MicroRNAs in Cardiometabolic Diseases
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BACKGROUND: MicroRNAs (miRNAs) are ~22-nucleotide noncoding RNAs with critical functions in multiple physiological and pathological processes. An explosion of reports on the discovery and characterization of different miRNA species and their involvement in almost every aspect of cardiac biology and diseases has established an exciting new dimension in gene regulation networks for cardiac development and pathogenesis.

CONTENT: Alterations in the metabolic control of lipid and glucose homeostasis predispose an individual to develop cardiometabolic diseases, such as type 2 diabetes mellitus and atherosclerosis. Work over the last years has suggested that miRNAs play an important role in regulating these physiological processes. Besides a cell-specific transcription factor profile, cell-specific miRNA-regulated gene expression is integral to cell fate and activation decisions. Thus, the cell types involved in atherosclerosis, vascular disease, and its myocardial sequelae may be differentially regulated by distinct miRNAs, thereby controlling highly complex processes, for example, smooth muscle cell phenotype and inflammatory responses of endothelial cells or macrophages. The recent advancements in using miRNAs as circulating biomarkers or therapeutic modalities, will hopefully be able to provide a strong basis for future research to further expand our insights into miRNA function in cardiovascular biology.

SUMMARY: MiRNAs are small, noncoding RNAs that function as post-transcriptional regulators of gene expression. They are potent modulators of diverse biological processes and pathologies. Recent findings demonstrated the importance of miRNAs in the vasculature and the orchestration of lipid metabolism and glucose homeostasis.
homeostasis. MiRNA networks represent an additional layer of regulation for gene expression that absorbs perturbations and ensures the robustness of biological systems. A detailed understanding of the molecular and cellular mechanisms of miRNA-mediated effects on metabolism and vascular pathophysiology could pave the way for the development of novel diagnostic markers and therapeutic approaches.

**KEYWORDS:** microRNA, lipid metabolism, glucose homeostasis, vascular endothelium, vascular smooth muscle, atherosclerosis

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### Introduction

Recent development of high-throughput genomic analyses has revolutionized biomedical research. It is not surprising that these cutting-edge technologies start to transform microRNA (miRNA/miR) investigation. Both gene-chip microarray and the next-generation RNA-sequencing technologies have been introduced for miRNA target identification.(1) In the last few years several groundbreaking studies have indicated that in addition to being relevant in cardiac remodeling and function, miRNAs exert dominant functions in vascular and metabolic disease as well.(2) Another discovery in miRNA biology that is developing with remarkable pace is the revelation that miRNAs are detectable and highly stable in plasma or serum. Circulating miRNAs appear to correlate with disease, opening up the possibility to use them as novel diagnostic biomarkers. For cardiovascular disease, circulating miRNAs so far have been shown to be potential biomarkers for acute myocardial infarction, heart failure, coronary artery disease (CAD), stroke, and type 2 diabetes.(2)

An important contribution of miRNAs to the regulation or alteration of lipid metabolism and glucose homeostasis may determine the predisposition to cardiometabolic disease and atherosclerosis. For instance, miR-33 controls cellular cholesterol export and fatty acid degradation, which are stimulated by its host genes, whereas miR-122 can limit cholesterol synthesis and lipoprotein secretion in the liver. (3) miRNAs regulate multiple aspects and functions of the vascular endothelial growth factor (VEGF) signaling pathway in vasculogenesis and angiogenesis, in particular providing insights into the role of miRNAs and downstream effectors in modulating VEGF output during development. (4)

Jaringan miRNA mencerminkan sebuah lapisan ekstra yang menyerap gangguan pada ekspresi gen dan menjamin kekokohan sistim biologi. Pemahaman yang lebih rinci tentang mekanisme selular dan molekular pengaruh mediasi miRNA pada metabolisme dan patofisiologi vaskular dapat membuka jalan untuk pengembangan marker diagnostik dan pendekatan terapi baru.

**KATA KUNCI:** microRNA, metabolisme lemak homeostasis glukosa, endotelium vaskular, otot polos vaskular, aterosklerosis

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### miRNAs

Disturbances in gene expression as a result of perturbed transcription or post-transcriptional regulation is one of the main causes of cellular dysfunction that underlies different disease states. The discovery of miRNAs in mammalian cells has renewed our focus on post-transcriptional regulatory mechanisms during pathogenesis.(7)

Usually described as inhibitory factors, they act by enhancing degradation(8), or inhibiting translation(9) of their target mRNAs. The human miRNA panel could regulate several thousands of genes.(10) Several miRNAs could regulate the same gene. Conversely, one miRNA could regulate several targets, involved in different physiological pathways.(11) As a consequence, gene regulation by miRNAs could occur in all physiological situations. Today, more than 1,000 human and 600 mouse miRNAs are listed in the miRBase database (http://www.mirbase.org).(12) A...
nomenclature system to classify miRNAs was established in 2003.(13)

MiRNAs act as rheostats that fine tune protein output. (14,15) Despite having a modest effect on individual targets, miRNAs can exert potent biological effects. A single miRNA is able to regulate the expression of multiple targets often within the same biological pathway. Recent evidence suggests that miRNAs function by generating thresholds in target gene expression.(16) Alternatively, miRNAs may act as both positive and negative regulators of cellular processes to ensure the precision and robustness of biological systems against perturbations.(17-19)

The effect of a particular miRNA on gene expression is likely to be dictated by the relative expression of the miRNA and its target genes, which can compete for the binding in their 3'-untranslated regions. Of note, one miRNA often regulates multiple genes that are involved in a specific signaling cascade or cellular mechanism, thus making miRNAs potent cellular regulators.(20,21)

Long primary miRNAs (pri-miRNAs) transcripts are often several thousand nucleotides long and undergo a first cleavage within the nucleus by the RNase III enzyme Drosha.(22) After processing of pri-miRNAs by the Drosha/ DiGeorge syndrome critical region gene 8 (DGCR8) complex, resulted pre-miRNAs are transported via exportin into the cytoplasm awaiting further modifications.(23)

Recently, several studies have highlighted the presence of miRNAs in the plasma. Plasma miRNAs are packaged in microvesicles (including exosomes) that protect them from degradation.(24) Moreover, recent reports have also identified these small RNAs associated with proteins, including the RNA-binding protein Argonaute 2.(25)

Extracellular miRNAs are also transported by lipoproteins, namely high-density lipoproteins (HDL) and low-density lipoproteins (LDL), both of which are highly abundant in plasma. Whereas exosomes and microparticles are composed of a bilayer-phospholipid shell and hydrophilic core, lipoproteins consist of a single layer of lipids, a hydrophobic core, and are defined by specific structure–function apolipoproteins.(26) Some miRNAs are enriched in the plasma under pathological conditions, including myocardial infarction (miR-208, miR-1, miR-133a and miR-21)(27), hepatic steatosis and hepatic injury (miR-122)(28), and hypertension (Let-7e)(29) or reduced, such as miR-126 in type 2 diabetes mellitus(30); therefore they can be used as disease biomarkers. Finally, Vickers, et al.(26) have also recently found miRNAs associated with lipoproteins. Interestingly, the HDL miRNA profile of normal subjects is significantly different from that of familial hypercholesterolemia subjects.(26)

