al. [5] found that experimental autoimmune uveoretinitis is reduced in severity in the initial stages in the Siglec-1 knockout (KO) mice, with a reduction in the proliferative capacity and IFN-gamma secretion of T cells from the KO mice draining lymph nodes after immunization with IRBP peptides. These results together suggest that Siglec-1 has a proinflammatory role in inflammatory and autoimmune diseases, and inhibition of Siglec-1 can attenuate disease severity and prevent development of these diseases.

Early study showed that Siglec-1 positive macrophages were seen in atherosclerotic plaque of apolipoprotein E3-Leiden (*APOE*3-Leiden) transgenic mice [6]. Our previous study [7] also found that Siglec-1 was highly expressed on circulating monocytes and may be considered as a potential risk marker for monitoring disease severity in coronary artery disease (CAD). Our recent work further elucidated that monocytes Siglec-1 from CAD patients can stimulate CD4+ and CD8+ T cells proliferation and activation *in vitro* [8]. However, the exact role of Siglec-1 in the pathogenesis of atherosclerosis *in vivo* remains unclear. As an adhesion molecule of Ig superfamily, Siglec-1 on monocytes may participate in the process of endothelial cells adhesion, lipid internalization, antigen presentation and pro-inflammatory cytokines secretion. Manipulating the expression of Siglec-1 on monocytes/macrophages may prevent disease progression and represent a new therapeutic option for atherosclerosis.

Accordingly, we desired to explore the role of Siglec-1 in the pathogenesis of atherosclerosis *in vivo*. Firstly, lentiviral vectors containing small interfering RNA (siRNA) targeting Siglec-1 were constructed and effective vector was injected intravenously into *Apoe*$^{-/-}$ mice for

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in vivo Siglec-1 inhibition; Secondly, mice were fed a Western diet to induce AS and early stage *ex vivo* monocytes-endothelial cells adhesion assay was performed. Meanwhile, chemokines (MCP-1, CXCL-2) expression on aorta and chemokine receptors (CCR2, CXCR2) expression on monocytes were determined; Thirdly, at the end of the experiment, mice were sacrificed and serum cytokines and plaque macrophages infiltration and chemokines expression were measured. Furthermore, the ability of oxLDL uptake by peritoneal macrophages was determined.

2. Methods

2.1. Lentiviral vector construction and virus packaging

PSCSi1 ~ PSCSi4 (see Supplemental Table s1) of mouse siglec-1 (NM_011426.3) was cloned into the *Age*I-*Eco*RI sites of the lentiviral vector pGCSIL-GFP (Shanghai GeneChem Co., Ltd., Shanghai, China. see Fig. s1) downstream of the U6 promoter. A nonspecific control PSC-NC: 5′-TTCTCCGAACGTGTCACGT-3′ was cloned into the same vector. All constructs were verified by DNA sequencing. Virus stocks were produced as described [9]. Viral titers were determined to be within the range of 1×10^8 to 1×10^9 TU/ml.

2.2. Mice

Apoe knock-out mice were purchased from Animal Center of Peking University and maintained in specific-pathogen free (SPF) condition. Protocols for animal experiments were approved by the institutional animal use committee of the Chengdu Military General Hospital and were performed conform the NIH guidelines (NIH Publication No. 85–23, Eighth Edition, revised 2011) for the care and use of laboratory animals. 4×10^7TU virus in 125 μl sterile saline or saline alone was injected intravenously into male *Apoe*$^{-/-}$ mice every four weeks and mice were fed a Western diet (Per 100 g containing normal mouse chow diet 60 g, lard 15.5 g, sucrose 9.3 g, egg yolk powder 9 g,

