REVIEW ARTICLE

MicroRNAs in Cardiometabolic Diseases

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Abstract

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

Abstrak

LATAR BELAKANG: *MicroRNA* (miRNA) adalah suatu *noncoding* RNA dengan 22 nukleotida yang memiliki fungsi penting dalam berbagai proses fisiologi dan patologi. Banyaknya laporan mengenai penemuan dan karakterisasi spesies miRNA, serta keterlibatan miRNA pada hampir semua aspek biologi dan penyakit jantung telah membuka dimensi baru dalam hal jaringan regulasi gen pada perkembangan dan patogenesis jantung.

ISI: Perubahan kendali metabolisme lemak dan homeostasis glukosa pada individu dapat memicu berkembangnya penyakit kardiometabolik seperti diabetes melitus tipe 2 dan aterosklerosis. Penelitian yang dilakukan pada dasawarsa terakhir telah menunjukkan bahwa miRNA memiliki peran penting dalam pengaturan proses fisiologi ini. Selain profil faktor transkripsi spesifik sel, ekspresi gen yang diregulasi oleh miRNA mempengaruhi tujuan akhir perkembangan dan keputusan aktivasi sel. Jadi, tipe sel yang terlibat dalam aterosklerosis, penyakit vaskular, dan kerusakan miokardial mungkin diregulasi secara berbeda oleh miRNA yang berlainan, melalui proses pengaturan yang sangat rumit, misalnya fenotipe sel otot polos dan respon inflamasi sel endotel atau makrofag. Perkembangan terakhir dalam hal penggunaan miRNA sebagai biomarker atau modalitas terapi diharapkan dapat menjadi dasar yang kuat untuk penelitian lebih lanjut, sehingga dapat memperkaya pemahaman kita mengenai fungsi miRNA pada biologi kardiovaskular.

RINGKASAN: MiRNA adalah *noncoding* RNA yang kecil, berfungsi sebagai pengatur *post*-transkripsi pada ekspresi gen. MiRNA merupakan modulator potensial pada berbagai proses dan patologi biologi. Penemuan yang ada menunjukkan pentingnya peran miRNA pada vaskulatur, alur metabolisme lemak, dan homeostasis glukosa.

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

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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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)

Myocardial infarction as a common and severe manifestation of advanced atherosclerosis is characterized by altered gene expression and dysregulation of underlying signaling pathways, which may involve an induction or repression of miRNAs affecting cell-specific downstream effects on cardiac function.(5)

The differential roles of distinct miRNAs during the pathogenesis of atherosclerosis, which encompass downregulation of miR-145 controlling smooth muscle cell differentiation, delivery of miR-126 in endothelial cell (EC)-derived microparticles to signal the need for endothelial repair, or an upregulation of miR-155 relevant in proinflammatory macrophage polarization. The identification of this miRNA triad sheds light on the current concepts of atherogenesis and establishes novel treatment options.(6)

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 biological 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

