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Induction of microRNA-10a using retinoic acid receptor-α and retinoid x receptor-α agonists inhibits atherosclerotic lesion formation

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ABSTRACT

Background and aims: MicroRNA (miR)-10a is a shear-regulated miR with the lowest expression in vascular endothelial cells (ECs) in athero-susceptible regions with oscillatory shear stress (OS). The aim of this study is to elucidate the relationship between EC miR-10a and atherosclerosis and develop a hemodynamics-based strategy for atherosclerosis treatment.
Methods: A combination of *in vitro* flow system and *in vivo* experimental animals was used to examine the functional roles of EC miR-10a and its clinical applications in atherosclerosis.
Results: *En face* staining showed that EC miR-10a is down-regulated in the inner curvature (OS region) of aortic arch in rats. Co-administration with retinoic acid receptor-α (RARα)- and retinoid X receptor-α (RXRα)-specific agonists rescued EC miR-10a expression in this OS region. These effects of OS and RARα/RXRα-specific agonists on EC miR-10a expression were confirmed by the *in vitro* flow system, and were modulated by the RARα-histone deacetylases complex, with the consequent modulation in the downstream GATA6/vascular cell adhesion molecule (VCAM)-1 signaling cascade. Animal studies showed that miR-10a levels are decreased in both aortic endothelium of atherosclerotic lesions and blood plasma from apolipoprotein E-deficient ($ApoE^{-/-}$) mice. *In vivo* induction of EC miR-10a by administration of RARα/RXRα-specific agonists protects $ApoE^{-/-}$ mice from atherosclerosis through inhibition of GATA6/VCAM-1 signaling and inflammatory cell infiltration.
Conclusions: Our findings indicate that down-regulation of miR-10a in aortic endothelium and blood serum is associated with atherosclerosis, and miR-10a has potential to be developed as diagnostic molecule for atherosclerosis. Moreover, EC miR-10a induction by RARα/RXRα-specific agonists is a potential hemodynamics-based strategy for atherosclerosis treatment.

1. Introduction

Hemodynamic forces generated from blood fluid flow can be characterized as pulsatile (PS) and oscillatory shear stresses (OS) [1]. In the arterial tree, OS develops preferentially in athero-susceptible regions, i.e. arterial branches, curvatures, and bifurcations, whereas PS prevails in athero-protected regions, i.e. the straight part of arteries. Several lines of evidence indicate that OS

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induces pro-atherogenic signaling to cause endothelial cell (EC) dysfunction, and thus is defined as pro-atherogenic flow. In contrast, PS enhances athero-protective signaling to promote EC function, and is defined as athero-protective flow [1–5]. These results indicate that hemodynamic forces play important roles in regulating the formation and progression of atherosclerosis.

MicroRNAs (miRs), small non-coding RNA molecules that can modulate gene expression through binding 3′-UTRs of target gene, are vital epigenetic factors to modulate EC function and atherogenesis [3–7]. Accumulating evidence indicates that several miRs can be regulated by hemodynamic forces to induce pro-atherogenic and athero-protective signals in ECs in response to OS and PS, respectively [3–5]. A previous study by Fang et al. [8] indicates that miR-10a is a miR with the lowest expression among 1139 miRs in endothelia of athero-susceptible *vs.* athero-protected regions in normal adult swine *in vivo* and contributes to the inhibition of EC pro-inflammatory phenotypes *in vitro* [8]. Although Fang's study used normal animal model to identify the differential expression of miR-10a at the arterial wall based on hemodynamic forces, the relationship between miR-10a and atherosclerotic lesion development is not clear. Moreover, the diagnostic and therapeutic applications of miR-10a in atherosclerosis remain to be identified.

Previous studies by Weiss et al. [9] showed that miR-10a can be regulated by retinoic acid receptors (RARs) to modulate pancreatic cancer metastasis. RARs (i.e., RARα, RARβ, and RARγ) are the members of nuclear hormone receptors, which have been identified as transcriptional factors to bind to RA-responsive element (RARE) in the regulatory region of target genes to modulate their transcription. Moreover, RARs hetero-dimerize with other nuclear hormone receptors retinoid X receptors (RXRs, i.e., RXRα, RXRβ, or RXRγ) to enhance their transcriptional activity [10–12]. In contrast, RARs associate with their co-repressors to recruit histone deacetylases (HDACs) to repress their transcriptional activity [13,14]. Our previous study demonstrated that RARα and RXRα can be induced by athero-protective PS to enhance miR-10a expression and inhibit its downstream GATA6/vascular cell adhesion molecule (VCAM)-1 signaling in ECs, whereas HDAC-3/5/7 are induced by pro-atherogenic OS to repress RARα-directed miR-10a signaling [15]. However, whether RARα- and RXRα-specific agonists can serve as therapeutic components to mimic the athero-protective effect of PS to induce miR-10a expression and repress OS-induced pro-atherogenic signaling in ECs remain unclear.