A multi-biomarkers panel consisting of biomarkers capturing different levels of information (e.g., miRNAs to assess endothelial and platelet activation, molecular lipid species to profile metabolic status, and proteolytic degradation products to assess vascular integrity) could outperform inflammatory biomarkers without vascular specificity in their ability of predicting cardiovascular risk. As atherosclerosis develops over decades, different biomarkers may be required for different stages of disease. Thus far, there is no simple blood test to directly assess the health of blood vessels or identify vulnerable patients.(31)

Given the ever-expanding number of noncoding RNAs, understanding their function represents a formidable task. Technologies, such as metabolomics and proteomics, allow a more comprehensive assessment of miRNA effects and provide exciting opportunities for new pathogenetic insights into cardiovascular diseases.(30) Novel therapeutic strategies will face the major challenge of developing standardized methods for miRNA inhibition that combine high transfection efficiency with targeted delivery.(32)

miRNAs in Lipid Metabolism

HDL play a central role in systemic cholesterol homeostasis by stimulating the efflux of excess cellular cholesterol and transporting it to the liver for biliary excretion. HDL has long been touted as the “good cholesterol” because of the strong inverse correlation of plasma HDL cholesterol levels with coronary heart disease.(33)

Reverse cholesterol transport (RCT), is a multistep process, beginning with the hydrolysis of cytoplasmic lipid droplet-associated cholesteryl esters by neutral cholesteryl ester hydrolases and/or autophagy mediated lysosomal acid lipase.(34) The resulting free cholesterol is then effluxed from the cell by passive diffusion of cholesterol, as well as active cholesterol transfer onto lipid-poor apoA-I and HDL by the adenosine triphosphate (ATP) binding cassette transporters A1 (ABCA1) and G1 (ABCG1), respectively. Not only are the ABC transporters required for active macrophage cholesterol efflux, but ABCA1 is essential for HDL biogenesis in the liver, while ABCA1 and ABCG1 have discrete and important roles in the maintenance of mature HDL in the plasma.(35)

Each of the steps noted above represent points of control of the HDL pathway. At the transcriptional level, the liver X receptors (LXRs) coordinate the cellular response to excess cholesterol by upregulating the expression of several genes in this pathway (e.g., ABCA1, ABCG1).(36) Therapeutic strategies to harness HDL’s protective effects have to date focused on enhancing (e.g., LXR, ABCA1) or
inhibiting (e.g., Cholesteryl ester transfer protein (CETP)) these factors. (37)

Multiple genes in the HDL pathway have now been shown to be under control of these miRNAs, including those affecting HDL biogenesis, cellular cholesterol efflux, selective cholesterol uptake from HDL, and bile transport (Figure 1). These studies have revealed how single miRNA (e.g., miR-33) can target multiple components of this pathway, and also identified key genes that are under control of multiple miRNAs (e.g., ABCA1). Adding to this complexity, HDL particles have also been shown to transport extracellular miRNAs, raising the possibility that HDL’s miRNA cargo may influence its many functions, including its ability to promote RCT and enhance vascular function, as well as its anti-inflammatory and anti-thrombotic effects. (33) miR-122 was the first miRNA to be identified as having a role in lipid metabolism. Nearly a decade ago, it was reported that miR-122 was abundantly expressed in the liver and was highly conserved across species, hinting at an important role for this miRNA in hepatic function. (38)

miR-122 plays important roles in a wide variety of liver functions, ranging from cholesterol metabolism, liver cancer, stress responses, and viral infection to circadian regulation of hepatic genes. (39-44) Two pioneering studies have shown that antisense targeting of miR-122 results in a significant reduction of plasma cholesterol levels. (39,45)

The first study shows that the effect on plasma cholesterol results most likely from decreased expression of many cholesterol biosynthetic genes, including 3-hydroxy-3-methylglutarylcoenzyme A reductase (HMGCR), the rate-limiting enzyme in the cholesterol biosynthesis pathway. (45)

The second study implements a similar antisense technology (2'-O-methoxyethyl phosphorothioate antisense oligonucleotides) against miR-122 in mice and not only confirms the effect on plasma cholesterol, but also reports a significant decrease in plasma triglycerides (TGs), as well as decreased hepatic steatosis, in high-fat diet-fed mice. (39) Altogether, these results demonstrate that miR-122 plays an important role in regulating serum cholesterol and TG levels by controlling cholesterol biosynthesis and very-low-density lipoprotein secretion in the liver. (3)

In 2010, several groups independently identified miR-33, an evolutionarily conserved miRNA, as a key regulator of cholesterol and fatty acid homeostasis. (46-48) miR-33 consists of 2 intronic miRNAs, miR-33a and miR-33b, which are encoded within the introns of the Sterol

Figure 1. Regulation of HDL homeostasis by miRNAs. (33) (Adapted with permission from the American Society for Biochemistry and Biomolecular Biology, Inc.).
Regulatory Element-Binding Protein (Srebp) 1 and Srebp2 genes, respectively.(46–50) Although miR-33a and miR-33b share their target activities, they differ in their patterns of evolutionary conservation.

Specifically, miR-33a has been shown to target genes involved in cholesterol export, such as the ABC transporters ABCA1 and ABCG1 (46–48) and the endolysosomal transport protein Niemann-Pick C1 (Npc1). (48) In agreement with the regulation of ABCA1 by miR-33, modulation of miR-33a levels results in encompassing effects in cholesterol efflux in macrophages, thus suggesting that miR-33 may participate in the regulation of HDL levels in vivo. Importantly, miR-33a and miR-33b contribute to the regulation of fatty acid metabolism by controlling the expression of carnitine O-octanyl transferase (Crot), carnitine palmitoyltransferase 1A (Cpt1a), and hydroxyacyl-coenzyme A dehydrogenase/3-ketoacyl-coenzyme A thiolase/enoyl-coenzyme A hydratase (trifunctional protein) β-subunit.(49,50)

In addition to the regulation of fatty acid oxidation, miR-33a and miR-33b have also been shown to control the expression of adenosine monophosphate (AMP)-activated kinase (AMPKα1) and sirtuin 6 (Sirt6), which are involved in the regulation of lipid and glucose metabolism.(49) AMPKα1 regulates key lipogenic enzymes, including HMGCR and acetyl-CoA carboxylase (ACC). Thus, inhibition of AMPKα1 by miR-33 may increase HMGCR and ACC to boost intracellular levels of cholesterol and fatty acids. Altogether, these results suggest a paradigm in which miR-33a and miR-33b act in concert with their host genes, Srebp1 and Srebp2, to increase intracellular cholesterol and fatty acid levels by balancing transcriptional induction and post-transcriptional repression of lipid metabolism genes. (3)

