AIMS Medical Science, 4(1): 99–112
DOI: 10.3934/medsci.2017.1.99
Received 8 November 2016
Accepted 17 February 2017
Published 27 February 2017

http://www.aimspress.com/journal/medicalScience

Review

miRNA Update: A Review Focus on Clinical Implications of miRNA in Vascular Remodeling

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Abstract: MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression at the post-transcriptional level. Through specific base pairing with their targets messenger RNAs (mRNA), miRNA can modify cell phenotype and function. Several miRNAs are aberrantly expressed in diseased arteries and may influence different features of vascular remodeling, including neointimal formation and diminished re-endothelialization. This review will discuss the clinical implications of miRNAs in the field of vascular remodeling and their potential role as diagnostic and therapeutic tools. miRNA modulation offers a promising strategy for therapeutic intervention to inhibit smooth muscle cell proliferation and enhance endothelial regeneration after percutaneous coronary intervention (PCI) in order to reduce restenosis and late thrombosis.

Keywords: MicroRNA; vascular remodeling; percutaneous coronary intervention; restenosis; re-endothelialization

Abbreviations:

AMI: acute myocardial infarction; BMS: bare metal stents; DES: drug-eluting stents; dsRNA: double-stranded RNA; ECs: endothelial cells; ISR: In-stent restenosis; mRNAs: messenger

RNAs; miRNAs: MicroRNAs; NO: nitric oxide; PCI: percutaneous coronary intervention; VSMCs: vascular smooth muscle cells

1. Introduction

miRNAs are a novel class of endogenous, small, non-coding RNAs that regulate gene expression via degradation, translational inhibition, or, under special conditions, translational activation of their target messenger RNAs (mRNAs). miRNAs can directly pair with complementary sites in protein-coding mRNA leading to the formation of double-stranded RNA (dsRNA). The presence of dsRNAs in the cytoplasm of eukaryotic cell triggers the cleavage of mRNA by the RNA-induced silencing complexes (RISCs) leading to complete mRNA degradation or preventing its translation, and therefore, silencing gene expression (Figure 1) [1]. As a result, miRNAs can regulate the expression of its multiple target genes and completely modify cell phenotype [2]. miRNAs are involved in multiple cell functions including proliferation, apoptosis, migration, differentiation and development [3]. Numerous studies have highlighted the role and function of miRNA in maintaining normal physiological conditions and disease development. In this review, we aim to summarize the clinical implications of miRNAs in the field of vascular remodeling.

