The magic and mystery of MicroRNA-27 in atherosclerosis

Wu-Jun Chen,a,b Kai Yin,a,c Guo-Jun Zhao,a,d Yu-Chang Fu,e Chao-Ke Tanga,*

a Institute of Cardiovascular Research, Key Laboratory for Atherosclerosis of Hunan Province, Life Science Research Center, University of South China, Hengyang, Hunan 421001, China
b Institute of Pharmacy and Pharmacology, University of South China, Hengyang 421001, China
c The Department of Diagnostics, University of South China, Hengyang 421001, China
d Department of Histology and Embryology, University of South China, Hengyang 421001, China
e Department of Nutrition Sciences, University of Alabama at Birmingham, Birmingham, AL 35294-0012, USA

ARTICLE INFO

Article history:
Received 10 November 2011
Received in revised form 10 January 2012
Accepted 11 January 2012
Available online 18 January 2012

Keywords:
miR-27
Inflammation
Lipid metabolism
Mystery
Atherosclerosis

ABSTRACT

Atherosclerosis (As) is now widely appreciated to represent a chronic inflammatory reaction of the vascular wall in response to dyslipidemia and endothelial distress involving the inflammatory recruitment of leukocytes and the activation of resident vascular cells. MicroRNAs (miRNAs) are a group of endogenous, small (~22 nucleotides in length) non-coding RNA molecules, which function specifically by base pairing with mRNA of genes, thereby induce translation repression of the genes within metazoan cells. Recently, the function of miR-27, one of the miRNAs, in the initiation and progression of atherosclerosis has been identified. In vivo and in vitro studies suggest that miR-27 may serve as a diagnostic and prognostic marker for atherosclerosis. More recently, studies have identified important roles for miR-27 in angiogenesis, adipogenesis, inflammation, lipid metabolism, oxidative stress, insulin resistance and type 2 diabetes, etc. In this review, we focus on the role of miR-27 in the development of vulnerable atherosclerotic plaques, potential as a disease biomarker and novel therapeutic target in atherosclerosis.

¢ 2012 Elsevier Ireland Ltd. All rights reserved.

Contents

1. Introduction ................................................................................................................. 314
   2.1. MiR-27 in angiogenesis ......................................................................................... 315
   2.2. MiR-27 in the regulation of adipocyte differentiation and obesity ...................... 315
   2.3. MiR-27 in inflammatory response and immune response ................................. 317
       2.3.1. MiR-27 may regulate inflammatory response in atherosclerosis by VEGF signaling pathways ................................................................. 317
       2.3.2. MiR-27 regulates inflammatory response through PPARs and RXRs .......... 317
       2.3.3. MiR-27 is responsive to inflammation and arteriosclerosis through regulating apoptosis .......................................................... 318
       2.3.4. MiR-27 may contribute to plaque formation in atherosclerosis through regulating the expression of matrix metalloproteinase 13 (MMP-13) ................................................................. 318
       2.3.5. MiR-27 regulates inflammatory response through NF-kB pathway .............. 319
   2.4. MiR-27 in lipid metabolism ................................................................................. 319
   2.5. MiR-27 may promote atherosclerosis through regulating G2A by Runx1-c/EBPs pathway ......................................................................................... 321
   2.6. Future directions and challenges ........................................................................ 321
Acknowledgments ........................................................................................................... 322
References ....................................................................................................................... 322

1. Introduction

Atherosclerosis (As) is one of the leading causes of mortality in the world. It has been identified as a chronic inflammatory disease of the artery wall. The development of native atherosclerosis is mechanistically considered as dysregulation of many biological phenomena; such as angiogenesis, adipogenesis, inflammatory
response and immune response, lipid metabolism, oxidative stress, insulin resistance and type 2 diabetes [1,2].

Molecular analysis of vulnerable plaques, lipid accumulation and inflammation in atherosclerosis can be performed at different levels (coronary syndromes, myocardial infarction or stroke), such as for instance proteomics, lipidomics, or RNA analysis. With respect to RNA analysis, the discovery and use of miRNAs may yield new diagnostic and even therapeutic options. Recent extensive studies have demonstrated that miRNAs are highly expressed in vascular walls, and their expression or function are dysregulated in diseased vessels. Thus, miRNAs are found to play important roles in atherosclerosis initiation and progression via regulating key vascular cellular events through their target genes [3].

miRNAs are a group of endogenous, conserved ~22 nucleotides non-coding RNAs that anneal to inexactly complementary sequences in the 3′UTR (Fig. 1) and 5′UTR of target mRNAs of protein-coding genes or protein-coding exon regions to cause mRNA cleavage or repression of the translational machinery for protein synthesis [3–5]. It is currently estimated that there may be 1000 miRNA genes in the human genome (www.sanger.ac.uk/Software/Flat/animals/mirna/). Indeed, human miRNAs are predicted to control the activities of more than 60% of all protein-coding genes. Therefore, miRNAs have now been implicated in the controls of a wide range of biological functions including development, differentiation, metabolism, growth, proliferation and apoptosis. They can act as an important regulator in inflammation as well as angiogenesis, both important characteristics of plaque vulnerability in atherosclerosis. In addition, importance of these small RNAs in regulating adipocyte differentiation, lipid metabolism and oxidative stress has only recently been uncovered [3,6].

These studies suggest that aberrant expression of miRNAs might be involved in atherosclerosis and cardiovascular diseases, and some miRNAs have been identified in these diseases, one of them is miR-27 [6–9].