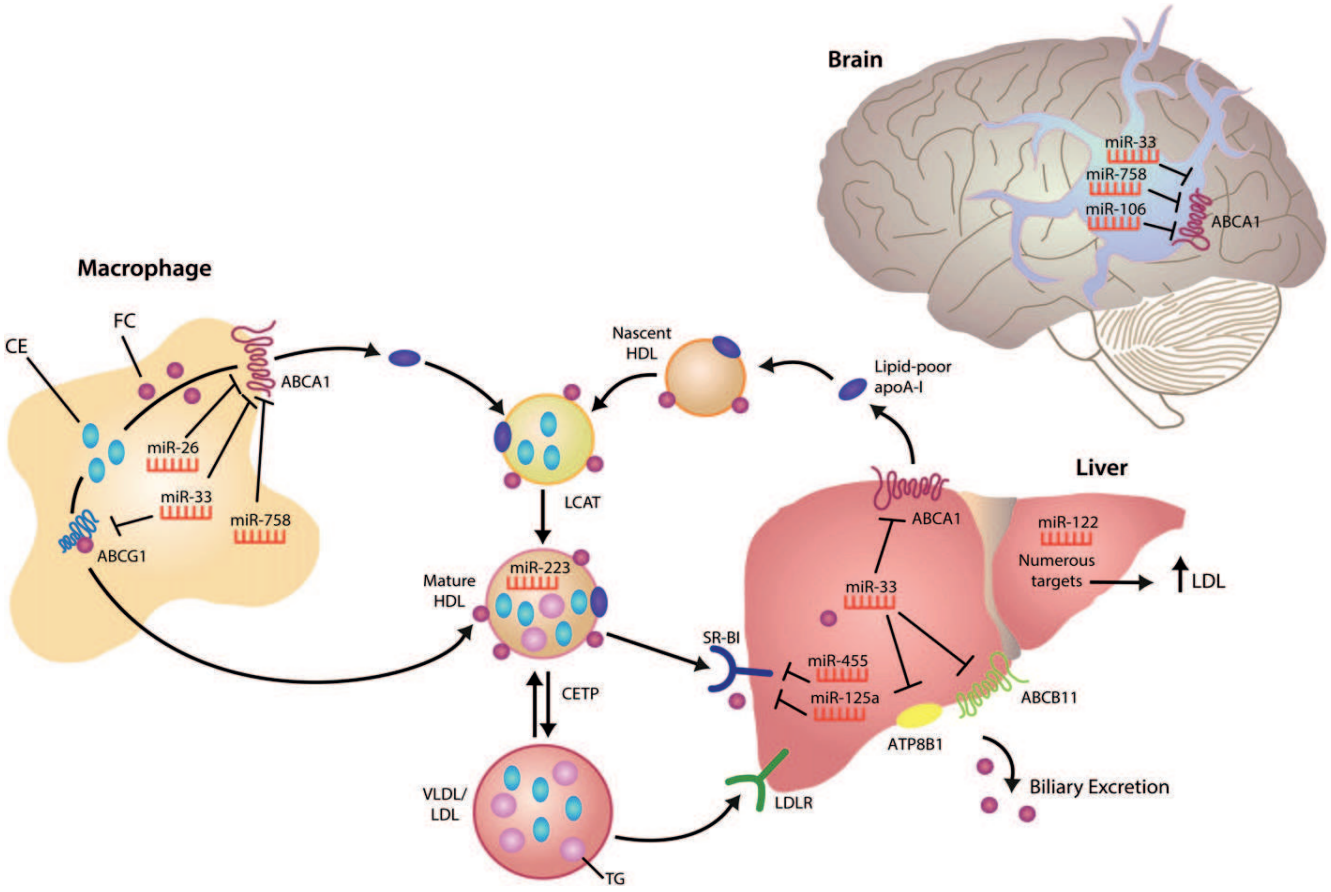

Figure 1. Regulation of HDL homeostasis by miRNAs.(33) (Adapted with permission from the American Society for Biochemistry and Biomolecular Biology, Inc.).

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 carboxilase (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)

miRNAs in Glucose Homeostasis

In addition to hormones, miRNAs have emerged as critical regulators of glucose metabolism by regulating insulin production and secretion, as well as insulin sensitivity. The global impact of miRNAs in glucose production and pancreatic β-cell functions was defined with the generation of pancreas-specific dicer knock-out mice.(56)