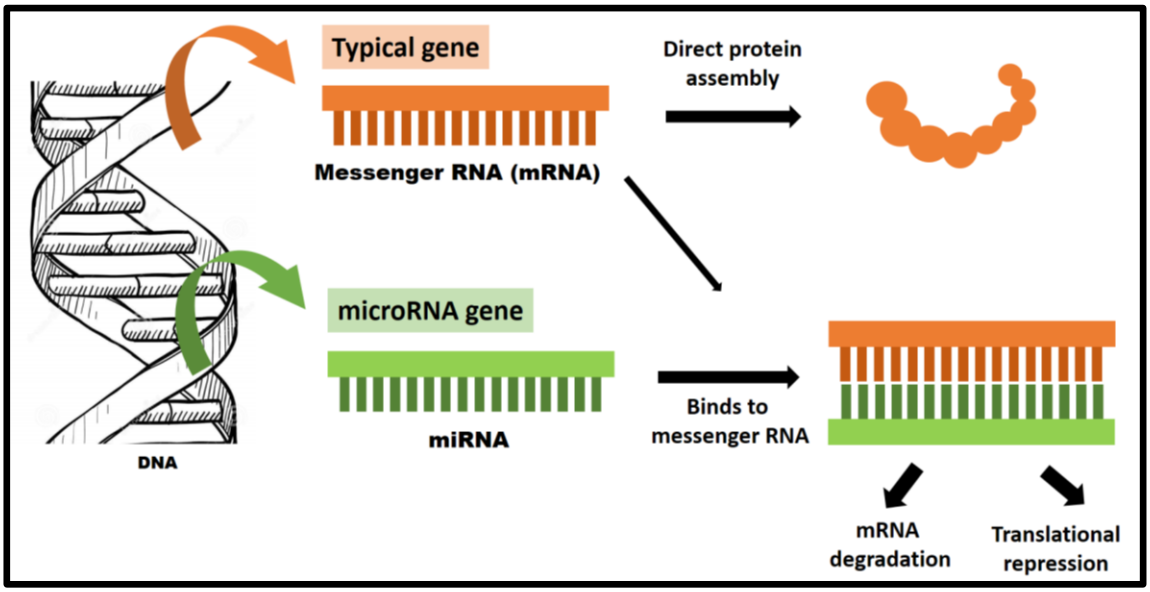

Figure 1. miRNAs regulation of protein synthesis leading to translational repression or degradation of the target mRNA (messenger RNA). miRNAs are a class of short, non-coding, single-stranded RNA molecules, approximately 22 nucleotides in length, that negatively regulate gene expression at the post-transcriptional level. They bind sequences in the target messenger RNA through complementarity and form RNA-RNA complex. This process leads to mRNA degradation or translational inhibition.

The existence of miRNA is a relatively recent discovery. The first miRNA was described in 1993 as a key player in the normal temporal control of diverse postembryonic developmental events in C. elegans (Figure 2) [4]. Nevertheless, it was not until the turn of the century that they were identified in human cells. In 2008, scientists found that miRNAs are present in human biofluids, including plasma and serum, in a remarkably stable form that is protected from endogenous RNase activity [5]. These findings made a major breakthrough in translational research, allowing researchers to compare levels of circulating miRNA between patients and healthy individuals and to assess their utility as disease biomarkers. Since then, scientific interest in this field has grown exponentially with current estimates suggesting the existence of approximately 2000 miRNAs that are active in humans [6,7].

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Figure 2. *Caenorhabditis elegans*. The first miRNA (lin-4) was discovered in *Caenorhabditis elegans* which controls the timing of the nematode larval development.

2. Role of miRNA in Vascular Diseases

miRNAs profile is expressed in a tissue-specific manner, and may vary according to different diseases [8]. These small non-coding RNAs are highly expressed in the cardiovascular system, including the vascular wall. In the vasculature, they are involved in endothelial dysfunction, ischemic angiogenesis, re-endothelialization, and vascular remodeling (Figure 3). Extensive evidence suggests that they play important roles in either vascular function or diseases regulating key signaling pathways through their target genes [9]. Studies have described aberrant miRNAs expression in diseased arteries and have suggested their association with neointimal formation and diminished re-endothelialization [10]. Both processes—neointimal proliferation and incomplete or delayed re-endothelialization—represent important challenges in the field of percutaneous coronary intervention (PCI) in patients with coronary artery disease.