In the miR-27 family, there are two isoforms, miR-27a and miR-27b. According to the miR database (http://microrna.sanger.ac.uk/), miR-27a is an intergenic miRNA, miR-27b is an intronic miRNA located within the 14th intron of the human C9orf3 host gene, and a mouse ortholog of C9orf3 also harbors miR-27b. They are homologous to each other, sharing 20 out of 21 nucleotides, and are highly conserved during evolution and present in mammals, birds, fish and reptiles. Recently, an integral role of miR-27 in pathogenesis of cardiovascular disease has begun to emerge and been extensively studied in vivo [7]. Many studies indicate that miR-27 is involved in all the known processes of atherosclerosis including angiogenesis, adipogenesis, inflammation, lipid metabolism, oxidative stress, insulin resistance and type 2 diabetes. Moreover, the level of miR-27 expression is significantly associated with clinic pathological factors and the prognosis of patients with atherosclerosis obliterans, suggesting that it might serve as a diagnostic and prognostic marker for human atherosclerosis. Furthermore, miR-27 dysregulation has been reported to be a predictor of vulnerable atherosclerotic plaques [7,9]. However, many studies suggest that miR-27 was involved in the development of cancer, and its role in atherosclerosis is rather young. So we discussed the magic and mystery of miR-27 in atherosclerosis to promote it in atherosclerosis study.


2.1. MiR-27 in angiogenesis

MiR-27 has been reported to influence angiogenesis. Importantly, angiogenesis has been recognized as an important process for the progression of atherosclerotic plaques [3,10]. MiR-27a/b are expressed at the highest levels in the lung, heart and ECs, which are highly vascularized tissues and cells, suggesting a potential role in EC function [11,12]. With regard to angiogenesis, the highly expressed miR-27b exerts pro-angiogenic effects as evidenced by the blockade of in vitro angiogenesis with 2′-O-methyl oligonucleotide inhibitors. Since miR-27b has been reported to be significantly downregulated by Drosha and Dicer siRNAs. Interestingly, silencing of Dicer expression additionally impaired in vivo angiogenesis, whereas deletion of Drosha did not exert significant effects [11]. Thus, the knockdown of Dicer exerted more profound effects on endothelial sprout formation in vitro and in vivo compared with Drosha. By using in silico prediction of targets for the highly expressed and Drosha/Dicer downregulated miR-27b, it has been demonstrated that miR-27b may regulate the expression of thrombospondin-1 (TSP-1), which was recently identified as an endogenous angiogenesis inhibitor. Moreover, the expression of TSP-1 increases during atherosclerosis in animals, and the absence of TSP-1 accelerates atherosclerotic plaque maturation in ApoE−/− mice [13,14]. Consistent with the predicted role of the miR-27b for TSP-1 expression, Drosha and Dicer silencing augmented TSP-1 expression. In addition, given that TSP-1 is a potent angiogenesis inhibitor, its upregulation might contribute to the impairment of angiogenesis after Drosha or Dicer downregulation [11]. Importantly, among the predicted target genes, multiple conserved binding sites for miR-27a/b are contained in the 3′UTR of TSP-1 mRNA, suggesting that miR-27 might accelerate arteriosclerosis development by reducing the mRNA and protein levels for TSP-1 gene (Fig. 3).

Vascular endothelial growth factor (VEGF) is considered as one of the key growth factors involved in angiogenesis. It is expressed by ECs, smooth muscle cells (SMCs), and macrophages within the microenvironment of an atherosclerotic plaque [15]. Many studies demonstrated that VEGF enhanced atherosclerotic plaque progression in ApoE−/− cholesterol-fed mouse model [16,17]. Interestingly, Zhou et al. found that the pro-angiogenic actions of miR-27 correlated with the repression of their target miRNAs encoded by Sprouty2 and Sema6A genes which negatively regulate MAPK and VEGF-R2 signaling pathways in response to angiogenic factors [12]. Loss of miR-27 functions impaired MAPK and VEGF-R2 signaling pathways in response to VEGF, and thereby repressed angiogenesis. The authors also found that miR-27 was enriched in ECs and other highly vascularized tissues. Knockdown of miR-27 in cultured aortic rings repressed EC outgrowth, proliferation and migration in response to VEGF, whereas adenoviral overexpression of miR-27 enhanced aortic ring EC sprouting. Silencing of miR-27 in ECs impaired vascular network formation on matrigel and suppressed the sprouting of vasculature in mice, suggesting that miR-27 has a more dominant role in angiogenesis [12]. Of important, the formation of atherosclerotic lesions is caused by the phenotypic modulation in VSMCs accompanied by accelerated migration, proliferation and production of extracellular matrix components. The pro-angiogenic properties of miR-27 may affect the transition of VSMCs from a differentiated phenotype to a dedifferentiated state, which plays a critical role in the pathogenesis of atherosclerosis. However, this speculation needs to be further proved by experimental evidence, and how miR-27 regulates VEGF through stimulating MAPK and VEGFR2 signaling pathways by directly targeting the SPRY2 and Sema6A 3′UTRs for repression in arteriosclerosis is yet to be investigated (Fig. 3).

2.2. MiR-27 in the regulation of adipocyte differentiation and obesity

Some studies have focused on miR-27 which appears to act as negative regulators of adipogenesis. Dysfunction or an
excess of perivascular adipose tissue is thought to directly induce inflammation of the adjacent arteries and it can be hypothesized that perivascular adipose tissue may thus be involved in the pathogenesis of atherosclerosis, atherothrombosis and plaque rupture [18]. Lin et al. studies demonstrated that the miR-27 gene family was functionally characterized using 3T3-L1 preadipocytes and OP9 mouse bone marrow mesenchymal stem cells [19]. Overexpression of miR-27 by transfecting miR-27 precursors before adipogenic stimulation specifically inhibited adipocyte differentiation. Mechanistically, miR-27 prevented the induction of peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT/enhancer-binding protein α (C/EBPα), the two master transcriptional regulators of adipogenesis [19]. In addition, overexpression of miR-27a in 3T3-L1 pre-adipocytes suppresses PPARγ expression and adipocyte differentiation. The 3’UTR of PPARγ harbors a putative miRNA binding site, which has been shown to specifically bind to miR-27a using a luciferase reporter assay [20]. Another family member, miR-27b, is also downregulated during adipocyte differentiation. MiR-27b can also bind to the 3’UTR of PPARγ and represses PPARγ protein levels in adipocytes [21]. Interestingly, miR-27a is expressed more abundantly in the stromal vascular fraction of murine adipose tissues than in mature adipocytes [20], indicating that miR-27a may be necessary to have more functions for atherosclerosis through perivascular adipose tissue hypertrophy. Another recent study showed that PPARγ was the target of miR-27b in cardiomyocytes and partially mediated cardiac hypertrophy that resulted from miR-27b overexpression [22]. These studies suggest the miR-27 family could be a useful anti-adipogenic target for fat accumulation and cell proliferation. In this case, potential of miR-27 molecular mimics could be used to regulate pre-adipocyte proliferation and differentiation.