Finally, insulin receptor substrate 2 (Irs2), an adaptor protein that controls insulin signaling in the liver, has also been shown to be a miR-33 target, thereby affecting the signaling of a complex downstream network of proteins, including protein kinase B (also known as Akt) phosphorylation and forkhead box O1 cytoplasmic localization.(49) Collectively, these data indicate that both isoforms of miR-33 participate in the regulation of relevant pathways that impact 3 of the primary risk factors of metabolic syndrome, namely insulin resistance, low HDL, and high very-low-density lipoprotein, and suggest that anti–miR-33 therapies may be an an attractive approach for treating metabolic diseases.(3,32,51)

Additional miRNAs (miR-106, miR-758, miR-26, miR-370, miR-378/378*, let-7, miR-27, miR34a, and miR-335) have been described to participate in the regulation of lipid metabolism. Among them, miR-758, miR-26, and miR-106b have been shown to regulate cellular cholesterol efflux by targeting ABCA1 in macrophages, hepatocytes, and neuronal cell lines, therefore indicating that the post-transcriptional regulation of ABCA1 expression is mediated by multiple miRNAs.(52–54) miR-370 has been shown to reduce fatty acid β-oxidation via its targeting activity toward Cpt1a.(55) In addition, miR-370 appears to participate in the regulation of miR-122 by increasing the expression of lipogenic genes, including Srebp1 and diacylglycerol acyltransferase (Dgat) 2.(55)

HDL research is rapidly evolving. The decades old therapeutic endeavor of raising HDL-cholesterol to confer cardioprotection has shifted focus toward increasing HDL flux and functionality. The recently discovered, prevailing effects of miRNAs on HDL homeostasis have opened new avenues to achieve this.(33)
MiR-34a and miR-146a are elevated in pancreatic islets from diabetic obese mice and significantly affect the survival of β-cells and insulin exocytosis. Activation of p53 upregulated miR-34a. The latter was proposed to mediate β-cell apoptosis and to impair nutrient-induced insulin secretion. Inhibition of miR-34a and miR-146 could partially rescue the apoptotic response but failed to restore normal insulin secretion.

Changes in cellular cholesterol content affect insulin secretion. In this regard, the ABCA1 transporter plays an important role in regulating cholesterol homeostasis in pancreatic β-cells. Indeed, β-cell–specific deletion or loss of function mutations in ABCA1 result in impaired glucose tolerance, insulin secretion, and β-cell dysfunction.

Altogether, these results suggest that miR-33 also plays an important role in regulating insulin secretion and glucose homeostasis.

Other miRNAs regulate insulin sensitivity in the liver and peripheral tissues by controlling the expression of many components of the insulin signaling pathway, including insulin-like growth factor receptor 1, insulin receptor, Irs2, phosphatidylinositol 3-kinase regulatory subunit-α (PIK3IP1), Akt2, tuberous sclerosis protein 1, caveolin-1, and rapamycin-insensitive companion of mTOR (RICTOR). Two independent groups have recently shown that the Let-7 family of miRNAs regulates glucose homeostasis and insulin sensitivity. In addition to Let-7, other miRNAs, including miR-33, miR-103, miR-107, and miR-29a/b, also regulate the insulin signaling pathway.

Overexpression of miR-107 results in an increase in fasting glucose and insulin levels. Conversely, silencing of miR-103/miR-107 enhances insulin sensitivity in the liver and in the adipose tissue. Mechanistically, miR-103/107 inhibition increases the expression of caveolin-1, a scaffold protein required for caveolae formation, and enhances insulin signaling by increasing insulin receptor stability in the cell membrane.

In summary, multiple miRNAs are able to control
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Figure 3. Pathways by which activated miR-33 may contribute to regulation of cholesterol, fatty acid and glucose metabolism, cell proliferation, and transcription.(71) (Adapted with permission from American Heart Association).

glucose metabolism by regulating a network of genes in the liver and peripheral tissues. The contribution of specific miRNAs will be determined by the tissue and metabolic state.

The therapeutic manipulation of miRNA-regulated pathways is emerging as a promising avenue for the treatment of dyslipidemia and other metabolic disorders. Given the role of miR-33a/b in repressing cholesterol efflux, fatty acid oxidation, and insulin signaling (Figure 3), pharmacological targeting of miR-33a/b may be a promising strategy to treat metabolic syndrome.(71) Major goals in the pursuit of novel therapies to target this residual risk have focused on raising levels of HDL to exploit its atheroprotective functions, lowering TGs, and improving insulin signaling. Whether miR-33 could be such a panacea awaits future studies.

miRNAs in Vascular Smooth Muscle Cells (VSMCs)

Recently, the function of miRNAs in the setting of vascular disease has gained increasing interest at both the basic science and translational levels.(72) The phenotype of VSMCs is dynamically regulated in response to various stimuli. In a cellular process known as phenotype switching, VSMCs alternate between a contractile and synthetic phenotype state. Deregulation of phenotype switching is associated with vascular disorders such as atherosclerosis, restenosis after angioplasty, and pulmonary hypertension. (73)

Vascular injury results in the activation of several mediators of VSMC phenotype, including growth factors and transcription factors, which act together to phenotypically alter VSMCs from a contractile to a synthetic state. The result is vessel remodeling mediated through multiple mechanisms including VSMCs proliferation and migration accompanied by inflammation and extracellular matrix deposition.(74,75)

Recently, miRNAs have been implicated in the regulation of VSMC phenotype through the modulation of transcription factors and other signaling molecules involved in proliferation and migration.(76-81) miR-143 and miR-145 are bicistronic miRNAs clustered on human chromosome 5. These miRNAs are enriched in VSMCs and implicated in the maintenance of a contractile VSMC phenotype. (76-78,82,83) Earlier studies demonstrated a reduction in the expression of miR-143 and miR-145 following acute vascular injury and the observation that inhibition of neointimal formation and promotion of contractile gene expression could be achieved by elevating the expression of these miRNAs.(76,77,82) Furthermore, miR-143 and miR-145 knockout mice are hypotensive and display abnormally
thin vessel walls and low actin stress fibre expression, indicating the importance of these miRNAs in fundamental smooth muscle cell (SMC) maintenance in vivo.(78,82-84)

Hergenreider, et al(85) presented a novel mechanism of VSMCs maintenance by miR-143 and miR-145. This was via miRNA-mediated cell–cell communication between VSMCs and the endothelium. Kruppel-like factor 2 (Klf2), a transcription factor induced in ECs in response to shear stress, was shown to directly bind to a putative Klf2-binding site in the miR-143/miR-145 promoter, activating transcription in human umbilical vein ECs (HUVECs).

