The Role of microRNA in the Development, Diagnosis, and Treatment of Cardiovascular Disease – Recent Developments*

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microRNA in Cardiovascular Disease

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Nonstandard abbreviations:
AAV: adeno-associated virus
AQ9: aquaporin-9
CAD: coronary artery disease
CaMKII: calmodulin-dependent protein kinase II
CDC: cardiosphere-derived cells
CV: cardiovascular
CVD: cardiovascular disease
DCM: diabetic cardiomyopathy
HF: heart failure
HFrEF: HF with preserved ejection fraction
IP₃R: inositol-3-phosphate receptor
LA: left atrium
LAD: left anterior descending
LNA: locked nucleic acid
LV: left ventricle
MAP: mitogen-activated protein
MI: myocardial infarction
miRISC: miRNA-induced silencing complex
miRNA: microRNA
NGAL: neutrophil gelatinase associated lipocalin
NOS: nitric oxide synthase
PI3K: phosphatidylinositol 3-kinase
PTEN: phosphatase and tensin homologue
TGF-β: transforming growth factor β
WRF: worsening renal function

Section assignment: Cardiovascular
Abstract

Since their discovery in 1993, microRNAs (miRNAs, miRs) have emerged as important regulators of many crucial cellular processes and their dysregulation have been shown to contribute to multiple pathological conditions, including cardiovascular disease (CVD). miRNAs have been found to regulate the expression of various genes involved in cardiac development and function, and in the development and progression of CVD. Many miRNAs are master regulators fine-tuning the expression of multiple, often interrelated, genes involved in inflammation, apoptosis, fibrosis, senescence, and other processes crucial for the development of different forms of CVD. This article presents a review of recent developments in our understanding of the role of miRNAs in the development of CVD and surveys their potential applicability as therapeutic targets and biomarkers to facilitate CVD diagnosis, prognosis, and treatment. There are currently multiple potential miRNA-based therapeutic agents in different stages of development, which can be grouped into two classes: miRNA mimics (replicating the sequence and activity of their corresponding miRNAs) and antagomiRs (antisense inhibitors of specific miRNAs). However, in spite of promising preliminary data and our ever-increasing knowledge about the mechanisms of action of specific miRNAs, miRNA-based therapeutics and biomarkers have yet to be approved for clinical applications.
Significance

Over the last few years microRNAs have emerged as crucial, specific regulators of cardiovascular system and in the development of CVD, by posttranscriptional regulation of their target genes. The minireview presents the most recent developments in this area of research, including the progress in diagnostic and therapeutic applications of microRNAs. microRNAs seem very promising candidates for biomarkers and therapeutic agents, though some challenges, such as efficient delivery and unwanted effects, need to be resolved.
Introduction

microRNAs (miRNAs or miRs) are short (18-26 nucleotides) RNA molecules coded in different regions of the genome, often in introns or sometimes exons of other genes (Boyd, 2008). They’re transcribed in the nucleus by RNA polymerase II as pri-miRNA molecules, which are subsequently processed by Drosha into pre-miRNA molecules, which are then exported to the cytoplasm and processed by Dicer to become miRNA duplexes (Han et al., 2004; Lee et al., 2004). The duplexes are incorporated into miRNA-induced silencing complex (miRISC), whose core component, Argonaute, guides miRNA maturation and incorporation of their target mRNAs, based on fully or partially complementary sequences, usually located in the 3’ untranslated region (3’UTR) of target mRNAs (Bridge et al., 2017) (Bartel, 2004). For detailed description of the miRNA biogenesis, including noncanonical pathways, see (Wronska et al., 2015), (Ha and Kim, 2014), and (Michlewski and Caceres, 2019).

miRNAs were discovered in 1993 in C. elegans (Lee et al., 1993) as novel regulatory elements mostly downregulating, but sometimes upregulating, expression of their target genes through mRNA degradation or translational repression. miRNAs have been shown to be important factors in many cellular pathways and physiological and pathophysiological processes and are highly cell- and tissue-specific. Since the first publication indicating their role in CVD, in 2006 (van Rooij et al., 2006), microRNAs have emerged as ever more important regulators of cardiovascular (CV) physiology as well as key players in the development of CVD (Table 1).

Single miRNAs often regulate and are regulated by multiple functionally related genes (Chavali et al., 2013). Some miRNAs form clusters, which can be regulated by specific factors, and each of the miRNAs in the cluster can have its own set of target genes (O’Brien et al., 2018). Many genes targeted by miRNAs regulate crucial cellular signaling pathways, which makes
them great fine-tuning tools, very responsive to specific changes in cellular environment. E.g. miR-486 targets phosphatase and tensin homologue (PTEN) and Foxo1a, which inhibit PTEN/AKT pathway (Small et al., 2010) and miR-1 regulates cardiac conduction by targeting Irx5, a transcription factor crucial for the expression of cardiac ion channels (Zhao et al., 2007), while miR-29 regulates fibrotic response through targeting various collagens (van Rooij et al., 2008).

Some of the most important miRNAs expressed in the heart are miR-1 and miR-133a, which drive the differentiation into cardiomyocytes (Ivey et al., 2008) and mediate cardiac conductance and action potential (Chistiakov et al., 2016), while miR-208a/b and miR-499 are specific for later stages of cardiac development, regulating the expression of cardiac contractile proteins and fast/slow muscle fiber specificity (Chistiakov et al., 2016) (Xiao et al., 2019). The expression of miR-1 is indirectly stimulated by the mammalian target of rapamycin (mTOR) (Sun et al., 2010), a known regulator of myogenesis (Figure 1). No wonder these miRNAs have been implicated in multiple aspects of the development of various CVD. The current review presents the advancements in our understanding of miRNA’s role in CV physiology, CVD development, and the utility of miRNAs as diagnostic, prognostic, and therapeutic agents since 2015, when a previous comprehensive review on this topic was published (Wronska et al., 2015).