Recently, Li et al. found that over-expressed miR-27a and 27b influenced fat accumulation and cell proliferation during rat hepatic stellate cell activation, furthermore, retinoid X receptor (RXRα) which is involved in multiple signaling pathways related to cell proliferation and differentiation, mainly as heterodimeric partner of several nuclear receptors, was also confirmed to be the target of miR-27a and 27b in these cells [23]. In another recent study [24], miR-27a was shown to be upregulated with hyperglycemia status in adipose tissue, and exposure of 3T3-L1 adipocytes to increased glucose concentration would upregulate the expression of miR-27a, suggesting that, at least in adipose tissue, increased expression of miR-27 may be involved in the initial cellular responses to hyperglycemia and be part of the early cellular events related to the pathogenesis of type 2 diabetes. In addition, the MAPK and insulin signaling pathways were also suggested by analysis of the combined list of predicted target genes of miR-27a. Since atherosclerosis is the leading cause of death in type 2 diabetes [25], these findings provide novel information on the molecular mechanisms that may account for miR-27 family playing a role in the accelerated atherosclerosis associated with type 2 diabetes. Furthermore, the PPARγ regulates gene expression upon heterodimerization with the RXRα by binding to peroxisome proliferator response elements (PPREs) in the promoter region of target genes [26], and C/EBPs were found to colocalize with PPARγ at the majority of its binding sites and to have cooperative effects on target gene transcriptions [27]. Therefore, miR-27 family may accelerate arteriosclerosis development though decreased mature adipocyte differentiation and then increased perivascular adipose tissue hypertrophy by reducing the expression of PPARγ, RXRα or C/EBPα (Fig. 3). However, even the role of miR-27-family-dependent downregulation of its target genes and adipogenesis in
arteriosclerosis has been proved to be important and their functions remain unknown.

2.3. MiR-27 in inflammatory response and immune response

Inflammatory response to vascular injury can lead to atherosclerotic plaque formation and instigated the process by rapid cell proliferation. Of note, inflammation is an important factor in the initiation, development, and progression of atherosclerosis [1,2].

2.3.1. MiR-27 may regulate inflammatory response in atherosclerosis by TSP-1 or VEGF signaling pathways

TSP-1 and VEGF are not only involved in angiogenesis but also in inflammatory phenotypes. Functional studies in Tsp1−/− mice have shown animal phenotypes, such as enhanced leukocyte infiltration, pneumonia with macrophages and neutrophils, and mild pancreatic inflammation [13,14]. As we described above, miR-27 may downregulate the expression of TSP-1 by directly binding to the TSP-1 3′UTR. Hence, TSP-1 seems equally important as miR-27 in inflammation. However, little is known on how miR-27 accelerated atherosclerotic plaque development by contributing to TSP1-dependent inflammatory response (Fig. 3).

VEGF mediate vascular permeability of various inflammatory processes, contributing to atherosclerotic plaque development and instability [16,17]. Indeed, the expression of miR-27 in human umbilical vein endothelial cells (HUVECs) was up-regulated when these cells were stimulated with lipopolysaccharide (LPS). When using DIANA-miPath from DIANAlab to predict the pathways regulated by miR-27, the data indicated that MAPK signaling pathway is also included in addition of the TGFBR1 and TGFBR2 signaling pathways [12]. However, further studies are needed to explain how miR-27 regulates inflammatory response in atherosclerosis through VEGF by directly targeting the SPROUTY2 and SEMA6A 3′UTRs for its repression regulation (Fig. 3).

2.3.2. MiR-27 regulates inflammatory response through PPARs and RXRα

PPARs activation in immune cells predominantly results in transrepression of proinflammatory gene expression. In vivo, PPARs can form a heterodimer with the RXRs, and then regulate inflammatory response (Fig. 2). Of important, expression of PPARs and RXRs has been demonstrated in atherosclerotic plaques, suggesting that PPAR-RXR activation may have beneficial effects on modulating inflammatory responses in atherosclerosis [26,27].

LPS, a classical pro-inflammatory stimulus, has been shown time-dependently reducing PPARγ mRNA and protein amounts in macrophages. Concerning the stimulation regime provoking M1 macrophage activation, one has to keep in mind that LPS treatment rapidly down-regulates PPARγ after 6–15 h by miR-27β-dependent mRNA destabilization [8]. The potential of miR-27 to decrease PPARγ mRNA is acknowledged by several laboratories. Previous studies demonstrated that overexpression of miR-27a and -27b play an important role during inflammation in the animals [8,28]. Jendrewin et al. observed that induction of miR-27b was at least partially nuclear factor-κB (NF-κB)-dependent, and they concluded that the NF-κB-dependent PPARγ mRNA decrease, could be the result at least in part from the NF-κB-dependent induction of miR-27b upon the LPS exposure [8]. In line, stimulation with TNFα also reduced PPARγ levels likely by miR-27b induction, while IFNγ (not activating NF-κB) rather induced PPARγ expression. Thus, overexpressing or inhibiting miR-27b would affect TNFα and IL-6 mRNA amounts. Interestingly, Inhibition of miR-27a showed no effect on PPARγ reduction upon LPS exposure, and
whether miR-27 family response to inflammatory response in atherosclerosis by target PPARγ-RXRα or RXRα is unknown (Fig. 3). Nevertheless, the roles of miR-27a and -27b during inflammation in mouse atherosclerotic plaques remain obscure. In another study [29], the expression of PPARα in human liver-derived cell lines was decreased by the overexpression of miR-27b, mechanistically, PPARα was confirmed to be the target of miR-27b. In fact, upon ligand activation, PPARα down-regulates diverse components of the pro-inflammatory response such as chemokines and cytokines by decreasing the expression of the Th1 transcription factor T-bet (T-box expressed in T cells) and increasing the expression of GATA-3 (guanosine adenine thymidine adenosine 3'), known as a positive regulator of Th2 cytokines. However, how miR-27 regulates inflammation in atherosclerosis by repressing PPARα expression is yet to be investigated (Fig. 3).