Serum response factor (SRF), along with its cofactors myocardin and myocardin-related transcription factors (MRTFs), is a major regulator of VSMC biology through binding to CArG box elements in the promoter regions of contractile genes and promoting gene expression.(86) SRF myocardin association has previously been shown to activate miR-143/miR-145 transcription by binding to the CArG box located approximately 5 kb upstream of the miR-143/miR-145 cluster.(76,78) More recently, signaling molecules such as transforming growth factor-β (TGFβ) and bone morphogenetic protein 4 (BMP4) were shown in pulmonary artery smooth muscle cells (PASMCs) to promote the expression of miR-143/miR-145 by independent signaling mechanisms involving alteration of expression of SRF cofactors myocardin and MRTFs.(87)

miR-143 was recently shown to be an important factor in the signaling pathway which promotes the expression of phosphatase and tensin homolog (PTEN).(88) Loss of PTEN expression in SMCs in vivo has previously been associated with an increase in inflammation and proliferation, resulting in a larger neointimal formation in response to vascular injury.(89) Taken together, these studies reveal that several of the major regulators of VSMC phenotype, that is, TGFβ, BMP4 and SRF-myocardin/MRTF, all regulate contractile gene expression through diverse signaling pathways which are dependent, at least in part, on the promotion of miR-143/miR-145 expression.

The role of miR-21 in the vasculature appears to be dependent on cell type with several studies reporting miR-21 to have prosynthetic and procontractile qualities depending on the context of the expression.(90-93) Recently, miR-21 expression was reported to be upregulated approximately 8-fold in human arteries presenting with arteriosclerosis. (91) Platelet-derived growth factor (PDGF)-induced human artery SMC proliferation and migration was shown to be significantly attenuated by miR-21 inhibition.

It is clear from these studies that miR-21 is important in the maintenance of VSMC phenotype both in health and disease. However, the exact role of miR-21 appears to depend on the microenvironment and cells responsible for the disease pathology. Targeting the diverse pathways involved in the modulation of VSMC phenotype is therefore a possibility to treat a number of diseases including atherosclerosis, vein graft failure and in-stent restenosis. (94)

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**miRNAs in Vascular ECs**

Many miRNAs have been described to play a key role in the cardiovascular system, controlling virtually all cellular processes.(95,96) The regulated response of ECs to signals in their environment is not only critical for the *de novo* formation of primordial vascular networks during early development (i.e., vasculogenesis), but is also required for the subsequent growth and remodeling of new blood vessels from preexisting ones (i.e., angiogenesis). VEGFs and their EC-specific receptors play a crucial role in nearly all aspects of blood vessel growth.(97)

Angiogenesis is a very tightly controlled process, in which ECs need to migrate and proliferate toward ischemic tissue. A long-known factor that provides a gradient for ECs to migrate toward is VEGF (98). Binding of VEGF to VEGF receptor 1 (fms-related tyrosine kinase 1 (FLT1)) does not result in proangiogenic signaling, which raised the concept that FLT1 acts as a trap or decoy for VEGF. Thus, FLT1 can negatively regulate VEGF signaling, and this is of crucial importance, for instance, to keep the cornea avascular,(99), but also aids in controlling the fine balance between pro- and anti-angiogenic factors.(100)

More recently, specific miRNAs with a role in angiogenesis have been identified, including miR-126, members of the miR-17-miR-92 cluster(101,102), and members of the miR-23-miR-27-miR-24 cluster(103-105). The current study by the Srivastava laboratory identifies that miR-10 is capable of modulating FLT1 levels, thereby affecting VEGF signaling and angiogenesis. Nitric oxide (NO) generated and released by endothelial NO synthase (eNOS) exerts multiple beneficial functions in vessels and plays a critical role in maintaining cardiovascular homeostasis.(106) Dysregulation of NO synthesis attributable to the abnormal activity or eNOS expression or both has been considered to be a major contributor to the pathogenesis of vascular diseases, such as hypertension and atherosclerosis.(107,108)

Because miRNAs inhibit gene expression through binding to the 3’ untranslated regions of their target mRNAs, miRNAs may be the important post-transcriptional modulators of eNOS expression. eNOS is a direct target of miR-155. Overexpression of miR-155 decreased, whereas inhibition of miR-155 increased. Inflammatory cytokines

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including tumor necrosis factor (TNF)α increased miR-155 expression. Inhibition of miR-155 reversed TNFα-induced downregulation of eNOS expression and impairment of endothelium-dependent vasorelaxation. Thus, Inhibition of miR-155 may be a new therapeutic approach to improve endothelial dysfunction during the development of cardiovascular diseases.(109)

Inflammation plays an essential role in vascular pathologies, including those associated with sepsis and atherosclerosis. In ECs, miR-18 1b plays a vital role in controlling inflammation by targeting importinα3, a regulator of NFκB nuclear import. These findings provide compelling evidence that modulation of miRNAs may be a useful therapeutic approach for inflammatory vascular diseases.(110) Grundmann, et al. demonstrate that miR-100 has an anti-angiogenic function and represses mTOR signaling in endothelial and VSMCs. Inhibition of miR-100 could be a novel approach for the modulation of blood vessel growth and other mTOR-dependent processes.(111) The adaptive growth of blood vessels is an important protective mechanism in patients with chronic vascular occlusive disease. The progressive occlusion of a major artery results in hemodynamic changes and downstream tissue ischemia, which induce both the proliferation of small preexisting collateral arteries and capillary sprouting in ischemic tissue. (112)

Atherosclerosis is a multifactorial disease driven, in part, by chronic inflammation in response to cholesterol accumulation in the arterial wall.(74) The first major event in the progression of the early atheroma is the loss of endothelial integrity. Endothelium dysfunction facilitates the subendothelial accumulation of cholesterol-bearing lipoproteins, compromises vasodilation, and is both proinflammatory and prothrombotic.(113,114) Circulating endothelial progenitor cells have been demonstrated to play an integral role in endothelial integrity due to their ability to reinforce the endothelium with new healthy ECs to replace damaged or apoptotic cells.(115,116) In a recent study, individuals with atherosclerosis, as defined by CAD, showed significantly higher expression of miR-221 and miR-222 in endothelial progenitor cells (EPC) compared with non-CAD individuals.(117) Statins, inhibitors of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, have previously been shown to increase circulating EPC numbers in individuals with CAD. (118,119) Consistent with these observations, atorvastatin was shown to decrease miR-221 and miR-222 expression in EPCs (117). The implications of this study are of significant merit as they illuminate miRNAs as possible mediators of statins’ observed pleiotropic beneficial effects. Collectively,
these studies suggest that miRNAs may have numerous roles in angiogenesis and endothelium integrity, both of which significantly contribute to the development and maturation of the atherosclerotic plaque.(120)