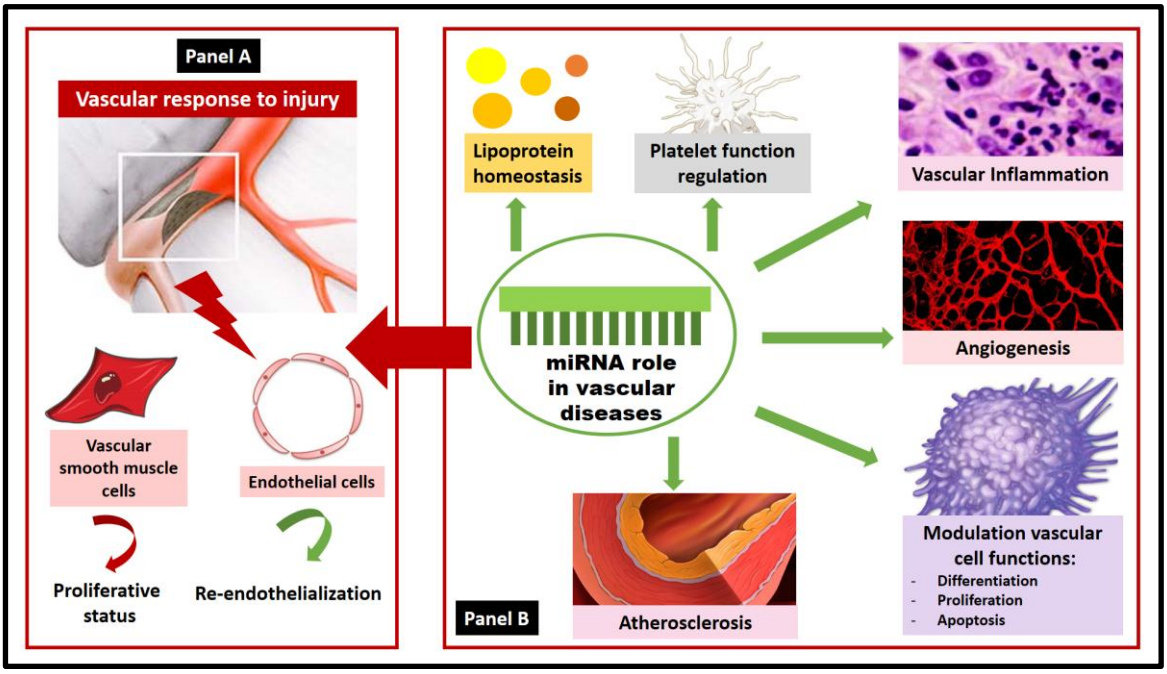

Figure 3. miRNA role in vascular diseases and vascular response to injury. During vascular injury (**Panel A)**, vascular smooth muscle cells (VSMCs) have a remarkable plasticity and undergo a phenotypic switch from a contractile to a synthetic (proliferative) state. Endothelial cells also undergo phenotype modification to increase proliferation and migration. miRNAs play a critical role in regulating both VSMC and endothelial phenotype towards regeneration. **Panel B** shows that miRNAs are also involved in vascular diseases due to they regulate key vascular processes like vascular inflammation, angiogenesis, atherosclerosis or lipoprotein homeostasis.

3. Implications of miRNA in Restenosis and Thrombosis

Restenosis is the development of luminal narrowing at the site of PCI and represents the response of the vessel to injury caused by uncontrolled neointimal proliferation. In-stent restenosis (ISR) is recognized as a major clinical problem in patients undergoing PCI, with rates ranging from 3% to 40%, depending on patient- and stent-related characteristics. The introduction of bare metal stents (BMS) to PCI procedure has diminished the rate of restenosis by attenuating early arterial recoil and contraction. Nonetheless, rate of repeat revascularization due to restenosis at one year remained relatively high at 25%. Currently, although antiproliferative drug-eluting stents (DES) significantly reduced the risk for ISR, there is still—especially for first-generation DES—a great risk of late and very late thrombosis related to the negative impact of nonselective antiproliferative drugs release on endothelial regeneration [10].

Vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) are key players implicated in the processes of ISR. Most of the current studies in PCI are targeted at finding optimal therapeutic

strategy to reduce VSMC proliferation (and restenosis) and to improve endothelial regeneration after stenting, to avoid late thrombosis related with DES [11] (Figure 4). miRNAs have a great potential to modulate several aspects of neointimal proliferation and diminished re-endothelialization after stenting because they regulate both VSMCs and ECs phenotype in distinct pathophysiological conditions, including vascular injury.