2.3.3. MiR-27 is responsive to inflammation and atherosclerosis through regulating apoptosis

In another study [30], overexpression of a miRNA complex including miR-27a had sensitized HEK293T cells to TNFα cytotoxicity and miR-27a negatively regulated the expression of FADD (Fas Associated protein with Death Domain) which cause apoptosis in mammalian cells. FADD-deficient mice have been examined to show enhanced vascular inflammation, and high rates of apoptosis are often observed in vascular lesions, including atherosclerotic vessels and vessels after angioplasty [30,31]. Furthermore, Culpan et al. studies has also shown that miR-27b was decreased in a dose-dependent manner after 15 min exposure to TNF-α, suggesting that miR-27b responded to TNFα [32]. TNFα has been shown to play an important role in atherosclerosis, mainly due to its direct effects on the vasculature. However, further work is needed to establish the miR-27a/b that are responsive to inflammation and atherosclerosis through directly targeting to TNFα and FADD (Fig. 3).

2.3.4. MiR-27 may contribute to plaque formation in atherosclerosis through regulating the expression of matrix metalloproteinase 13 (MMP-13)

Akhtar et al. have also shown that increased expression of MMP-13 correlated with down-regulation of miR-27b [33]. In silico analysis to identify a sequence in the 3'UTR of MMP-13 mRNA complementary to the seed sequence of miR-27b, overexpression of miR-27b suppressed the activity of a reporter construct containing the 3'UTR of human MMP-13 mRNA and inhibited the IL-1β-induced expression of MMP-13 protein in cells, and NF-κB and MAPK activation down-regulated the expression of miR-27b. However, no changes in miR-27a expression were noted, which suggest that miR-27a is not an IL-1β-responsive gene [33]. Importantly, Deguchi laboratory and others found that MMP-13 deficiency, rather than alterations in lesion sizes of these mice, is correlated with changes in collagen deposition and collagen fibers alignment in atherosclerotic plaques, suggesting that MMP-13 activity actually reduces plaque size in the processing atherosclerosis [34,35]. Genetic silencing of Dicer and Drosha led to significantly reduced miR-27 expression in ECs [11], indicating that miR-27 may contribute to plaque formation in atherosclerosis through regulating the expression of MMP-13 in ECs. Indeed, database analysis of proinflammatory stimuli-upregulated miRNAs revealed potential NF-κB binding sites in the promoter elements of miR-27 gene.
MiR-27b was transactivated through promoter binding of the NF-kB p65 subunit following by proinflammatory stimuli. Proinflammatory stimuli increases transcription of pri-miR-27b cluster, as well as the host gene transcript, C9orF3, via promoter binding of p65 to a binding site at -1254 of the immediate upstream of the gene [36]. NF-kB p65-dependent regulation may increase IL-8 mRNA expression and binding p65 to the promoter of IL-8 gene in cells exposed to miR-27, suggesting a role for miR-27 in linking inflammation to NF-kB in atherosclerosis. However, how miR-27 regulates inflammation in atherosclerosis through MMP-13 and NF-kB p65-dependent gene is yet to be investigated (Fig. 3).

2.3.5. MiR-27 regulates inflammatory response through NF-kB pathway

Reactive oxygen species (ROS) produced in macrophages are critical for microbial killing, but they also take part in inflammation and antigen presentation functions. Furthermore, ROS generated by the mitochondria are important in the regulation of signaling pathways (such as inducing NF-kB pathway) that contribute to atherosclerosis development [37,38]. Previous studies have shown that the expression of miR-27a* and miR-27b* in RAW 264.7 cells were downregulated in response to H_{2}O_{2} which was an example of ROS [39]. The authors observed that the translocation of p65 subunit which is the transactivating subunit in the canonical NF-kB pathway was significantly affected in the cells transfected with miR-27b* when compared with untransfected cells or cells transfected with control mimics. In addition, overexpression of miR-27b* mimics might negatively regulate its target proteins, which may be critical for NF-kB activation and the absence of these proteins in cells transfected with miR-27b* mimics might have led to either failure of NF-kB activation or active suppression of NF-kB. Interestingly, overexpression of miR-27b* cannot alter NF-kB-dependent gene expression, such as IL-1β, IL-6, and TNFα, but Ccl2 mRNA expression was lower in the cells transfected with miR-27b* mimics as compared with that of control mimics [39]. The presence or absence of specific pathways in specific cell types should be also highlighted since NF-kB is expressed in most cell types and can be markedly induced in some of them. It is believed that miR-27 and miR-27a* are both mature miR-27 molecules arising from the same precursors. miR-27* is less abundant than miR-27 and they are likely to be non-functional. Importantly, ROS-mediated repression of Sp and Sp-dependent genes involves downregulation of miR-27a, and ROS-dependent effects on the miR-27a promoter, demonstrate that ROS suppresses miR-27a and miR-27 was involved in oxidative stress [40–42]. These previous reports suggest that the oxidative stress-responsive miR-27 family may accelerate atherosclerotic plaque development via NF-kB pathway but the identification of putative target proteins that modulate this pathway remains to be determined (Fig. 3).