Dicer-deficient β-cells show a significant decrease in insulin synthesis and secretion, which is associated with the upregulation of basic helix-loop-helix family member e22 (*Bhlhe22*) and *Sox6*, 2 transcriptional repressors of the insulin gene. Interestingly, 4 miRNAs, including miR-24, miR-26, miR-182, and miR-148, regulate *Bhlhe22* and *Sox6* expression at the post-transcriptional level and are significantly down-regulated in dicer-deficient pancreatic β-cells.(57) miR-375 is one of the most abundant miRNAs in the pancreas and regulates insulin secretion independently of changes in plasma glucose levels.(58) Overexpression of miR-375 suppressed glucose-induced insulin secretion, and conversely, inhibition of endogenous miR-375 function enhanced insulin secretion suggesting that miR-375 is a negative regulator of β-cell exocytosis.(32) miR-375 also regulates the expression of a cluster of genes controlling cellular growth and proliferation, including caveolin-1 (*Cav1*), inhibitor of DNA binding 3 (*Id3*), Ras-dexometasone-induced-1 (*Rasd1*), and the human antigen D/embryonic lethal abnormal vision-like 4 (*HuD/Elavl4*). (59)

In addition to miR-375, other miRNAs have been shown to regulate insulin release, including miR-124a, miR-9, miR- 96a, and miR-33.(60-63) miR-124a regulates insulin secretion by controlling the expression of Ras

associated protein (Rab) 27a which is involved together with its effector, granuphilin/synaptotagmin-like protein 4-a (Slp4), in the exocytosis of insulin-containing secretory granules in pancreatic β-cells.(60) miR-9 is mandatory for maintaining appropriate granuphilin levels and optimal secretory capacity in β-cells. Onecut-2 (OC2) (Figure 2), the granuphilin repressor, is a direct target of miR-9.(61) Likewise, miR-96 increases granuphilin, but independently of OC2. Additionally, miR-96 decreases Noc2, a Rab effector and positive regulator of insulin secretion.(60) Rabphilins (Rab proteins) represent a family of small guanosine triphosphate (GTP)-binding proteins that facilitate exocytosis.

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.(64) 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.(65) Altogether, these results suggest that miR-33 also plays an important role in regulating insulin secretion and glucose homeostasis.(3)

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.(66,67) In addition to Let-7, other miRNAs, including miR-33, miR-103, miR-107, and miR-29a/b, also regulate the insulin signaling pathway.(49,68-70)

Overexpression of miR-107 results in an increase in fasting glucose and insulin levels.(70) 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.(70)