Atherosclerosis is a condition caused by lipid-induced inflammation of the vessel wall orchestrated by a complex interplay of various cell types, such as ECs, SMCs and macrophages. Downregulation of miR-145, which controls differentiation of SMCs, promotes lesion formation, whereas the EC-specific miR-126 signals the need for endothelial repair through its transfer from apoptotic ECs in microvesicles. Elevated miR-155 levels are characteristic of proinflammatory macrophages and atherosclerotic lesions. (121) Patients with CAD have reduced levels of miR-126, miR-145, and miR-155 in the circulation.(122) Moreover, decreased levels of miR-126 in patients with diabetes mellitus or insulin resistance have been reported.(30) These studies indicate that circulating levels of miR-126, miR-145, and miR-155 may indicate the presence or absence of atherosclerosis or endothelial dysfunction and thus may play a role as novel biomarkers.(123)

A therapeutic strategy based on the functional role of miR-126, miR-145 and miR-155 in atherosclerosis would need to increase the levels of miR-126 and miR-145, and to inhibit miR-155 in macrophages at least in advanced stages of atherosclerosis. miRNAs mimics need to be packaged in liposomes or nanoparticles for therapeutic delivery.(124) Alternatively, the endogenous packaging of miR-126 in apoptotic bodies and of miR-143/miR-145 in shear stress-induced microvesicles may provide a template for bioengineered vesicles for the therapeutic delivery of miRNAs.(6,85) Accordingly, treatment with Klf2-induced endothelial microvesicles containing miR-143/145 effectively reduced atherosclerosis.(85)

miRNAs in Myocardial Infarction

Acute myocardial damage attributable to ischemia is a result of cellular hypoxia and the subsequent cascade of cellular events, such as an increase in reactive oxygen species during early reperfusion, EC activation, and production of proinflammatory chemokines and cytokines in the damaged area, ultimately priming and recruiting neutrophils and other inflammatory cells to the infarcted region.(125,126) The cascade of maladaptive signaling triggers further release of oxidants and proteolytic enzymes(127), leading to infarct size extension, cardiomyocyte death, and endothelial capillary impairment.

Myocardial infarction (MI) is characterized by strongly altered gene expression, deregulation of underlying signaling pathways, and crucial participation of several miRNAs in this context. Mechanistically, miRNA induction or repression after myocardial infarction triggers downstream events in a cell-type–specific manner, and interference with endogenous miRNA expression might regulate overall cardiac function.(5) In addition, several studies indicated a crucial role for miRNA – dependent regulation of cardiac angiogenesis, fibrosis, and cardiomyocyte hypertrophy upon MI.(101,105,128,129) These observations clearly link cardiac ischemic disease to altered miR expression. However, miRNA deregulation also offers a new therapeutic entry point to counteract MI-induced cardiac dysfunction. (5)

Cardiac injury as it occurs after acute myocardial infarction increases the circulating levels of several myocardial-derived miRNAs (eg, miR-1, miR-133, miR-499, miR-208), whereas patients with CAD or diabetes showed reduced levels of endothelial-enriched miRNAs, such as miR-126.(123) Several groups have studied the hypothesis that heart-specific miRNAs leak into the circulation during an acute myocardial infarction (AMI) and can be used to detect and monitor myocardial injury. Four cardiac miRNAs (miR-208a, miR-499, miR-1, and miR-133) are found to be consistently elevated in plasma of AMI patients within hours after the onset of infarction.(130-138) Of these 4 miRNAs, miR-208a, which is encoded by an intron of the αMHC gene, is to the best of our knowledge the only heart-specific miRNA.(139) The other 3 miRNAs (miR-499, miR-1, and miR-133), besides being highly expressed in the heart, are also expressed in skeletal muscle. (140,141) Another miRNA downregulated after murine ischemia–reperfusion injury and in human myocardial infarction is miR-494. Likewise, myocardial infarct size was significantly reduced in transgenic hearts on ischemia–reperfusion injury when compared with wild-type hearts. Thus miR-494 might constitute an interesting target for the treatment of ischemic heart disease.(142)

Overexpression of miR-320 enhanced cardiomyocyte death and apoptosis, whereas its knockdown was cytoprotective.(143) In line, transgenic mice with cardiac-specific overexpression of miR-320 showed an increased extent of apoptosis and infarction size on ischemia–reperfusion injury, whereas in vivo treatment with an antagonist against miR-320 reduced infarct size.(143) The miR-21 is also highly expressed in cardiac fibroblasts, where it improves cell survival, leading to enhanced – cardiac fibrosis. During chronic cardiac remodeling, inhibition of miR-21 via specific antagonists attenuated fibrosis development and improved cardiac function.(23)

Treatment of MI and its consequences is an enormous task and needs careful consideration. Besides the use of
standard pharmacological approaches, miRNA-based therapies offer new challenging avenues. The identification of MI-associated miRNAs is of great interest to develop miRNA therapeutics, and herein discussed miRNAs offer certain therapeutic value.

**Conclusion**

In summary, miRNA profiling and functional testing will certainly play a significant role in future cardiovascular science discovery expedited by the rapid development of novel strategies and tools for working with miRNAs. The extensive impact of miRNA—mediated gene regulation and the relative ease of *in-vivo* applicable modifications highlight the enormous potential of miRNA-based therapeutics in cardiometabolic diseases.