2.4. MiR-27 in lipid metabolism

Previous studies have shown that miR-27 gene was involved in lipid metabolism [6,43]. In fact, the dysregulation of lipid metabolism, such as triglyceride and cholesterol, has an important role in the onset and outcome of atherosclerotic process. Alisi et al. studies showed that miR-27 was significant down-regulated in HFD (high-fat diet), SD-HF (standard diet enriched with fructose) and HFD-HF (high-fat diet enriched with high content of fructose) fed rats. In these animal models, the downregulation of miR-27a appeared mainly associated to the inflammatory molecules (p38MAPK and LITAF) and fatty acid metabolism (LPL and CYP4A1) [44]. In addition, more recent studies also show that miR-27a and miR-27b were novel negative regulators of the LPL expression [20,21]. Overexpression of miR-27a and miR-27b blunted early induction of LPL and repressed triglyceride accumulation in cells and adipogenesis. Several genes implicated in lipid metabolism, including PPARγ, RXRα, adiponectin, CD36, FASN, FABP4, GLUT4, and SREBP1c, have been shown to be repressed by miR-27α and miR-27b in cells [20,21]. LPL is the rate-limiting enzyme in the hydrolysis of triglycerides in chylomicron (CM) and very low density lipoprotein (VLDL) in the plasma. Many studies have confirmed that the LPL deficiency may be proatherogenic, however, it was conflicting reports [45,46]. Previous studies from our laboratory and others have also shown that increasing LPL activity in skeletal muscle resulted in decreased fat accumulation, and long-term administration of ibrolipim which is an effective LPL activator protected against the development of experimental atherosclerosis in animals [47,48]. In addition, PPARα was also confirmed to be the target of miR-27b, as we described above, overexpression of miR-27b showed down-regulated expression of PPARα [29]. PPARα, which is mainly expressed in the liver, regulates the expression of many genes [such as LPL, ATP-binding cassette transporter A1 (ABCA1) and G1 (ABCG1), etc.] involved in lipid metabolism [26]. ABCA1 and ABCG1 regulate circulating HDL levels through their roles in HDL biogenesis and cellular cholesterol efflux. Our previous studies have demonstrated that ABCA1 and ABCG1 play critical roles in preventing cholesterol accumulation in macrophages. In mice combined deficiency of ABCA1 and ABCG1 in macrophages, the phenotypes of double knockout animals presented impaired cellular cholesterol efflux in vitro and a massive increase of lipid accumulation in macrophage in vivo, and accelerated atherosclerotic plaque maturations [49–52]. In addition, PPARγ-dependent expression of liver X receptor (LXRα) also showed a decrease of intracellular cholesterol accumulation in mice. As a heterodimer in combination with the RXRα, LXRα usually induces expression of the ABCA1, ABCG1 and SR-B1, causing lipid export from macrophages and thus counteracting foam cell generation [26]. Since LPL is a target gene of PPARγ and PPARγ increases LPL activity. The objective of these studies was to investigate whether or not miR-27 family might downregulate the expression of LPL, ABCA1 and ABCG1 through directly repressing the expression of PPARα, PPARγ or RXRα, and then to promote atherosclerosis progressing [53] (Fig. 3). In another study [54], Nishi et al. have also shown that the binding sites of miR-27a in the 3′ UTR of thyroid hormone receptor β1 (TRβ1) could mediate translational repression by miR-27a. Overexpression of miR-27a can decrease TRβ1 protein levels without affecting TRβ1 mRNA levels in cells, and the miR-27a decay increased TRβ1 protein levels. These findings indicated that TRβ1 is a target of miR-27 both in rat and human cells. Importantly, TRβ is responsible for the cholesterol lowering. Erion et al. studies show that targeting TRβ agonists to the liver reduces cholesterol and triglycerides and improves the therapeutic index [55], suggesting that miR-27 may promote lipid accumulation and atherosclerotic plaque development by targeting TRβ1 in cells (Fig. 3). However, the functions of TRβ1 in arteriosclerosis have not been extensively studied.

Interestingly, to search for a possible involvement of miR-27 in posttranscriptional regulation of targets prediction information, we use databases: TargetScan (http://www.targetscan.org), microRNA.org (http://www.microrna.org/microrna/home.do), RNAhybrid (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/welcome.html) and PicTar (http://pic tar.mdc-berlin.de/), etc. Our results have shown that the 3′UTR sequences of the PPAR, RXR, ABCA1, LDL, LDLR, 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMGCGR), low density lipoprotein receptor-related protein 6 (LRP6), oxysterol binding protein-like 10 (OSBPL10) and 11 (OSBPL11), etc., which are associated with lipid metabolism, were predicted to bind to miR-27 family. However, only a few targets of miRNA-27, including the transcription factors PPARα, PPARγ and RXRα, have been currently validated (Table 1). In contrast to
these findings, one interesting possibility is that miR-27 family are able to regulate lipid metabolism by binding to the 3′UTR of LPL and ABCA1, at any rate selectively modulating lipid metabolism through downregulation the expression of LPL and ABCA1 by PPAR/RXR-LXR/RXR signaling pathways [53] (Fig. 3). More intriguingly, recent unpublished data from our laboratory indicate that miR-27a/b represent critical regulator of the cholesterol efflux. We recently made the surprising discovery that anti-miR-27a/b (loss of function) attenuated oxLDL-induced lipid accumulation in macrophages; conversely, overexpression of miR-27a/b enhanced lipid accumulation in THP-1 macrophages. This reduction in intracellular lipid accumulation was also evidenced by a decrease in cellular level of cholesterol efflux. In addition to a transcriptional mechanism, our group has also shown that a post-transcriptional mechanism mediated by miR-27a/b are likely to be involved in the abolition of ABCA1 protein. Moreover, miR-27a/b are enriched in THP-1 macrophages, suggesting a potential role in THP-1 macrophage function [12]. This data suggest that miR-27a/b may target the ABCA1 cholesterol efflux pump for translational repression/mRNA degradation, resulting in increased intracellular cholesterol. Of major importance, this work was supported by grants from the National Natural Science Foundation of China in this year (MiR-27a/b regulate reverse cholesterol transport by downregulating the expression of ABCA1, 81170278). In this research project, we have fused a fragment of the human ABCA1 3′UTR harboring the predicted miR-27 target sequences to a luciferase reporter plasmid for our experiments. Accordingly, we will observe the intracellular lipid, cellular cholesterol content, ABCA1 expression and cholesterol efflux in THP-1, HepG cells and peritoneal macrophages through overexpression of miR-27 or knockdown of miR-27. Importantly, we will investigate the plasma lipid level, the area of atherosclerotic plaque and thickness of endomembrane, ABCA1 expression and circulating HDL levels [the rate of RCT (reverse cholesterol transport)] in apoE−/− mice through overexpression of miR-27 or knockdown of miR-27. In addition, data from our laboratory and other investigators revealed that ABCA1 can function as an anti-inflammatory receptor to suppress the expression of inflammatory factors, suggesting that ABCA1 may be the molecular basis for the interaction between inflammation and RCT [56,57]. Based on these findings, miR-27 plays an important role in the macrophage pro-inflammatory response which may be selectively mediated by its targeting of ABCA1 to elevated levels of LDL in a high-fat diet animal model.