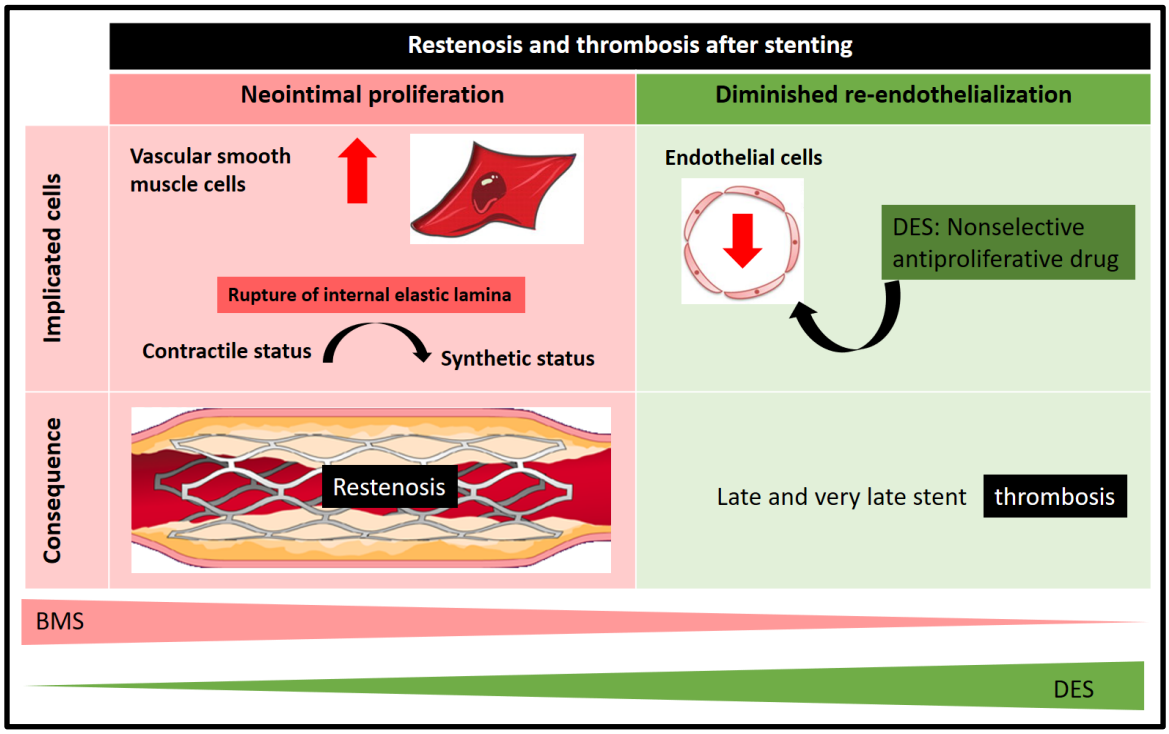

Figure 4. Restenosis and thrombosis after stenting. Vascular smooth muscle cells (VSMCs) and endothelial cells (ECs) are implicated in the processes of restenosis due to neointimal proliferation and late and very late stent thrombosis due to diminished re-endothelialization after stenting. The use of BMS is limited by a high rate of in-stent restenosis. DES increases the risk of late and very late thrombosis because of the negative impact of nonselective antiproliferative drugs released. BMS: bare metal stents; DES: drug-eluting stents.

4. miRNA and VSMC in Neointimal Formation

Neointimal proliferation in response to the rupture of internal elastic lamina leads to an excessive proliferation of the VSMC culminating in vessel lumen narrowing [12]. Under normal conditions, VSMCs in adult blood vessel present a contractile phenotype, characterized by low rate of proliferation. VSMCs phenotype is tightly regulated, and it is constantly oscillating between a contractile and a synthetic status. Deregulation of these phenotypes switching is associated with vascular disorders, including atherosclerosis and restenosis. After a stimulus, such as balloon injury,

the VSMC suffer a phenotypic switch from a quiescent contractile state to a synthetic and proliferative status, causing the lumen reduction [13].

Table 1. miRNA involved in the modulation of vascular smooth muscle cell's biology and phenotypic switch from contractile to a synthetic/proliferative status.

	Biological effect	Modulation after injury	Target
miRNA 21	Proproliferative (pro-synthetic status)	Upregulated	PTEN; BMPR2; WWP1
miRNA146a	Proproliferative (pro-synthetic status)	Upregulated	KLF-4
miRNA 221/222	Proproliferative (pro-synthetic status), promigratory and anti-apoptotic	Upregulated	p27, p57
miRNA 424	Proproliferative, differentiation, promigratory	Upregulated	Cyclin D164, 65; STIM 66; Calumenin 67
miRNA 145	Pro-contractile. Inhibition of migration and proliferation	Downregulated	KLF-5; KLF-4; calmodulin kinase
miRNA 143	Pro-contractile. Inhibition of migration and proliferation	Downregulated	KLF-5; Elk 1
miRNA 133a	Pro-contractile. Inhibition of proliferation	Downregulated	Sp-1
miRNA 195	Inhibition of migration and proliferation	Downregulated	Cdc-42; FGF1, Cyclin D1
miRNA 23b	Inhibition of migration and proliferation	Downregulated	FOXO4, uPA, SMAD3
miRNA 125	Inhibition of migration and proliferation	Downregulated	Ets-1

BMPR2 indicates bone morphogenetic protein receptor type II; Cdc-42: cell division control protein 42; FOXO4: Forkhead box protein O4; KLF-4: Kruppel-like factor 4; KLF-5: Kruppel-like factor 5; PTEN: phosphatase and tensin homolog; SMAD3: small mother against decapentaplegic; STIM: stromal interaction molecule; uPA: urokinase-type plasminogen activator; WWP1: NEDD4-like E3 ubiquitin-protein ligase.