**References**

1. Lim LP, Lau NC, Garrett-Engele P, Grimson A, Schelter JM, Castle J, et al. Microarray analysis shows that some microRNAs downregulate large numbers of target mRNAs. Nature. 2005; 433: 769–73.
2. Van Rooij E. Introduction to the series on MicroRNAs in the cardiovascular system. Circ Res. 2012; 110: 481–2.
3. Fernandez-Hernando C, Ramirez CM, Goedeke L, Suarez Y. MicroRNAs in metabolic disease. Arterioscler Thromb Vasc Biol. 2013; 33: 178–85.
4. Weber C. MicroRNAs from basic mechanisms to clinical application in cardiovascular medicine. Arterioscler Thromb Vasc Biol. 2013; 33: 168–9.
5. Fiedler J, Thum T. MicroRNAs in myocardial infarction. Arterioscler Thromb Vasc Biol. 2013; 33: 201–5.
6. Zernecke A, Bidzhekov K, Noels H, Shagdarsuren E, Gan L, Denecke B, et al. Delivery of microRNA-126 by apoptotic bodies induces CXCL12-dependent vascular protection. Sci Signal. 2009; 2: ra81.
7. Abdellatif M. Differential expression of microRNAs in different disease state. Circ Res. 2012; 110: 638–50.
8. Zile MR, Mehurg SM, Arroyo JE, Stroud RE, DeSantis SM, Spinale FG. Relationship between the temporal profile of plasma microRNA and left ventricular remodeling in patients after myocardial infarction. Circ Cardiovasc Genet. 2011; 4: 614–9.
9. Mukherji S, Ebert MS, Zheng GX, Tsang JS, Sharp PA, van Oudenaarden A. MicroRNAs can generate thresholds in target gene expression. Nat Genet. 2011; 43: 854–9.
10. Stark A, Brennecke J, Bushati N, Russell RB, Cohen SM. Animal microRNAs confer robustness to gene expression and have a significant impact on 3’UTR evolution. Cell. 2005; 123: 1133–46.
11. Bartel DP, Chen CZ. Micromanager genes: the potentially widespread influence of miRNAs on metazoan microRNAs. Nat Rev Genet. 2004; 5: 396–400.
12. Farh KK, Grimson A, Jan C, Lewis BP, Johnston WK, Lim LP, et al. The widespread impact of mammalian microRNAs on mRNA repression and evolution. Science. 2005; 310: 1817–21.
13. Ambros V. The functions of animal microRNAs. Nature. 2004; 431: 350–5.
14. Selbach M, Schwannhäuser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. Nature. 2008; 455: 58–63.
15. Baek D. The impact of microRNAs on protein output. Nature. 2008; 455: 64–71.
39. Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, et al. miR-122 regulation of lipid metabolism revealed by *in vivo* antisense targeting. Cell Metab. 2006; 3: 87–98.

40. Hsu SH, Wang B, Kota J, Yu J, Costinean S, Kutay H, et al. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. J Clin Invest. 2012; 122: 2871-83.

41. Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science. 2010; 327: 198–201.

42. Tsai WC, Hsu SD, Hsu CS, Lai TC, Chen SJ, Shen R, et al. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. J Clin Invest. 2012; 122: 2884–97.

43. Gafitfield D, Le Martelot G, Vajne CR, Gerlach D, Schaad O, Fleury-Olela F, et al. Integration of microRNA miR-122 in hepatic circadian gene expression. Genes Dev. 2009; 23: 1313–26.

44. Kojima S, Gatfield D, Esau CC, Green CB. MicroRNA-122 modulates the rhythmic expression profile of the circadian deadenylase Nocturnin in mouse liver. PLoS ONE. 2010; 5: e11264.

45. Kritzfeldt J, Rajewsky N, Braich R, Rajev KG, Tsuich T, Manoharan M, et al. Silencing of microRNAs in vivo with ‘antagomirs’. Nature. 2005; 438: 685–9.

46. Marquart TJ, Allen RM, Ory DS, Baldán A. miR-33 links SREBP-2 induction to repression of sterol transporters. Proc Natl Acad Sci USA. 2010; 107: 12228-32.

47. Najafi-Shoushtari SH, Kristo F, Li Y, Shioda T, Cohen DE, Gerszten RE, et al. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis and insulin sensitivity. Proc Natl Acad Sci USA. 2011; 108: 21075-80.

48. Najafi-Shoushtari SH, Kristo F, Li Y, Shioda T, Cohen DE, Gerszten RE, et al. MicroRNA-33 and the SREBP host genes cooperate to control cholesterol homeostasis and insulin sensitivity. Proc Natl Acad Sci USA. 2011; 108: 21075-80.

49. Dávalos A, Goedeke L, Smibert P, Ramírez CM, Warrier NP, Andreo U, et al. MicroRNA-33a regulates ABCA1 expression, cholesterol accumulation, and insulin secretion in pancreatic islets. Diabetes. 2012; 61: 653–8.

50. Rayner KJ, Suárez Y, Dávalos A, Parathath S, Fitzgerald ML, Tamehiro M, et al. Silencing of microRNAs in vivo with ‘antagomirs’. Nature. 2005; 438: 685–9.

51. Fernandez-Hernando C, Suarez Y, Rayner KJ, Moore KJ. MicroRNAs in lipid metabolism. Curr Opin Lipidol. 2011; 22: 86-92.

52. Krumhansl JT, Allen RM, Ory DS, Baldán A. miR-33 links SREBP-2 induction to repression of sterol transporters. Proc Natl Acad Sci USA. 2010; 107: 12228-32.

53. Ramírez CM, Dávalos A, Goedeke L, Salerno AG, Warrier N, Cirera-Salinas D, et al. MicroRNA-758 regulates cholesterol efflux through regulating tropomyosin 1. Arterioscler Thromb Vasc Biol. 2011; 31: 1941-2.

54. Gerin I, Clerbaux LA, Haumont O, Lanthier N, Das AK, Burant CF, et al. Expression of miR-33 from an SREBP2 intron inhibits cholesterol export and fatty acid oxidation. J Biol Chem. 2010; 285: 33652–61.

55. Fernandez-Hernando C, Suarez Y, Rayner KJ, Moore KJ. MicroRNAs in lipid metabolism. Curr Opin Lipidol. 2011; 22: 86-92.

56. Kim J, Yoon H, Ramírez CM, Lee SM, Hoe HS, Fernández-Hernando C, et al. MicroRNA-106b impairs cholesterol efflux and increases Aβ levels by repressing ABCA1 expression. Exp Neurol. 2012; 235: 476–83.

57. Ramírez CM, Dávalos A, Goedeke L, Salerno AG, Warrier N, Cirera-Salinas D, et al. MicroRNA-758 regulates cholesterol efflux through posttranscriptional repression of ATP-binding cassette transporter A1. Arterioscler Thromb Vasc Biol. 2011; 31: 2707–14.

58. Sun D, Zhang J, Xie J, Wei W, Chen M, Zhao X. MiR-26 controls LXRe-dependent cholesterol efflux by targeting ABCA1 and ARL7. FEBS Lett. 2012; 586: 1472-9.

59. Iliopoulos D, Drosatos K, Hiyama Y, Goldberg JJ, Zannis VI. MicroRNA-370 controls the expression of microRNA-122 and Cpt1alpha and affects lipid metabolism. J Lipid Res. 2010; 51: 1513–23.

60. Lynn FC, Skewes-Cox P, Kosaka Y, McManus MT, Harfe BD, German MS. MicroRNA expression is required for pancreatic islet cellogenesis in the mouse. Diabetics. 2007; 56: 2938–45.

61. Melkman-Zehavi T, Oren R, Kredo-Russo S, Shapira T, Mandelbaum AD, Rivkin N, et al. miRNAs control insulin content in pancreatic β-cells via downregulation of transcriptional repressors. EMBO J. 2011; 30: 835–45.