Recently, our previous studies have demonstrated that TGF-β up-regulated expression of ABCA1, ABCG1 and SR-BI through LXRs signaling pathway in THP-1 macrophage-derived foam cells, then cellular cholesterol content was decreased while cholesterol efflux was increased [58]. Interestingly, miR-27 may downregulate the expression of TSP-1 by directly binding to the TSP-1 3′UTR, and TSP-1 is a major activator of latent TGF-β that displayed anti-atherosclerosis effect [13,59]. These findings raise the possibility that miR-27a and miR-27b may be able to selectively downregulate ABCA1, ABCG1 and SR-BI expression through TSP-1/TGF-β/LXRs signaling pathway, then depress the activity of this cholesterol-removal pathway and promote atherosclerosis progressing (Fig. 3). However, it is not clear whether the effects of

### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gen name</th>
<th>Function</th>
<th>Verification</th>
<th>miRNA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPROUTY2</td>
<td>Sprouty homolog 2 (Drosophila)</td>
<td>Anti-angiogenesis, Anti-inflammatory, MAPK and VEGF R2 signaling</td>
<td>protein</td>
<td>miR-27a/b</td>
<td>[12]</td>
</tr>
<tr>
<td>SEMA6A</td>
<td>Semaphorin 6a</td>
<td>Anti-angiogenesis, Pre-inflammatory, MAPK and VEGF R2 signaling</td>
<td>protein</td>
<td>miR-27a/b</td>
<td>[12]</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Peroxisome proliferator-activated receptor γ</td>
<td>Pro-adipogenic, Anti-inflammatory, Lipid metabolism</td>
<td>mRNA, protein</td>
<td>miR-27b</td>
<td>[8,19–22]</td>
</tr>
<tr>
<td>C/EBPα</td>
<td>CCAAT/enhancer-binding protein α</td>
<td>Pro-adipogenic, Anti-inflammatory</td>
<td>protein</td>
<td>miR-27a/b</td>
<td>[19,63]</td>
</tr>
<tr>
<td>RXRα</td>
<td>Retinoid X receptor</td>
<td>Pro-adipogenic, Anti-inflammatory, Lipid metabolism</td>
<td>mRNA, protein</td>
<td>miR-27a/b</td>
<td>[23]</td>
</tr>
<tr>
<td>FADD</td>
<td>Fas associated protein with death domain</td>
<td>Cell apoptosis, Anti-inflammatory</td>
<td>protein</td>
<td>miR-27a</td>
<td>[30]</td>
</tr>
<tr>
<td>PPARα</td>
<td>Peroxisome proliferator-activated receptor α</td>
<td>Anti-inflammatory, Lipid metabolism</td>
<td>mRNA, protein</td>
<td>miR-27b</td>
<td>[29]</td>
</tr>
<tr>
<td>MMP-13</td>
<td>Matrix metalloproteinase 13</td>
<td>Anti-angiogenesis, Anti-inflammatory</td>
<td>protein</td>
<td>miR-27b</td>
<td>[33]</td>
</tr>
<tr>
<td>NF-KB</td>
<td>NFκB nuclear factor kappa B</td>
<td>Pro-inflammatory</td>
<td>NF-κB p65 promoter</td>
<td>miR-27b</td>
<td>[36]</td>
</tr>
<tr>
<td>p38MAPK</td>
<td>p38 map kinase</td>
<td>cytokine/chemokine-mediated signaling pathway</td>
<td>miR-27a</td>
<td>miR-27a</td>
<td>[44]</td>
</tr>
<tr>
<td>LITAF</td>
<td>Lipopolysaccharide-induced TNF factor</td>
<td>cytokine/chemokine-mediated signaling pathway</td>
<td>miR-27a</td>
<td>miR-27a</td>
<td>[44]</td>
</tr>
<tr>
<td>LPL?</td>
<td>Lipoprotein lipase</td>
<td>Lipid metabolism</td>
<td>protein</td>
<td>miR-27a/b</td>
<td>[20,21,44]</td>
</tr>
<tr>
<td>CYPA1?</td>
<td>Cytochrome P450, family 4, subfamily a, polypeptide 1</td>
<td>fatty acid metabolism</td>
<td>miR-27a</td>
<td>miR-27a</td>
<td>[44]</td>
</tr>
<tr>
<td>Adiponectin, CD36,FASN, FABP4,GLT4, and SREBP1c?</td>
<td>Lipid metabolism</td>
<td>protein</td>
<td>miR-27a/b</td>
<td>miR-27a</td>
<td>[20,21,44]</td>
</tr>
<tr>
<td>ABCA1, ABCG1 and SR-B1?</td>
<td></td>
<td>Cholesterol efflux</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRβ 1</td>
<td>thyroid hormone receptor β 1</td>
<td>Cholesterol metabolism</td>
<td>protein</td>
<td>miR-27a</td>
<td>[54]</td>
</tr>
<tr>
<td>Runx1</td>
<td>Runt related transcription factor 1</td>
<td></td>
<td>protein</td>
<td>miR-27a/b</td>
<td>[63]</td>
</tr>
</tbody>
</table>
the TSP-1 on development of atherosclerosis would be mediated through changes in cholesterol efflux by TGF-β/LXRα signaling pathway. More intriguingly, Wang et al. has recently observed that TGF-β significantly inhibited miR-27b at the transcriptional level [22], suggesting that a possible link mutual influence between miR-27 and TGF-β. The authors also found that TGF-β signaling might protect cardiomyocytes from hypertrophic growth by downregulating miR-27 that causes cardiac hypertrophy when overexpressed. Most importantly, PPARY which is a well-known critical regulator during cardiac hypertrophy was the target of miR-27b and partially mediated cardiac hypertrophy that resulted form miR-27b overexpression [22]. As we described above, TGF-β protects against the development of atherosclerosis by promoting the induction of the LXRs-ABC metabolic cascade that governs cholesterol removal from cells [58]. However, the detailed mechanism for ABCA1 induced by TGF-β is still unclear. Based on our studies and others, we hypothesized that TGF-β might protect foam cells formation and the atherogenesis through increasing the expression of ABCA1 and cholesterol efflux by downregulating miR-27, at any rate selectively modulating activation of the PPARY-LXRs-ABC transcriptional cascade by downregulation the expression of miR-27. However, further studies in this area have to be investigated.