In summary, multiple miRNAs are able to control

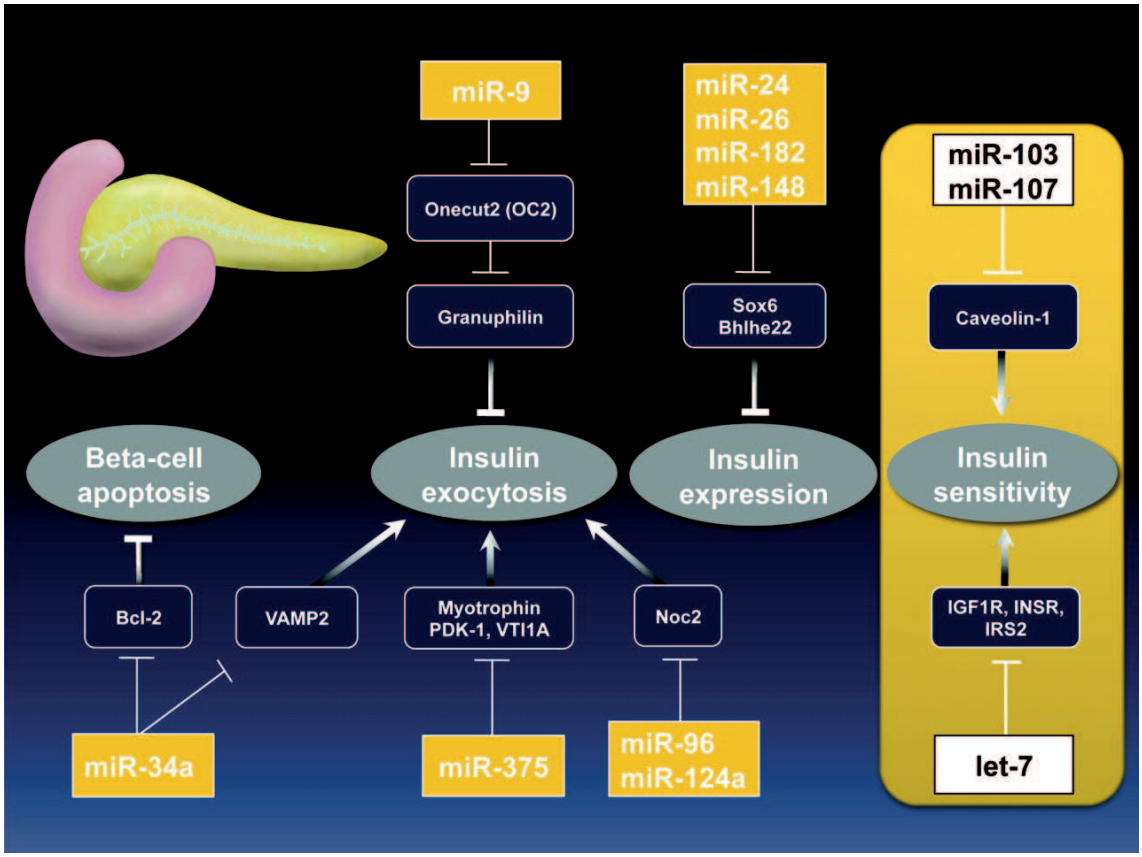

Figure 2. MiRNAs involved in glucose homeostasis. (32) (Adapted with permission from American Heart Association).