62. Poy MN, Eliasson L, Kutzfeld J, Kuwajima S, Ma X, Macdonald PE, et al. A pancreatic islet-specific microRNA regulates insulin secretion. Nature. 2004; 432: 226–30.

63. Poy MN, Hauers J, Trajkovski M, Braun M, Collins S, Rorsman P, et al. miR-375 maintains normal pancreatic alpha- and beta-cell mass. Proc Natl Acad Sci USA. 2009; 106: 5813–8.

64. Rayner KJ, Suárez Y, Dávalos A, Parathath S, Fitzgerald ML, Tamehiro M, et al. Silencing of microRNAs in vivo with ‘antagomirs’. Nature. 2005; 438: 685–9.
controls vascular neoimtimal lesion formation. Circ Res. 2009; 105: 158–66.
78. Xin M, Small EM, Sutherland LB, Qi X, McAnally J, Plato CF, et al. MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. Genes Dev. 2009; 23: 2166–78.
79. Chen J, Yin H, Jiang Y, Radhakrishnan SK, Huang ZP, Li J, et al. Induction of microRNA-1 by myocardin in smooth muscle cells inhibits cell proliferation. Arterioscler Thromb Vasc Biol. 2011; 31: 368–75.
80. Liu X, Cheng Y, Zhang S, Lin Y, Yang J, Zhang C. A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. Circ Res. 2009; 104: 476–87.
81. Ji R, Cheng Y, Yue J, Yang J, Liu X, Chen H, et al. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of microRNA in vascular neoimtimal lesion formation. Circ Res. 2007; 100: 1579–88.
82. Elia L, Quintavalle M, Zhang J, Contu R, Cossu L, Latronico MV, et al. The knockout of miR-143 and -145 alters smooth muscle cell maintenance and vascular homeostasis in mice: correlates with human disease. Cell Death Differ. 2009; 16: 1590–8.
83. Boettger T, Beetz N, Kostin S, Schneider J, Krüger M, Hein L, et al. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster. J Clin Invest. 2009; 119: 2634–47.
84. Quintavalle M, Elia L, Condorelli G, Courteidege SA. MicroRNA control of podosome formation in vascular smooth muscle cells in vivo and in vitro. J Cell Biol. 2010; 189: 13–22.
85. Hergenreider E, Heydt S, Tréguer K, Boettger T, Horrevoets AJ, Zeiher AM, et al. Atherosprotective communication between endothelial cells and smooth muscle cells through miRNAs. Nat Cell Biol. 2012; 14: 249–56.
86. Miano JM, Long X, Fujiwara K. Serum response factor: master regulator of the actin cytoskeleton and contractile apparatus. Am J Physiol Cell Physiol. 2007; 292: C70–81.
87. Davis-Dusenbery BN, Chan MC, Reno KE, Weisman AS, Layne MD, Lagna G, et al. Down-regulation of Kruppel-like factor-4 (KLF4) by microRNA-143/145 is critical for modulation of vascular smooth muscle cell phenotype by transforming growth factor-beta and bone morphogenetic protein 4. J Biol Chem. 2011; 286: 28097–110.
88. Horita HN, Simpson PA, Ostriker A, Furgeson S, Van Putten V, Weiser-Evans MC, et al. Serum response factor regulates expression of phosphatase and tensin homolog through a micro-RNA network in vascular smooth muscle cells. Arterioscler Thromb Vasc Biol. 2011; 31: 2909–19.
89. Nemenoff RA, Horita H, Ostriker AC, Furgeson SB, Simpson PA, VanPutten V, et al. SDF-1α induction in mature smooth muscle cells by inactivation of PTEN is a critical mediator of exacerbated injury-induced neoimtimal formation. Arterioscler Thromb Vasc Biol. 2011; 31: 1300–8.
90. Kang H, Davis-Dusenbery BN, Nguyen PH, Lal A, Lieberman J, Van Aelst L, et al. Bone morphogenetic protein 4 promotes vascular smooth muscle cell contractility by activating microRNA-21 (miR-21), which down-regulates expression of family of dedicator of cytokinesis (DOCK) proteins. J Biol Chem. 2012; 287: 3976–86.
91. Wang M, Li W, Chang QQ, Ye CS, Ou JS, Li XX, et al. MicroRNA-21 regulates vascular smooth muscle cell function via targeting tropomysin 1 in arteriosclerosis obliterans of lower extremities. Arterioscler Thromb Vasc Biol. 2011; 31: 2044–53.
92. Yang S, Banerjee S, Freitas AD, Cui H, Xie N, Abraham E, et al. miR-21 regulates chronic hypoxia-induced pulmonary vascular remodeling. Am J Physiol Lung Cell Mol Physiol. 2012; 302: L521–9.
93. Caruso P, MacLean MR, Khanin R, McClure J, Soon E, Southgate M, et al. Dynamic changes in lung microRNA profiles during the development of pulmonary hypertension due to chronic hypoxia and monocrotaline. Arterioscler Thromb Vasc Biol. 2010; 30: 716–23.
94. Robinson HC, Baker AH. How do microRNAs affect vascular smooth muscle cell biology? Curr Opin Lipidol. 2012; 23: 405-11.
95. Bonauer A, Boon RA, Dimmeler S. Vascular microRNAs. Curr Drug Targets. 2010; 11: 943–9.
96. Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. Nature. 2011; 469: 336–42.
97. Dang LH, Lawson ND, Fish JE. MicroRNA control of vascular endothelial growth factor signaling output during vascular development. Arterioscler Thromb Vasc Biol. 2013; 33: 193-200.
98. Ferrara N. Vascular endothelial growth factor. Arterioscler Thromb Vasc Biol. 2009; 29: 789–91.
99. Ambati BK, Nozaki M, Singh N, Takeda A, Jani PD, Suthar T, et al. Corneal avascularity is due to soluble VEGF receptor-1. Nature. 2006; 443: 993–7.
100. Boon RA. MicroRNAs control vascular endothelial growth factor signaling. Circ Res. 2012; 111: 1388-90.
101. Bonauer A, Carmona G, Iwasaki M, Mione M, Koyanagi M, Fischer A, et al. MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science. 2009; 324: 1710–3.
102. Doebele C, Bonauer A, Fischer A, Scholz A, Reiss Y, Urbich C, et al. Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. Blood. 2010; 115: 4944–50.
103. Zhao Q, Gallagher R, Ufret-Vincenty R, Li X, Olson EN, Wang S. Regulation of angiogenesis and chordoidal neovascularization by members of microRNA-23–27–24 clusters. Proc Natl Acad Sci USA. 2011; 108: 8287–92.
104. Urbich C, Kaluza D, Frömel T, Knau A, Bennewitz K, Boon RA, et al. MicroRNA-27a/b controls endothelial cell repulsion and angiogenesis by targeting semaphorin 6A. Blood. 2012; 119: 1607–16.