2.5. MiR-27 may promote atherosclerosis through regulating G2A by Runx1-c/EBPs pathway

Bolick et al. and others found that loss of G2A contributes to the deterioration of the atherosclerotic lesion of mice and human [60,61]. In addition, Murakami et al. results demonstrated that transcription of G2A was dependent on both chromatin structure around TSS, and binding of the transcription factors (c/EBPα and β, and Runx1) to their cis-elements, located at the core promoter just upstream of TSS [62]. Interestingly, in another study [63], the reporter assays revealed that miR-27a and -27b targeted the 3’UTR of the Runx1. Overexpression of pre-miR-27a or -27b reduced the level of endogenous Runx1 protein levels in several hematopoietic cell lines. Furthermore, expression profiles of pri-miR-27a and Runx1 in granulocytes/macrophage progenitors (GMP) and differentiated granulocytes in mice paralleled those observed in 32D.c13s differentiation in vitro. Thus, in addition to a transcriptional mechanism, a post-transcriptional mechanism mediated by miR-27a and -27b is likely to be involved in the abolition of Runx1 protein. In addition, overexpression of miR-27a or -27b can reduce the levels of endogenous c/EBP protein in adipocyte [19]. However, the 3’UTR of c/EBP that can bind to miR-27 family is unknown. These previous studies demonstrated that miR-27 family might promote atherosclerosis through regulating G2A by Runx1-c/EBPs pathway (Fig. 3). However, G2A that protects effects against atherogenesis by activating of Runx and c/EBP function synergistically have to be proved in the future.

2.6. Future directions and challenges

In fact, the dysregulation of angiogenesis, adipogenesis, inflammatory response and immune response, lipid metabolism, oxidative stress and insulin signaling, have important roles in the onset and outcome of atherosclerosis. Li et al. showed for the first time, that significant increase of miR-27 expression was observed in the serum samples of atherosclerosis patients [7]. The expression of miR-27 in sera of atherosclerosis patients was positively correlated with fontaine stages, and miR-27 with different stages of atherosclerosis tells us that they may, at least in part, reflect the progression of atherosclerosis. These results demonstrated that the serum levels of miR-27 can serve as risk factors or diagnostic markers for atherosclerosis [7,9]. In addition, most evidence suggests that miR-27 family may be a genuine proatherogenic-gene and that it may play an important role in the regulation of angiogenesis, adipogenesis, inflammation, lipid metabolism, oxidative stress and insulin signaling. Although the function of miR-27, especially its role in atherosclerosis, has not been extensively studied, there is a good reason for us to propose that miR-27 may serve as potential indicators for atherosclerosis.

Many clinical studies have shown that combination therapy based on a patient’s molecular profile can deliver better responses. In order to restore or inhibit, the function of downregulated proatherogenic miRNAs, or upregulated antiatherogenic miRNAs, the application of miRNA agonists or antagonists has already been successfully demonstrated in experimental atherosclerosis mouse models (LDLR−/− or apoE−/−). The significant therapeutic benefit may be due to the potential of miRNAs to modulate a cohort of gene networks. For miRNAs with proatherogenic capabilities, potential therapies include anti-miRNA oligonucleotides, microRNA mimetics (agonomirs), antagonists [locked nucleic acid (LNA), antagonomirs], miRNA sponges, miRNA masking and small molecule inhibitors. For example, recent studies have revealed the safety and efficacy of LNA-antisense approaches. LNA-miR-122 antisense oligonucleotides against the cholesterol-regulating miR-122, which has also been implicated in the propagation of hepatitis C virus (HCV), are currently in human phase II trials for HCV [64,65]. In addition, recent advances in the understanding of lipid metabolism have revealed that miR-33, an intronic microRNA located within the SREBF2 gene, suppresses expression of the cholesterol transporter ABCA1, ABCG1 and lowers HDL levels [66,67]. Accordingly, anti-miR53 can increase circulating HDL, enhances the RCT pathway, and regresses established atherosclerosis in LDLR−/− mice and African green monkeys, indicating that antagonism of this microRNA might be a promising clinical approach for raising HDL in the treatment of cardiovascular disease [66–68]. In another study [69], Ramirez et al. have identified miR-758 as a novel microRNA that posttranscriptionally controls ABCA1 levels in different cells and regulates macrophage cellular cholesterol efflux to apoA1, opening new avenues to increase apoA1 and raise HDL levels in patients with cardiovascular disease.