By using *in vitro* flow system and *in vivo* experimental rat and apolipoprotein E-deficient ($ApoE^{-/-}$) mouse models, our present study demonstrated that down-regulation of miR-10a in both aortic endothelium and blood serum is highly associated to atherogenesis, implicating that miR-10a has potential to be developed as a diagnostic molecule for atherosclerosis. Our findings also indicate that *in vivo* induction of EC miR-10a by the administration of RARα/RXRα-specific agonists is a promising hemodynamics-based strategy for the treatment of atherosclerosis.

2. Materials and methods

2.1. Rat experiments and en face staining

Normal rats were intraperitoneally injected with vehicle control DMSO or specific agonists of RARα (AM580, 1 mg/kg) and RXRα (CD3254, 1 mg/kg) (Tocris Bioscience) for 2 weeks. The experimental protocol was approved by Institutional Ethics Committee (NHRI-IACUC-103004-A). Animals were euthanized with CO_2 and transcardially perfused with 150 mL of saline, followed by 500 mL of 10% neutral-buffered zinc-formalin (Thermo Scientific). The inner (OS region) and outer (PS region) curvatures of aortic arch and the straight segment of thoracic aorta (PS region) were harvested and post-fixed in the fixative solution for 1 h, and then subjected to *en face* immunostaining for miR-10a and von Willebrand factor (vWF).

2.2. Flow apparatus

Human aortic ECs (HAECs) were subjected to OS (0.5 ± 4 dynes/cm^2) in a parallel-plate flow chamber [16]. Detailed procedures are described in online supplemental document.

2.3. $ApoE^{-/-}$ mouse experiments

Twelve-weeks-old $ApoE^{-/-}$ mice received vehicle controls (DMSO + CLmiR), RARα/RXRα-specific agonists and CLmiR (RAα+RXα AGO + CLmiR), or RARα/RXRα-specific agonists and antagomiR-10a (AMR-10a) (RAα+RXα AGO + AMR-10a), together with western diet (WD) for 12 weeks ($n = 6$ each). DMSO + CLmiR group received intraperitoneal injections of olive oil plus 5% DMSO for 6 days per week (except Sunday) and tail-vein injections of miR-invivofectamine mixture of CLmiR twice per week. RAα+RXα AGO + CLmiR and RAα+RXα AGO + AMR-10a groups received intraperitoneal injections with the combination of RARα- and RXRα-specific agonists (1 mg/kg body wt each) daily for 6 days per week (except Sunday) and tail-vein injections of miR-invivofectamine mixture of CLmiR or AMR-10a twice per week. The experimental protocol was approved by the Institutional Ethics Committee (NHRI-IACUC-103125-M3-A).

Detailed procedures of experimental methods used in this study are described in Supplemental Data.

3. Results

3.1. OS-inhibition of EC miR-10a is rescued by the combined effects of RARα/RXRα-specific agonists in vivo and in vitro

Our previous study and others have shown that miR-10a is a shear-regulated miR [8,15], and RARα and RXRα can be activated by athero-protective PS to induce EC miR-10a expression [15]. In this study, we examined whether RARα- and RXRα-specific agonists could mimic PS effect to up-regulate miR-10a and down-regulate OS-induced pro-atherogenic signaling in ECs. *En face* immunostaining on the aortic arch and the straight segment of thoracic aorta of normal rats showed that miR-10a is down-regulated in ECs in the inner curvature of aortic arch, where OS occurs (Fig. 1A, left panel). In contrast, high levels of miR-10a were present in ECs in the outer curvature and the straight segment of thoracic aorta, where PS exists. This OS-induced down-regulation of EC miR-10a in the native circulation was rescued by co-administrations of RARα- and RXRα-specific agonists (Fig. 1A, right panel). *In vitro* flow experiments confirmed that OS inhibits EC miR-10a expression, and this OS-inhibition of EC miR-10a is totally rescued by the co-addition of RARα- and RXRα-specific agonists (Fig. 1B).

Since our previous study has demonstrated that pro-atherogenic OS can induce associations of HDAC-3/5/7 with RARα to repress RARα-RARE binding and up-regulate GATA-6/VCAM-1 signaling [15], here we investigated whether RARα- and RXRα-specific agonists can inhibit these OS-induced pro-atherogenic signaling cascades in ECs. Co-immunoprecipitation assays showed that RARα- or RXRα-specific agonist alone only achieved partial inhibition, but together they had additive effects to abolish the formation of RARα-HDAC-3/5/7 complex (Fig. 1C). Chromatin immunoprecipitation (ChIP) assay showed that co-addition of RARα- and RXRα-specific agonists abolished OS-inhibition of RARα-RARE binding, whereas addition of RARα- or RXRα-specific agonist alone had only minor effects (Fig. 1D).