Recent research has described the potential role of miRNA on VSMC fate in vessel development, function and disease [14,15], and suggest their role in the mechanisms involved in VSMC switch and neointimal formation. Ji *et al* observed a complex modulation of miRNA profile at 7, 14, and 28 days after vascular injury, with a time-course of both upregulation and downregulation of 140 out of 180 miRNAs expressed in the vessel wall [16]. This intricate modulation of miRNA levels suggests that miRNAs play a relevant role in modulating vascular response to injury [17]. Among miRNAs described, miR-221 and miR-222 play a role as modulators

of VSMCs functions and neointimal lesion formation. Both are increased in proliferative VSMCs and they have differential effects on VSMCs an ECs. They have pro-proliferative, pro-migratory, and anti-apoptotic effects on VSMCs; in contrast, the same miRNA can exert opposite effects in ECs [18]. Overexpression of the miRNA 221/222 cluster result in uncontrolled neointimal growth, whereas their downregulation leads to a 40% reduction of neointima formation [19]. miR-146a is another miRNA implicated in VSMCs phenotypic switch [20] and to promote VSMCs proliferation *in vitro* and vascular neointimal hyperplasia *in vivo* [21]. Chan *et al*. described that the inhibition of miR-24 prevents agonist-induced switch of VSMC from contractile to a synthetic phenotype [22]. A summary of the miRNAs expressed in VSMCs and ECs involved in vascular response to injury is shown in Figure 5, and the most relevant miRNA related to VSMCs switch are described in Table 1.

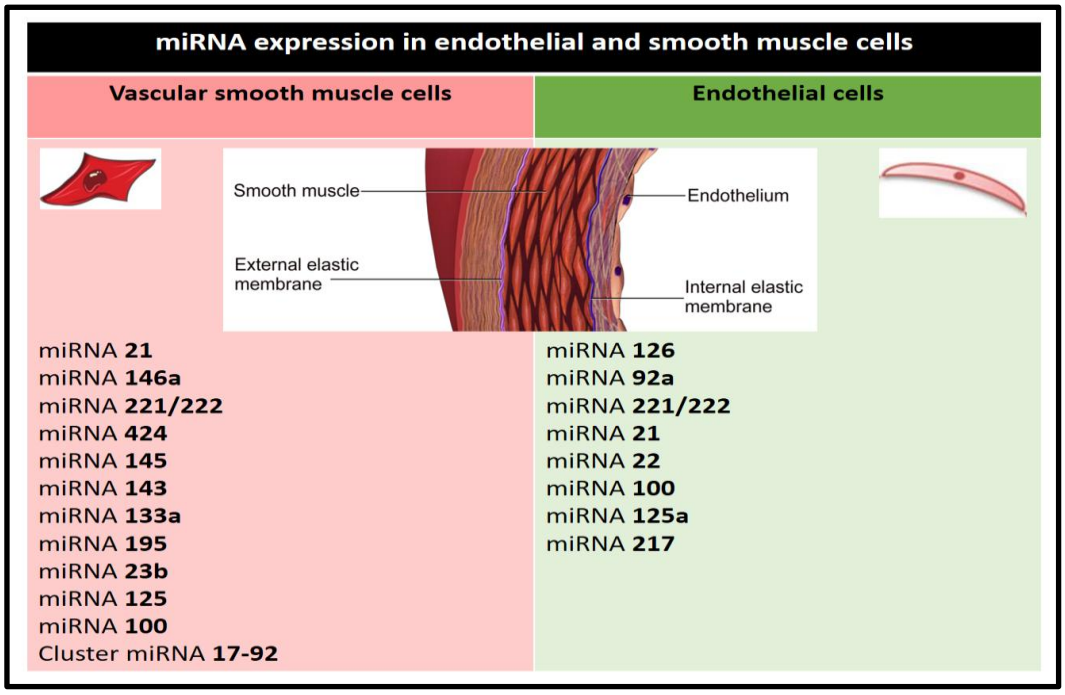

Figure 5. Expression of miRNAs in vascular smooth muscle cells and endothelial cells involved in vascular response to injury. The figure lists the most relevant miRNA involved in the modulation of vascular smooth muscle cells biology (left) and endothelial cells (right).