105. Fiedler J, Jazbutyte V, Kirchmaier BC, Gupta SK, Lorenzen J, Hartmann D, et al. MicroRNA-24 regulates vascularity after myocardial infarction. Circulation. 2011; 124: 720–30.
106. Schulz R, Rassaf T, Massion PB, Kelm M, Balligand JL. Recent advances in the understanding of the role of nitric oxide in cardiovascular homeostasis. Pharmacol Ther. 2005; 108: 225–56.
107. Balligand JL, Feron O, Desy C. ENOS activation by physical forces: from short-term regulation of contraction to chronic remodeling of cardiovascular tissues. Physiol Rev. 2009; 89: 481–534.
108. Förstermann U, Münzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation. 2006; 113: 1708–14.
109. Sun XZ, Zen DY, Li RT, Pang RP, Yang H, Hu YL, et al. Essential role of microRNA–155 in regulating endothelium-dependent vasorelaxation by targeting endothelial nitric oxide synthase. Hypertension. 2012; 60: 1407-14.
110. Fish JE, Cybulsky MI. Taming endothelial dysfunction activation with a microRNA. J Clin Invest. 2012; 122: 1967-70.
111. Grundmann S, Hans FP, Kinmary S, Heinke J, Helbing T, Bluhm F, et al. MicroRNA–100 regulates neovascularization by suppression of mammalian target of rapamycin in endothelial and vascular smooth muscle cells. Circulation. 2011; 123: 999-1009.
112. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. Nat Med. 2000; 6: 389–95.
113. Widlansky ME, Gokce N, Keaney JF Jr, Vita JA. The clinical implications of endothelial dysfunction. J Am Coll Cardiol. 2003; 42: 1149–60.
114. Endemann DH, Schiffrin EL. Endothelial dysfunction. J Am Soc Nephrol. 2004; 15: 1983–92.
115. Zampetaki A, Kirkton JP, Xu Q. Vascular repair by endothelial
progenitor cells. Cardiovasc Res. 2008; 78: 413–21.
116. Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, Li T, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science. 1997; 275: 964–7.
117. Minami Y, Satoh M, Maescawa C, Takahashi Y, Tabuchi T, Itoh T, et al. Effect of atorvastatin on microRNA 221/222 expression in endothelial progenitor cells obtained from patients with coronary artery disease. Eur J Clin Invest. 2009; 39: 359–67.
118. Dimmeler S, Aicher A, Vasa M, Mildehn-Rihm C, Adler K, Tiemann C, et al. Effect of atorvastatin on microRNA 221/222 expression in endothelial progenitor cells obtained from patients with coronary artery disease. Eur J Clin Invest. 2009; 39: 359–67.
119. Vasa M, Fichtlscherer S, Adler K, Aicher A, Martin H, Zeiher AM, et al. Increase in circulating endothelial progenitor cells by statin therapy in patients with stable coronary artery disease. Circulation. 2001; 103: 2885–90.
120. Vickers KC, Remaley AT. MicroRNAs in atherosclerosis ad lipoprotein metabolism. Curr Opin Endocrinol Diabetes Obes. 2010; 17: 150-5.
121. Wei Y, Nazari–Jahantigh M, Neth P, Weber C, Schober A, et al. MicroRNA–126, -145, and –155: A therapeutic triad in metabolism. Curr Opin Endocrinol Diabetes Obes. 2010; 17: 150-5.
122. Fichtlscherer S, De Rosa S, Fox H, Schwietz T, Fischer A, Liebetrau C, et al. Circulating microRNAs in patients with coronary artery disease. Circ Res. 2010; 107: 677–84.
123. Fichtlscherer S, Zeiher A, Dimmeler S. Circulating microRNAs: Biomarkers or mediators of cardiovascular diseases?. Arterioscler Thromb Vasc Biol. 2013; 33: 449-54.
124. Wang X, Zhang X, Ren XP, Chen J, Liu H, Yang J, et al. MicroRNA-494 targeting both proapoptotic and antiapoptotic proteins protects against ischemia/reperfusion-induced cardiac injury. Circulation. 2010; 122: 1308–18.
125. Cheng Y, Tan N, Yang J, Liu X, Cao X, He P, et al. A translational study of circulating cell-free microRNA-1 in acute myocardial infarction. Clin Sci (Lond). 2010; 119: 87–95.
126. Frangogiannis NG, Smith CW, Entman ML. The inflammatory response in myocardial infarction. Cardiovasc Res. 2002; 53: 31–47.
127. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007; 87: 315–424.
128. Wang J, Huang W, Xu R, Nie Y, Cao X, Meng J, et al. MicroRNA-24 regulates cardiac fibrosis after myocardial infarction. J Cell Mol Med. 2012; 16: 2150–60.
129. Qian L, Van Laake LW, Huang Y, Liu S, Wendland MF, Srivastava D. miR-24 inhibits apoptosis and represses Bim in human cardiomyocytes. J Exp Med. 2011; 208: 549–60.
130. Wang GK, Zhu JQ, Zhang JT, Li Q, Li Y, He J, et al. Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. Eur Heart J. 2010; 31: 659–66.
131. Corsten MF, Dennert R, Jochems S, Kuznetsova T, Devaux Y, Hofstra L, et al. Circulating microRNA-208b and microRNA-499 reflect myocardial damage in cardiovascular disease. Circ Cardiovasc Genet. 2010; 3: 499–506.
132. Ji X, Takahashi R, Hiura Y, Hirokawa G, Fukushima Y, Iwai N. Plasma miR-208 as a biomarker of myocardial injury. Clin Chem. 2009; 55: 1944–9.
133. D’Aleessandra Y, Devanna P, Limana F, Straino S, Di Carlo A, Brambilla PG, et al. Circulating microRNAs are new and sensitive biomarkers of myocardial infarction. Eur Heart J. 2010; 31: 2765–73.
134. Bostjancic E, Zidar N, Stajer D, Glavac D, MicroRNAs miR-1, miR-133a, miR-133b and miR-208 are dysregulated in human myocardial infarction. Cardiology. 2010; 115: 163–9.
135. Kuwabara Y, Ono K, Horie T, Nishi H, Nagao K, Kinoshita M, et al. Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. Circ Cardiovasc Genet. 2011; 4: 446–54.
136. Adachi T, Nakamichi M, Otsuka Y, Nishimura K, Hirokawa G, Goto Y, et al. Plasma microRNA 499 as a biomarker of acute myocardial infarction. Clin Chem. 2010; 56: 1183–5.
137. Ai J, Zhang R, Li Y, Pu J, Lu Y, Jiao J, et al. Circulating microRNA-1 as a potential novel biomarker for acute myocardial infarction. Biochem Biophys Res Commun. 2010; 391: 73–7.