It is important to note, to evaluate the effect of miR-27 overexpression in vivo, Wang et al., first generated two transgenic mouse lines carrying the mouse miR-27b DNA under the control of α-MHC (alpha-myosin heavy chain) promoter. These transgenic mice survived to adulthood and were fertile. However, 30% of these mice died suddenly between 6 and 12 months of age. These mice have been also used to examine the effect of antagonism-miR27b administration in vivo, and the authors found that mature miR-27b was significantly downregulated in hearts of transverse aortic constriction (TAC) model mouse [22]. However, different animal models have different characteristics according to their genetic backgrounds. So far, there is no evidence about whether the mature forms of miR-27a/b and its host gene C0r0f3 appear to be co-expressed or co-transcribed. Furthermore, there is no information available yet about knockout of C0r0f3 expression in mouse models. For these reasons, knockout of miR-27 in mouse models are probably difficult in vivo and have not been attempted. Clinical studies in human have revealed that miR-27 may play important roles in atherosclerosis. However, many more target genes of miR-27 have not been described in cardiovascular system thus far (Table 1). MiR-27 is also expressed in the cells of atherosclerotic lesion or macrophage cell lines (such as HUVEC, SMC, THP-1 and RAW 264.7 cells). Unfortunately, no studies have been performed in this research area yet. Currently, our understanding of the role exerted by specific miR-27 in the initiation and progression of atherosclerosis is still at an early stage. The therapeutic potential of miR-27 is still unexplored and miR-27-based atherosclerosis gene therapy offers the theoretical appeal of targeting multiple gene networks that are controlled by a single miR-27. Therefore,
suppression of miR-27 in atherosclerosis animal models has not been achieved, but many researchers will have a particular interest in this area.

In the future, it would be conceivably beneficial to examine the function of miR-27 in the initiation and progression of atherosclerosis. The combination of computational analysis, bioinformatics, and experimental approaches in atherosclerosis animal model will be needed to perform for the miR-27 target gene study. Bioinformatic predictions and experimental approaches have indicated that a single miRNA could target more than 100 mRNAs. Similarly, a single mRNA could be regulated by many miRNAs [66–69].

The way of how the single miR-27 tightly regulated multi-target genes in vivo and vitro should be precisely identified in the future, including whether the co-transfection of different miRNAs had an additive inhibitory effect on the single target gene expression. More importantly, there is much to be learned concerning the mechanisms of miR-27 that contribute to progress of atherosclerosis by targeting different signaling pathways in different cells or tissues. There is still no report regarding the in vivo pharmacokinetics of miR-27. Also, the effective delivery of synthetic therapeutic miR-27 to the desired target tissues will be a challenge. In addition, the upstream signaling pathways of miR-27 also should be investigated in the future. The question of how miR-27 and C9orf3 expression are co-regulated is remained to be clarified. Importantly, with the improvement of techniques and the development of new antagonist and mimic approaches, it may become easier to target individual miR-27 species with high specificity (knockdown). The rAAV9-EGFP (recombinant adeno-associated virus Serotype 9 mediated enhanced green fluorescent protein) was expressed at the highest levels in cardiovascular system, while the other tissues had a little or no expression [70], indicating that the co-transfection of miR-27 antisense oligonucleotides (ASO) with rAAV9-EGFP was ideal for investigating the role of miR-27 in cardiovascular system. However, the safety and pharmacokinetic profile of suppressing miR-27 in atherosclerosis animal models should be tested, and these speculation need to be proved by experimental evidence. In the future, important issues still remain if miR-27-based atherosclerosis therapy is to be taken into clinical practice. A better understanding of the mechanisms that underlie miRNA-mediated drug resistance/sensitivity of cells, coupled with improvements in drug delivery technology, will enable miR-27-mediated therapy to open a new era in atherosclerosis care.

Acknowledgments

The authors gratefully acknowledge the financial support from the National Natural Sciences Foundation of China (81170278, 81101213, 81070220), the Heng Yang Joint Funds of The Hunan Provincial Natural Sciences Foundation of China (10JJ9019), the Aid Program for Science and Technology Innovative Research Team in Higher Educational Institutions of Human Province, China (2008–244) and the Science and Technology Department Funds of Heng Yang of Hunan Province Grant (2010k17).

References

[4] Lytle JR, Vario TA, Steitz JA. Target mRNAs are repressed as efficiently by microRNA-binding sites in the 3’UTR as in the 3’UTR. Proc Natl Acad Sci USA 2007;104:9667–72.


Erion MD, Cable EE, Ito BR, et al. Targeting thyroid hormone receptor-beta agonists to the liver reduces cholesterol and triglycerides and improves the therapeutic index. Proc Natl Acad Sci USA 2007;104:15490–5.


学霸图书馆

www.xuebalib.com

本文献由“学霸图书馆-文献云下载”收集自网络，仅供学习交流使用。

学霸图书馆（www.xuebalib.com）是一个“整合众多图书馆数据库资源，提供一站式文献检索和下载服务”的24小时在线不限IP图书馆。

图书馆致力于便利、促进学习与科研，提供最强文献下载服务。

图书馆导航：

图书馆首页 文献云下载 图书馆入口 外文数据库大全 疑难文献辅助工具