5. miRNA and Endothelial Cells: Implications in Re-endothelialization and Endothelial Dysfunction

miRNAs are also important modulators of endothelial cell phenotype. The miRNA with the highest expression in ECs is miR-126 and it is involved in the inflammatory response and endothelial dysfunction. miR-126 has an essential role in maintaining vascular integrity and homeostasis after tissue damage by enhancing endothelial repair capacity [23]. In addition, miR-126 exerts an

inhibitory effect on atherosclerosis formation, likely related to its endothelial action. Interestingly, circulating levels of miR-126 is markedly diminished in the coronary, but not in peripheral, circulation of patients with myocardial injury, suggesting its uptake during transcoronary passage through the infarcted myocardium [24]. Another endothelium-specific miRNA, miR-92a, negatively regulates EC-derived production of nitric oxide (NO) and its upregulation has been associated with atherosclerosis and myocardial injury. On the contrary, miR-92a inhibition may play an important role in preventing neointimal hyperplasia since NO production by ECs inhibits VSMCs proliferation [25].

Besides abnormal VSMC proliferation during vascular injury, endothelial denudation also leads to exposure of subintimal components such as collagen, Von Willebrand factor, fibronectin, and laminin. This phenomenon causes platelet adherence and aggregation and increases the risk of late stent thrombosis. To date, approaches to reduce restenosis are aimed solely at reducing VSMC proliferation and still present high risk of thrombosis. There is currently a growing interest for processes to speed up the recovery of endothelial tissue to avoid all the consequences of endothelial denudation. Recent research has proposed the use of miRNA-based therapy specifically targeting the EC to improve re-endothelialization following vascular injury. In animal model of arterial stenting, in vivo administration of anti-miR-92a significantly enhances re-endothelialization in injured carotid arteries and diminishes neointimal formation [26]. Besides, recent studies have identified that the cluster miR-221/222 has significant inhibitory effects on EC migration, tube formation, and wound healing in cultured endothelial cells; despite its proliferative role in VSMC [27,28]. The recognition that miR-221/222 cluster is a sensitive regulator of EC function, and that they display opposite effects in VSMCs have shed much light in the field of vascular remodeling and may enable the development of novel strategies to concomitantly decrease neointimal proliferation and improve re-endothelization. The most relevant miRNAs on EC function are summarized in Table 2.

Table 2. Most relevant microRNA involved in the modulation of endothelial cell's biology.

miRNA related to modulation of endothelial cells			
	Biological effect	**Effect of miRNA upregulation on cell growth**	**Target**
miRNA 126	Vascular integrity and angiogenesis	+	Spred-1; VCAM-1
miRNA 92a	Angiogenesis, proliferation and migration	+	ITGalpha5; KLF-4; MKK4
miRNA 221/222	Migration and proliferation	−	c-kit

KLF-4: Kruppel-like factor; MKK4: mitogen-activated protein kinase kinase 4; VCAM-1: vascular cell adhesion molecule 1.

6. Diagnostic and Prognostic Potential of miRNA: Clinical Implications in Cardiology

The distinct patterns of miRNA expression change between cardiovascular diseases and other pathological conditions, suggesting that miRNA expression profiles could be a useful biomarker in cardiology. In addition to miRNA expressed in vessels and heart, circulating miRNA are also found in various body fluids, including plasma and serum, and they remain stable even during extended storage through association with lipids, proteins or microparticles. All these facts make miRNAs good candidates to be used as biomarkers to predict the risk and prognosis of cardiovascular disease [29]. Recent studies have demonstrated that circulating miRNAs could be used as biomarkers for acute myocardial infarction (AMI) [30], post-ischemic cardiac remodeling [31] and heart failure [32] in humans.

