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MiR-130b attenuates vascular inflammation via negatively regulating tumor progression locus 2 (Tpl2) expression

Peng Wang[a,1], Xue Zhang[b,1], Fulun Li[c], Kai Yuan[a], Maoran Li[a], Jiwei Zhang[a], Bin Li[c,d,*], Wei Liang[a,**]

[a] Department of Vascular Surgery, South Campus, Renji Hospital, School of Medicine, Shanghai Jiao Tong University, China
[b] Department of Vascular Surgery, Shanghai Pudong Hospital, Fudan University Pudong Medical Center, China
[c] Department of Dermatology, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200437, China
[d] Institute of Dermatology, Shanghai Academy of Traditional Chinese Medicine, Shanghai 201203, China

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ABSTRACT

Endothelial cell (EC) activation and dysfunction have been linked to a wide variety of vascular inflammatory diseases. However, the role of microRNAs in EC activation and inflammation remains largely unknown. In this study, we found that miR-130b was significantly decreased in human umbilical vein endothelial cells (HUVECs) after lipopolysaccharides (LPS) treatment. Forced expression of miR-130b inhibited the LPS-induced activation of extracellular signal-regulated kinase (ERK) and the inflammatory genes expression, such as interleukin (IL)-6 and tumor necrosis factor alpha (TNF-α). Furthermore, we identified that tumor progression locus 2 (Tpl2) is a direct target of miR-130b. Finally, in vivo overexpression of miR-130b via miR-130b agomir attenuates acute lung vascular inflammation in the LPS-induced sepsis mouse model. Taken together, our data demonstrated that miR-130b represses vascular inflammation via targeting Tpl2, suggesting that miR-130b mimics might be a promising therapeutic strategy for treatment of vascular inflammatory diseases.

1. Introduction

The vascular endothelium, which is composed of a monolayer of endothelial cells (ECs) represents a crucial interface between blood and all tissues [1,2]. EC is a typical multifunctional cell that has the critical basal and inducible metabolic and synthetic functions [3,4]. When exposed to the physical and chemical signals, ECs are able to produce a wide range of factors that can regulate multiple physiological processes, including blood flow, vascular tone, cellular adhesion, thromboresistance and smooth muscle cell proliferation [5–11].

The abnormally activation and dysfunction of endothelial cells link to various kinds of vascular inflammatory diseases, such as sepsis, atherosclerosis, diabetes, rheumatoid arthritis, and inflammatory bowel disease [12–20]. During inflammation, the vascular endothelium cells are activated and express various kinds of adhesion molecules that are critically involved in the recruitment of leukocytes to inflammation sites [21,22]. In particular, E-selectin, vascular cell adhesion molecule 1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1) regulate leukocyte attachment and rolling on the endothelial at the early stage, followed by the inflammatory response within tissues [21,23].

microRNA is a class of highly conserved, single-stranded, small noncoding RNA that regulates gene expression at a posttranscriptional level by typically binding to targeting mRNAs with the seed sequence. The emerging evidence reveals microRNA as a new regulator of multiple inflammatory and immune responses [24,25]. Abnormal expression of microRNA contributes to a variety of inflammatory diseases, such as acute liver injury [26] and inflammatory bowel disease [27]. Over 1800 human mature microRNA sequences are recorded in the web database-miRbase, however only a small number have been characterized as functional regulators of EC activation and inflammatory responses [28–32]. Thus, in this study, we aimed to identify the key miRNAs in regulating HUVEC activation and inflammation.

* Correspondence to: B. Li, Department of Dermatology, Yueyang Hospital of Integrated Traditional Chinese and Western Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200437, China.
** Correspondence to: W. Liang, Department of Vascular Surgery, South Campus, Renji Hospital, 2000 Jiangyue Road, Shanghai 201112, China.
E-mail addresses: libin@shyueyanghospital.com (B. Li), liangwei1375@renji.com (W. Liang).
[1] Wang[a] and Zhang[b] contributed equally to this study.

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Table 1
The sequences of primers.

GENES	Sense (5′-3′)	Antisense(5′-3′)
Hsa-RPL13a	CCTGGAGGAGAAGAGGAAAGAGA	TTGAGGACCTCTGTGTATTTGTCAA
Hsa-IL-6	GATGAGTACAAAAGTCCTGATCCA	CTGCAGCCACTGGTTCTGT
Hsa-TNFα	CAGCCTCTTCTCCTTCCTGAT	GCCAGAGGGCTGATTAGAGA
Hsa-*E*-selectin	CCCGTGTTTGGCACTGTGT	GCCATTGAGCGTCCATCCT
Hsa-Tpl2	CAAGTGAAGAGCCAGCAGTTT	GCAAGCAAATCCTCCACAGTTC
Mus-GAPDH	CAGAACATCATCCCTGCATC	CTGCTTCACCACCTTCTTGA
Mus-IL-6	GATGGATGCTACCAAACTGGAT	CCAGGTAGCTATGGTACTCCAGA
Mus -TNFα	GCAGGTCTACTTTGGAGTCATTGC	TCCCTTTGCAGAACTCAGGAATGG
Mus -E-selectin	GTGCGGTGTACGTCCTCTTGG	GACTTGTAGGTGAATTCTCCAGTAGT
Mus -Tpl2	TCAGTCCCCAGAATGGCCGCT	AGAACAGACCCTCCCTCGCCG
Tpl2 3'UTR	CCGCTCGAGCCGAGGAATCTGAGATGCTC	ATAAGAATGCGGCCGCTCAGTTGATTTGACCAACCAC

