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Micro RNA-19a suppresses IL-10 in peripheral B cells from patients with atherosclerosis

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ABSTRACT

Background and aims: The interleukin (IL)-10-production B cells play an important role in the pathogenesis of atherosclerosis (Asro) with unknown mechanism. Micro RNA (miR)-17-92 cluster has strong immune regulatory activities. This study tests a hypothesis that miR-17-92 cluster suppresses IL-10 expression in B cells of Asro patients.

Methods: Patients with Asro were recruited into this study. Peripheral blood samples were collected from the patients. B cells were isolated from the blood samples and analyzed to elucidate the role of miR-17-92 in the regulation of IL-10 expression.

Results: Peripheral B cells from patients with Asro show lower levels of IL-10 than that from healthy subjects. The IL-10 expression in the B cells is negatively correlated with the expression of miR-19a in the B cells. The serum levels of tumor necrosis factor (TNF)-α, interferon (IFN)-γ and interleukin (IL)-4 in Asro patients were higher than healthy subjects. Exposure to TNF-α or IFN-γ or IL-4 suppressed IL-10 expression in B cells via increasing the expression of miR-19a in B cells, which could be abolished by Inhibition of miR-19a.

Conclusions: TNF-α or IFN-γ or IL-4 suppresses IL-10 in B cells via up regulating miR-19a expression.

1. Introduction

Atherosclerosis (Asro, in short) is the main cause of coronary heart disease, cerebral infarction and peripheral vascular disease. Asro is a lipid metabolic disorder, characterized by lipid and carbohydrate accumulation on the intima of artery first; followed by hemorrhage, thrombosis, fibrous tissue hyperplasia and calcinosis; arterial medial layer gradually degenerated and calcification, leading to arterial wall thickening and hard, the vessel lumen stenosis. Lesions often involve large and medium-sized muscular arteries. Once the lesion develops large enough to block the lumen of the artery, the local tissue or organ suffers ischemia or necrosis. Yet, the pathogenesis of Asro has not been fully understood.

Published data indicate that immune inflammation plays a critical role in the pathogenesis of Asro. From the initial recruitment of circulating leukocytes to the arterial wall to eventual rupture of the unstable plaque, inflammatory cytokines, including tumor necrosis factor (TNF)-α, IL-1α, IL-1β, IL-4 and interferon-γ (IFN-γ) were found in the local tissue and in the peripheral system [1,2]. Although it is known that the major sources of these cytokines are quite clear, T helper (Th)1 cells produce TNF-α, IL-1 and IFN-γ; Th2 cells produce IL-4, macrophages and mast cells produce TNF-α [3,4], the regulation of these inflammatory cytokines in the pathogenesis of Asro is to be further investigated.

IL-10 is a cytokine with multiple functions in the regulation of immune responses [5]. By suppressing immune cell activities, IL-10 inhibits inflammatory responses [5]. Inflammation is one of the pathological features of Asro. Lower serum levels of IL-10 were identified in Asro patients [6]. Factors interfering with IL-10 expression in the peripheral system have not been fully elucidated.

It is reported that micro RNA (miR) is associated with the pathogenesis of Asro [7]. miRs are a class of endogenous non-coding RNA molecules of ~22 nucleotides in length that modulate differentiation, growth, apoptosis and proliferation of cells by inhibiting translation of mRNAs into proteins via binding to specific sites in the 3′ untranslated region (3′ UTR) of their target mRNAs [8,9]. Published data indicate that high serum levels of IL-4, low serum levels of IL-10, are associated with Asro [6,10]; the miR-17-92 cluster is involved in asthma, a disease with Th2 polarization [11]. Thus, we hypothesize that miR-17-92 cluster is involved in the interaction of IL-4 and IL-10 in Asro patients. In this study, we analyzed the expression of miR-17-92 cluster and IL-10 in peripheral B cells. The results showed that miR-19a, one of the 6 members of miR-17-92 cluster, was markedly higher in the peripheral B cells

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as compared with healthy controls. miR-19a mediated the effect of IL-4 on suppressing IL-10 in peripheral B cells in the culture.

2. Materials and methods

2.1. Patients

This study was conducted in accordance with the Declaration of Helsinki; the experimental procedures were approved by the Human Ethic Committee at Harbin Medical University. An informed written consent was obtained from each human subject. Twenty patients (10 females and 10 males; 49–64 years old) were diagnosed as having Asro after coronary angiography for suspected or known coronary Asro.