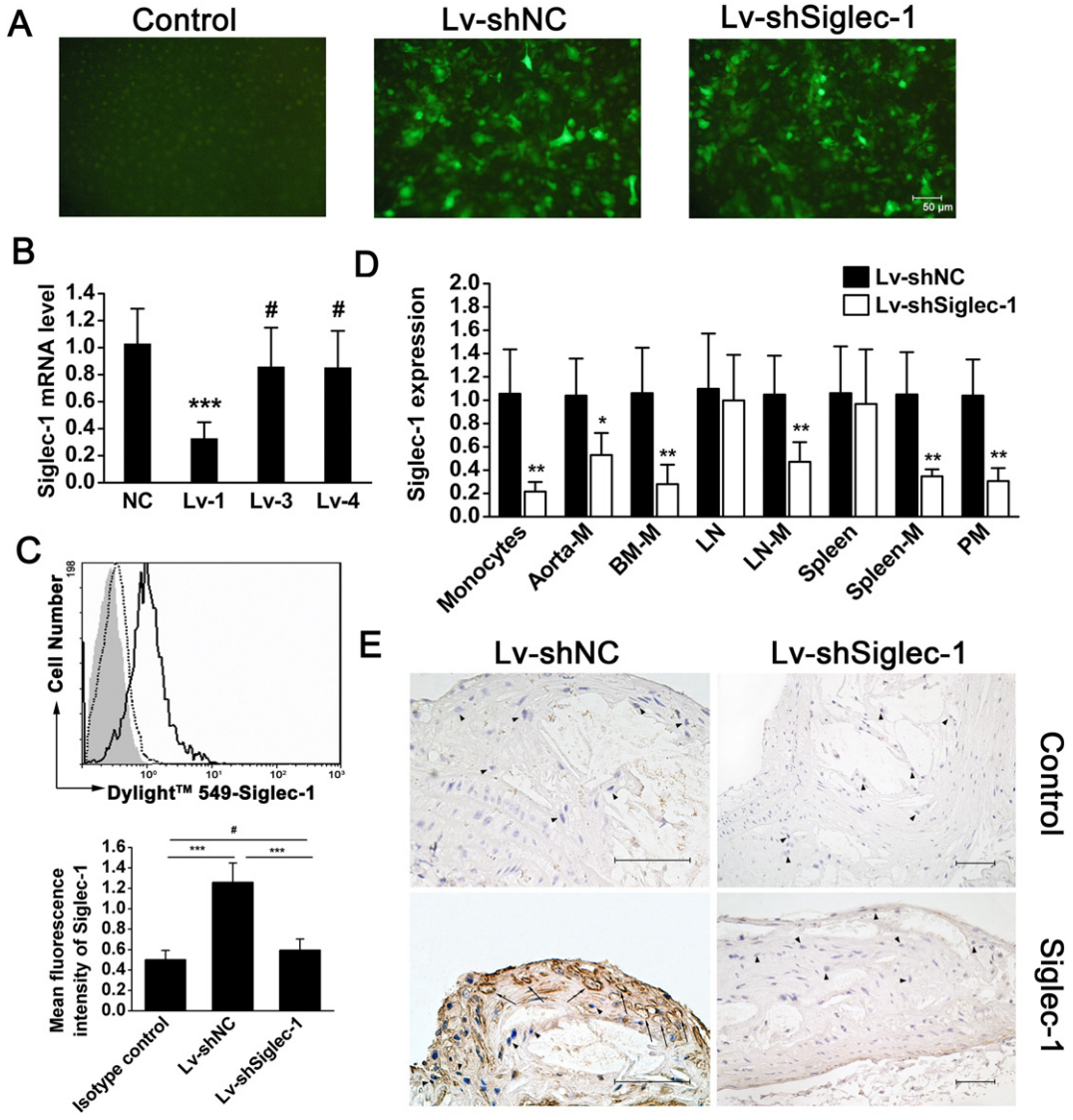

Fig. 1. Siglec-1 suppression by Lv-shSiglec-1 *in vitro* and *in vivo*. oxLDL (100 μg/ml, 48 h) stimulated BMMs were transduced with lentivirus at MOI of 50. Four days later transduction efficiency was determined by fluorescence microscopy and Siglec-1 inhibition was examined by QRT-PCR and FACS. (A), Transduction efficiency in Lv-shNC and Lv-shSiglec-1 group were about 70%–90% (×200). (B), In 3 packaged candidate viral vectors only the Lv-1 (we call it Lv-shSiglec-1) can inhibit Siglec-1 mRNA expression. Data showed mean ± SD ($n = 9$ for each group). $^{***}P < 0.001$, $^{\#}P > 0.05$ *vs.* Lv-shNC group. (C), Flow cytometry analysis. Shadow areas indicate isotype control, dotted lines indicate Lv-shSiglec-1 and solid lines indicate Lv-shNC. Statistic results of Siglec-1 mean fluorescence intensity (MFI) were shown. Data showed mean ± SD and $n = 9$. $^{***}P < 0.001$, $^{\#}P > 0.05$. (D), At 10wk, QRT-PCR was performed to determined *in vivo* Siglec-1 inhibition. Aorta-M, aorta macrophages; BM-M, bone marrow monocytes; LN, lymph node; LN-M, lymph node monocytes; Spleen-M, spleen macrophages; PM, peritoneal macrophages. Data represent mean ± SD ($n = 6$ for each group). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* Lv-shNC group. (E), At 22wk, Immunohistochemistry was used to examine Siglec-1 suppression in aortic plaques (×400), scale bars, 100 μm. Long arrows indicate Siglec-1(+) cells and arrowheads indicate Siglec-1(−) cells.