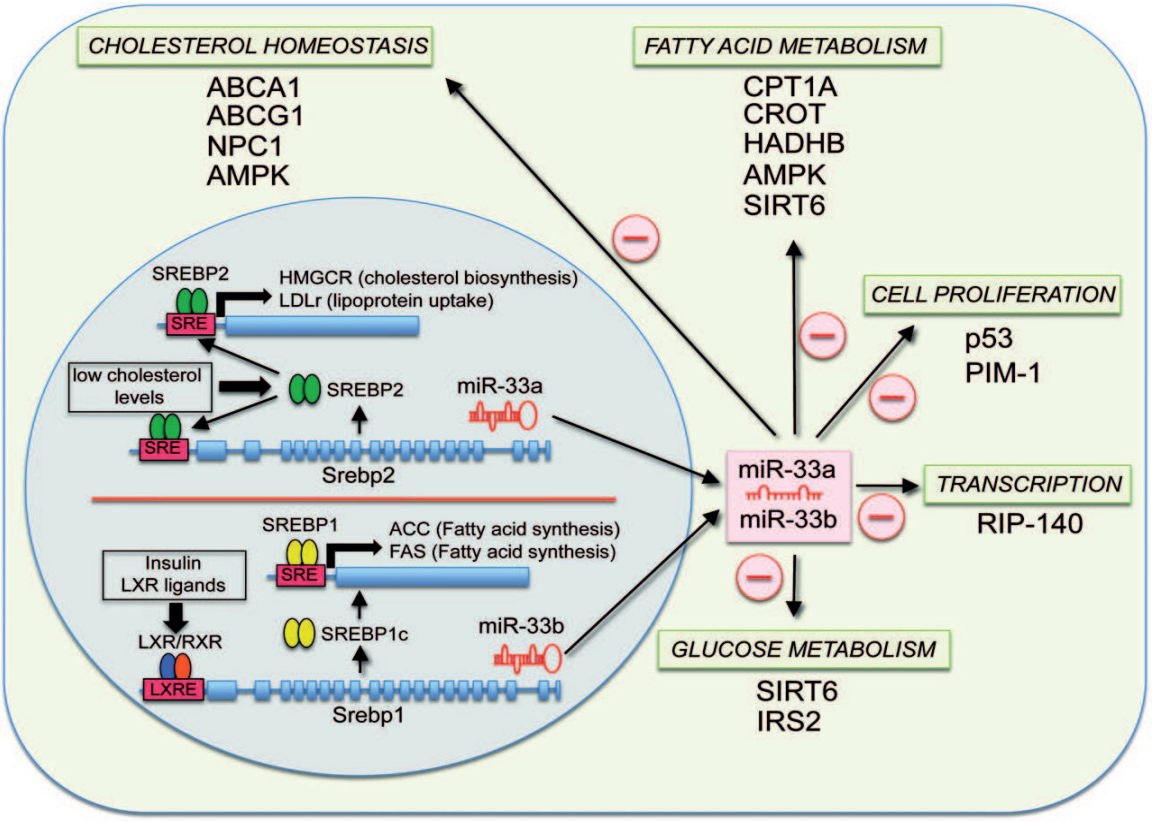

Figure 3. Pathways by which activated miR-33 may contribute to regulation of cholesterol, fatty acid and glucose metabolism, cell proliferation, and transcription.(71) (Adapted with permission from American Heart Association).

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)

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

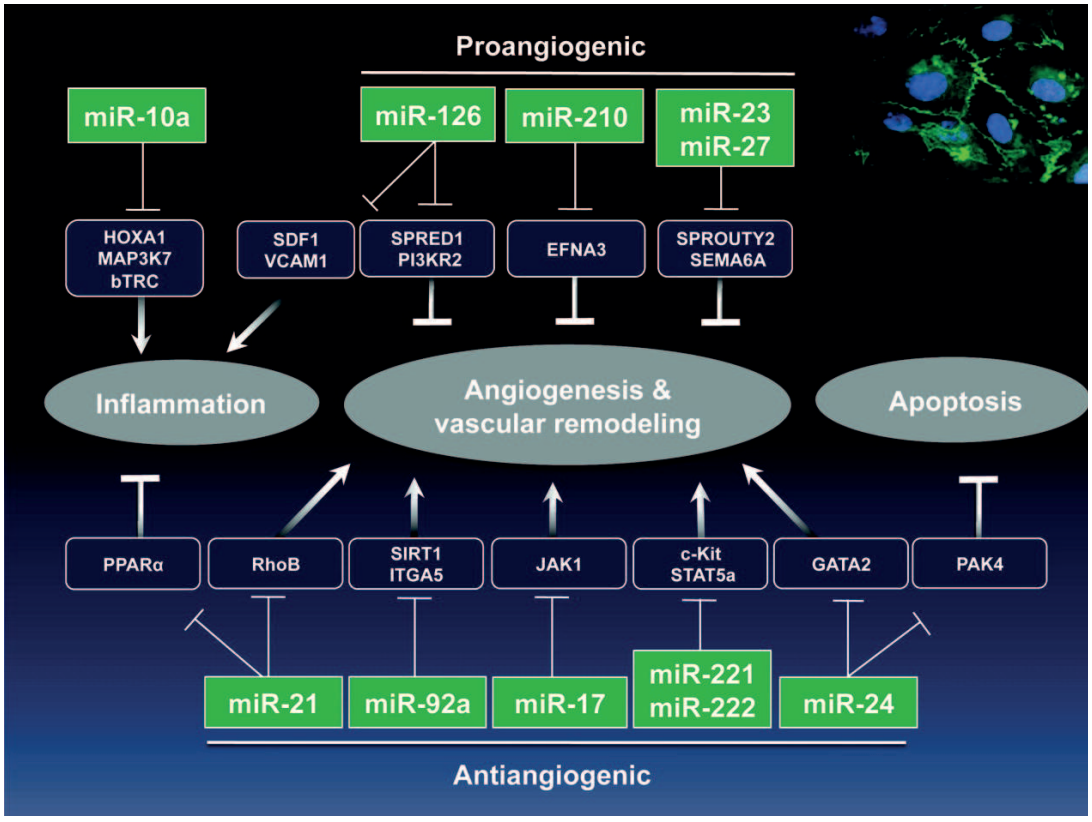

Figure 4. MiRNAs involved in EC function.(32) (Adapted with permission from American Heart Association).

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)

miRNAs in Atherosclerosis

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 antagomir 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 antagomirs 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.

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