As for vascular remodeling, some miRNAs have been identified as potential biomarkers of ISR. He *et al*. observed that circulating miR-21, miR-100, miR-143, and miR-145 levels were closely associated with the occurrence of ISR after coronary DES implantation. Compared with the non-ISR patients, circulating miR-21 significantly increased in the ISR patients, while the miR-100, miR-143, and miR-145 decreased. Moreover, it has been found that miR-21 levels were significantly higher, while miR-100, miR-143, and miR-145 levels were much lower in patients with diffuse ISR than in those with focal ISR. The miR-143 and miR-145 had the higher sensitivity and specificity in association with the occurrence of ISR, and their conclusion were that both can serve as novel noninvasive biomarkers for ISR [33].

Matsumoto *et al*. found that serum levels of miR-155 and miR-380 at the time of discharge after AMI are higher in patients who subsequently died of cardiac cause within 1-year of discharge than in those who did not experience cardiovascular events during the 3-year follow-up period [34]. Furthermore, there are other studies that indicate that circulating miRNAs have the potential to predict prognosis in patients with cardiovascular disease. Karakas *et al*. evaluate the predictive role of 8 circulating miRNA (miR-19a, miR-19b, miR-132, miR-140-3p, miR-142-5p, miR-150, miR-186 and miR-210) for cardiovascular mortality in patients with documented coronary artery disease. This prospective study identified an independent association between circulating levels of miRNA—especially miR-132, miR-140-3p and miR-210- and the risk of cardiovascular death [35].

Altogether, these data suggest that miRNA are promising biomarkers which may allow cardiologists in the coming years to 1) stratify risk in coronary artery disease; 2) guide secondary preventive therapies; 3) perform noninvasive identification of stent restenosis; 4) identify those patients with higher propensity to develop restenosis; 5) be a new generation of biomarkers in heart failure. Patients with high risk miRNA profile may benefit of closer follow-up and more personalized intensive medical management [36,37].

7. Therapeutic Applications of miRNA: Potential in Preventing PCI Complications

Given the importance of miRNAs in vascular remodeling, creating therapeutic strategies to enhance or inhibit miRNA expression is a promising approach. The use of miRNA in therapeutics has several theoretical advantages: 1) miRNAs and "anti-miRNAs" to inhibit endogenous miRNA are short, therefore localized administration may be relatively easy; 2) cell- and disease- specific miRNAs expression can attenuate side effects of drug action in other cells; 3) the regulatory effects of miRNA and their therapeutic levels of modulation can allow a faster and personalized interference to the molecular mechanism of cardiovascular disease.