2.2. Collection of peripheral blood samples and isolation of B cells

Peripheral blood samples were obtained from 20 Asro patients and 41 healthy subjects via ulnar vein puncture. Peripheral mononuclear cells (PBMC) were isolated from the blood samples by gradient density centrifugation. $CD19^+$ B cells were purified from the PBMCs by magnetic cell sorting with commercial reagent kits (Miltenyi Biotech) following the manufacturer's instructions. The purity of the isolated B cells was greater than 98% and did not contain $CD3^+$ T cells as checked by flow cytometry (Fig. 1).

2.3. Cell culture

B cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 2 mM glutamine. The cell viability was assessed by Trypan blue exclusion assay that was greater than 99% before using for further experiments.

2.4. Detection of miR-17-92 cluster and IL-10 mRNA by real time RT-PCR

Total RNA was extracted from isolated B cells by Trizol (Invitrogen) according to the manufacturer's instructions. Complementary DNA was synthesized from total RNA samples using the NCode Vilo miRNA cDNA Synthesis Kit (Life Technologies). Real-time PCR was performed with the mini Opticon real-time PCR system (Bio-Rad) using SYBR Green qPCR Master Mix (Invitrogen) with miR-17-92 cluster primers (Beijing Yijie Biotech; Beijing, China) according to the manufacturer's protocol. All miRs were normalized to a small nucleolar RNA, RNU48. For quantification, the fold-change of miRNA in experimental relative to control samples was determined by the $2^{-\Delta\Delta Ct}$ method. The sequences of IL-10 primers for PCR are gttctttggggagccaacag and gctccctggtttctcttcct. RNU48 expression was not modulated by LPS, ± IL-4, IFN-γ or TNF.

2.5. Western blotting

The total proteins were extracted from the B cells and quantified with the BCA method. The proteins were fractioned (80 μg/well) by SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and transferred onto a PVDF membrane. The membrane was blocked with 5% skim milk for 30 min, incubated with the primary antibodies (IL-10, purchased from Santa Cruz Biotech, clone V-15) overnight at 4 °C and followed by incubation with the secondary antibodies (labeled with peroxidase) for 1 h at room temperature. Washing with TBST (Tris-buffered saline Tween 20) was performed with the membrane after each time of incubation. The membrane was developed with ECL (enhanced chemiluminescence). The results were photographed with a UVI image system (Cambridge, UK).

2.6. Detection of serum cytokines by enzyme-linked immunosorbent assay (ELISA)

The sera were isolated from collected blood samples by centrifugation. The levels of IL-4, IL-10, IL-13, IFN-γ, TNF-α and IL-1β in the sera were determined by ELISA with commercial reagent kits (R&D Systems) following the manufacturer's instructions.

2.7. Regulation of miR-19a expression in B cells

$CD19^+$ B cells were isolated from blood samples collected from healthy subjects. The B cells were cultured in the presence of IL-4 or IFN-γ or TNF-α at gradient concentrations for 48 h. The cells were collected at the end of culture and processed to determine the miR-19a expression by RT-qPCR as described above.

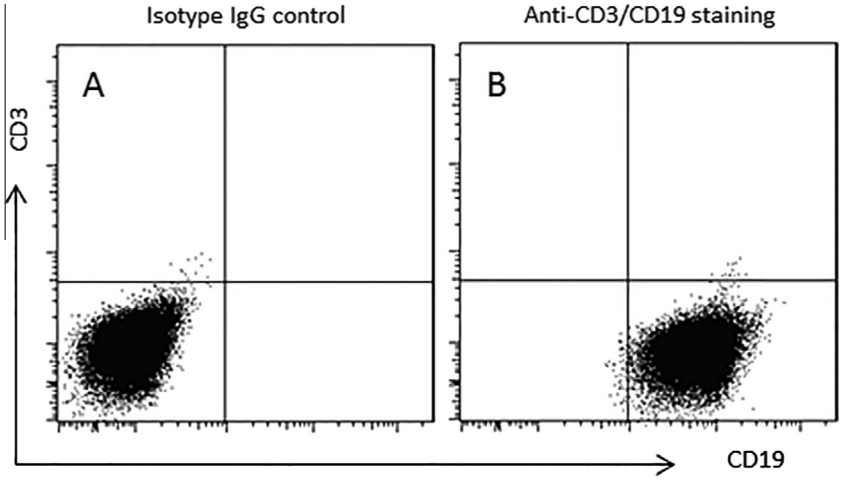

Fig. 1. Assessment of the purity of isolated B cells. $CD19^+$ B cells were purified from PBMCs by magnetic cell sorting with commercial reagent kits following the manufacturer's instructions. The isolated B cells were stained with anti-CD3 and anti-CD19 antibodies or isotype IgG and checked by flow cytometry. The dot plots show the frequency of $CD19^+$ B cells.