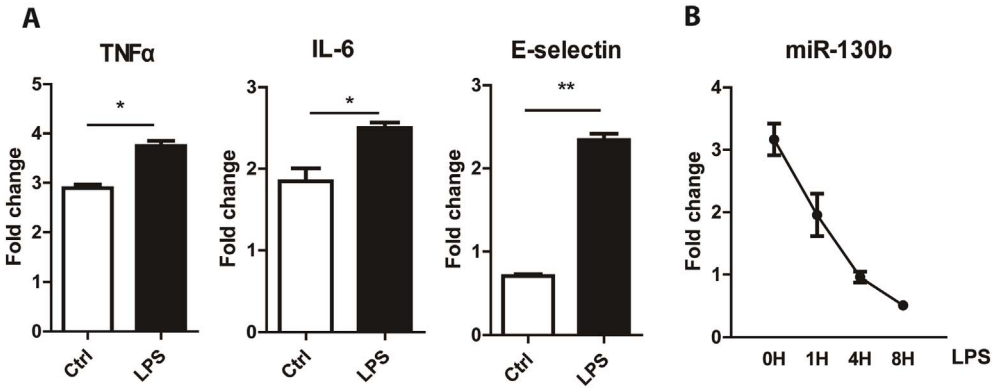

Fig. 1. Reduced levels of miR-130b in HUVECs after LPS stimulation. (A) The expression of inflammatory genes (TNFα, IL-6, and E-selectin) in HUVECs at 6 h after LPS treatment. (B) The kinetic change of miR-130b levels in HUVEC stimulated by LPS. Bars represent the means ± SEM. *P < 0.05, **P < 0.01.

2. Methods

2.1. Mice

Wild type C57BL/6J (B6) mice were purchased from SLAC laboratory, Shanghai, China). All animals were propagated and housed in a specific-pathogen-free barrier facility, with 12-hour light/dark cycle and allowed free access to food and water. 8–10 weeks old male mice were used for experiments. All experimental protocols involving animals were approved by the Animal Studies Committee of Shanghai Renji Hospital.

2.2. LPS induced lung injury

LPS (Sigma Chemical Co, St. Louis, MO) was prepared freshly by dissolving the compound in pre-warmed phosphate buffered saline (PBS, pH 7.4) before injection. For miR-130b intervention assay, mice were given (i.v.) negative control (NC) agomir or miR-130b agomir (RIBO, China) with Entranster™-in vivo Transfection Reagent (Engreen, Beijing, China) at a dose of 1000 nmol/kg body weight 24 h before LPS administration. All mice were euthanized 4 h after LPS administration (40 mg/kg). Lung tissues were harvested, and fixed with 4% paraformaldehyde (PFA) for 24 h to 48 h until processed for histological analysis.

2.3. Cell culture and transfection

HUVECs were purchased from ATCC and cultured in ATCC-formulated complete growth medium. Cells passaged < 5 times were used for all experiments. Lipofectamine RNAiMAX and 2000 transfection reagent was used for transfection, following the manufacturer's instructions. microRNA negative control or miR-130b mimic were transfected at 100 nM concentration, and microRNA inhibitor negative control or miR-130b inhibitor were transfected at 200 nM except where indicated. Cells were cultured for 24 h before treatment of 1 μg/ml LPS.

2.4. Taq-man human microRNA array analysis

Total RNA was extracted from the HUVECs using TRIzol (Invitrogen, San Diego, CA). Samples for the TaqMan Human MicroRNA Arrays (Life Technologies, Gaithersburg, MD) were reverse transcribed using Megaplex RT Primers and the TaqMan MicroRNA Reverse Transcription kit. The cDNAs were produced in pre-amplification reactions for 12 cycles using the Megaplex PreAmp Primers and TaqMan PreAmp Master Mix. The miRNA expression was then detected by real-time polymerase chain reactions with the TaqMan Universal PCR Master Mix and TaqMan MicroRNA Array.

2.5. RNA extraction, cDNA synthesis, and quantitative real-time PCR

Total RNA was extracted from the lung tissue. The expression of miR-130b was determined using a TaqMan miRNA assay kit (Life Technologies, Gaithersburg, MD). U6, a reference small nuclear RNA, was used as internal controls. RNA was converted to cDNA with the PrimeScript RT Reagent Kit (Takara Bio, Otsu, Japan). To examine mRNA expression level, quantitative real-time PCR were performed using a SYBR Premix Ex Taq RT–PCR kit (Takara Bio, Otsu, Japan) for 40 cycles with denaturation at 95 °C for 5 s and annealing at 60 °C for 30 s. RPL13a and GAPDH were determined as the internal controls for human and mouse, respectively. The sequences of real-time PCR primers were listed in Table 1.

2.6. Western blotting

Lung tissues were lyzed by RIPA buffer (Thermo Scientific, Rockford, IL). Protein amounts were determined by BCA assay (Thermo Scientific, Rockford, IL) and then normalized before western blot experiment. Lysate proteins were separated by SDS-PAGE, transferred to PVDF membranes, and probed with primary antibodies directed against phosphorylated (Ser) ERK (Cell Signaling, 1:1000), ERK (Cell Signaling, 1:1000), β-Tubulin (Abcam, 1:5000), and Tpl2 (Abcam, 1:1000) for