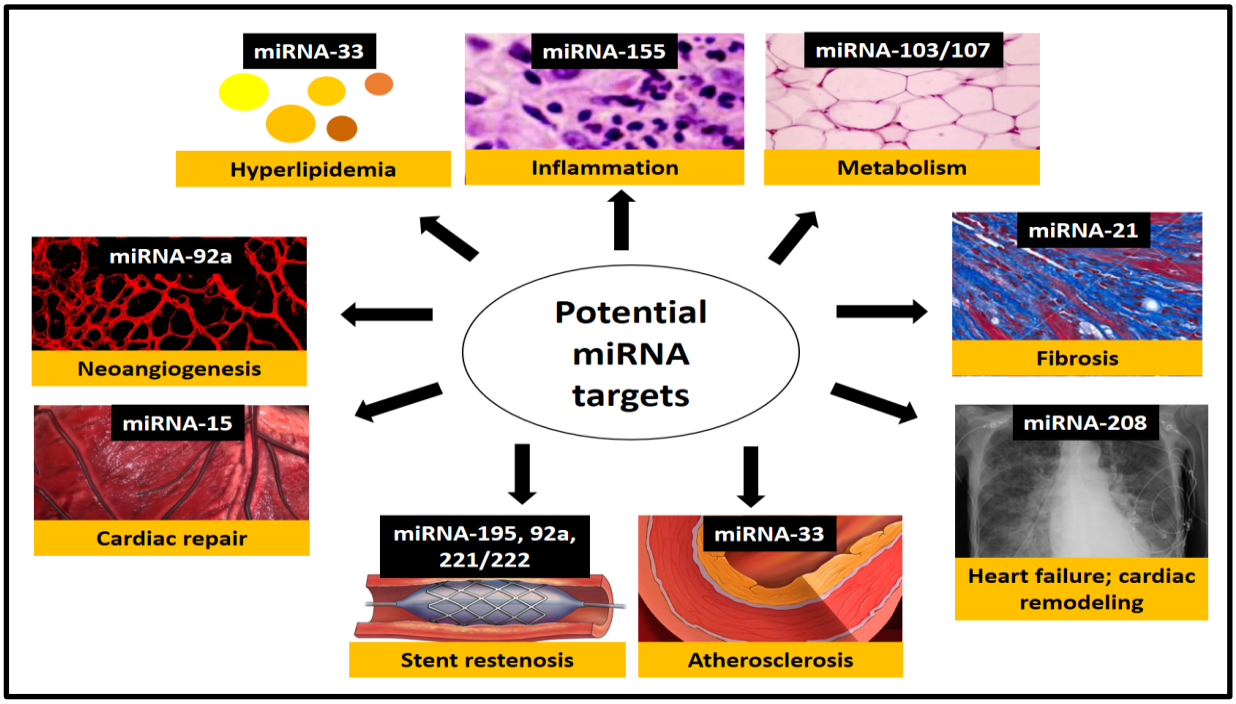

Figure 6. Potential drug targets in microRNA based therapy in the field of cardiovascular diseases. Specific miRNAs that are being studied as clinical therapeutic targets. The inhibition of these miRNA has shown therapeutic promise and are currently being pursued as clinical candidates.

In some diseases, such as different types of cancer and hepatitis C, the therapeutic potential of miRNAs is already being tested in terms of clinical trials [38–41]. As for cardiovascular disease, miRNAs have great potential as therapeutic target to prevent restenosis and stent thrombosis, as tissue- and cell- specific miRNAs could inhibit VSMCs proliferation at the same time they may empower endothelial regeneration. Although all miRNAs modified in the setting of neointimal hyperplasia are of therapeutic interest, those that show cellular specificity to EC or VSMC are of greater interest. Recent studies have employed the use of miRNA-based therapy for prevention of ISR. Local delivery of specific miRNA or anti-miRNAs oligonucleotides in experimental models of arterial injury have shown promising results in reducing neointimal formation and have opened the

door to the incorporation of miRNAs and anti-miRNAs into the arsenal of therapeutic strategies for use with DES [42–45]. In this regard, the local application of miRNA or anti-miRNA in the vascular wall is a feasible and promising strategy in clinical cardiology based on the possibility to create "miRNA-eluting-stents" to increase the re-endothelialization and decrease proliferation of the VSMC. Besides, specific miRNA expression depending on pathophysiological condition (sex, age, and risk factors) opens a field to a wide range (Figure 6) of personalized therapies for cardiovascular disease that should be pursued in the next few years.

8. Limitations of the Use of miRNAs in Diagnostic and Therapeutic

Despite the hype on miRNA research and their potential application in cardiology, there are still several challenges related to technical aspects that may be overcome before their introduction into clinics. For instance, methods for miRNAs normalization and their interaction with circulating drugs remain unresolved [46]. The best-known housekeeping genes commonly used for normalization of miRNA detection may vary depending on the sample and disease studied [47]. Besides, different collection tubes revealed divergent concentrations of circulating miRNAs, and tubes with lithium-heparin were found unsuitable for miRNA quantification [48].

Another issue, is that duration and cost of the whole procedure of miRNA detection are superior when compared to several useful and well-accepted biochemical and immunochemical assays used. Current miRNA detection techniques are time consuming and do not allow the rapid diagnosis required in patients with cardiovascular diseases [49]. Moreover, the protocols available for detecting miRNA from body fluids require extraction methodologies and analysis from sophisticated platforms [50], making them more expensive and slower than normal biochemical protocols. Currently no biomarker available have been shown to be associated with immediate acute cardiac event [51], and their use have been mostly associated to long-term outcomes. Therefore, all circulating miRNA findings require further steps of validation and a proper standardization of all preanalytical and analytical procedures before translation into clinical practice [52].

Conflict of Interest

All authors declare no conflicts of interest in this paper.

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