One of the therapeutic approaches potentially opening up thanks to our increasing understanding of the role of miRNAs in CVD is the regulation of the Notch pathway. Due to its specialized, context-dependent mechanism in different cell types (Luxan et al., 2016), the Notch pathway would have to be regulated in a very cell- and process-specific manner (Marracino et al., 2021). There is a complicated interplay between Notch and multiple miRNAs and dysregulation of this pattern at any point can contribute to the development of CVD, leading to
atherosclerosis, myocardial ischemia, heart failure (HF), and arrhythmias (Marracino et al., 2021). Upregulation of Notch signaling, in most instances, is associated with increased survival of the cells, so one of the potential approaches to treating early CVD would be through miRNAs known to regulate Notch, such as miR-121, miR-126-5p, miR-133 miR-143/145, and miR-155 in atherosclerosis; miR-34a-5p, miR-92a, miR-146a, miR-155, miR-210, miR-363, miR-381, and miR-449a in MI; miR-25; miR-34a, miR-99a, miR-195-5p, miR-199a, and miR-212/132 family in HF; and miR-1, miR-34a, and miR-208a in arrhythmias (reviewed in (Marracino et al., 2021)). Multiple therapeutic factors, which can be grouped into mimics (replicating the sequence and activity of their corresponding miRNAs) and antagomiRs (antisense inhibitors of specific miRNAs), based on some of these miRNAs showed promising results in in vitro and preclinical studies (Chakraborty et al., 2021).
miRNAs in Myocardial Infarction

Myocardial infarction (MI), popularly referred to as heart attack, is still the leading cause of mortality and morbidity in humans, in spite of significant progress in its prevention and treatment (Dani et al., 2022). Increased apoptosis and the activation of inflammatory response triggered by MI lead to the loss of cardiomyocytes and cardiac fibrosis, which significantly lower cardiac function, leading to HF and, ultimately, death (Heusch et al., 2014). Some microRNAs are known to contribute to morbidity and mortality associated with MI through stimulating fibrosis, which can ultimately lead to HF (reviewed in (Maries et al., 2021)).

Multiple studies have shown the important role of exosomes, 30-150 nm vesicles released from all cell types, as the transportation media for miRNAs, enabling miRNAs to exert their effects through a paracrine (on neighboring cells), autocrine (upon the intake back by the cells of origin) or endocrine (when released to the circulation) modes (Figure 2). The content of the exosomes reflects the conditions of the source cells; among other, specific miRNAs are enriched or depleted in response to physiological and pathophysiological conditions the cells are undergoing (Emanuelli et al., 2015).

De Couto at al. (de Couto et al., 2017) have shown a cardioprotective effect of exosomes secreted by cardiosphere-derived cells (CDC). According to their studies in pigs and rats with MI induced by ischemia/reperfusion, the beneficial effect of the intracoronary infusion of CDC-secreted exosomes (CDCexo) in these animal models was brought by a specific set of miRNAs enriched in the exosomes, mostly miR-181b, which, through targeting protein kinase C delta type (PKCδ), mediated an anti-inflammatory macrophage polarization.

Accordingly, Vaskova et al. (Vaskova et al., 2020) have shown that sacubitril/valsartan (neprilysin inhibitor/angiotensin receptor blocker) decreased myocardial fibrosis in a rat chronic
MI model, after permanent left anterior descending (LAD) coronary artery ligation. The mechanism of this salutary effect was through the increased production of exosomes with specifically downregulated miR-181a, which led to attenuation of myocardial fibrosis and pathological hypertrophy. Interestingly, rats treated with miR-181a antagomiR showed a marked improvement in left-ventricular (LV) function and cardiac remodeling, while addition of miR-181a mimic to sacubitril/valsartan treatment cancelled the beneficial effects of the latter, validating the specificity of miR-181a. It is known that cells under stress, such as hypoxia, increase cellular endosomal production, leading to increased exosome release, which mediates intercellular communication. Thus, miR-181a could be a valuable early biomarker of cardiac ischemia and/or its downregulation by a specific antagomiR could be developed into therapeutic applications.

Some miRNAs have been shown to protect the heart after MI through the phosphatidylinositol 3-kinase (PI3K)/AKT signaling pathway. E.g. miR-212 suppressed cardiomyocyte apoptosis and improved vascularization after MI, ultimately leading to improved cardiac function, by downregulating the expression of aquaporin-9 (AQ9) and subsequently activating the PI3K/AKT pathway, which resulted in a lower level of caspase-mediated apoptosis (Ren and Wang, 2018).

miR-144 have been shown to protect against deleterious post-MI remodeling in both ischemia/reperfusion and non-reperfused MI mouse models (Li et al., 2018). Repeated intravenous injections of miR-144 led to a reduced infarct size and improved cardiac function in the LAD coronary artery ligation MI mouse model. It has been shown that miR-144 exerts its protective function through both acute cardioprotection and by reducing chronic MI-induced remodeling. From clinical application perspective it is worth noting that intravenous application
was viable thanks to the association of the injected miRNA-144 with Argonaute-2, which protects it from digestion by RNase in the plasma (Li et al., 2018). Such delivery method, however, does not protect against any potential off-target and systemic effects.

Gao et al. have shown an important role of miR-19a/19b, which are members of the miR-17-92 cluster, in the prevention of HF after MI (Gao et al., 2019). In their MI mouse model of permanently ligated LAD coronary artery, an intracardiac injection of either miR-19a or miR-19b mimics reduced infarct site, preserved cardiac function during 5 days to 9 weeks post-MI, and increased survival. Members of the miR-17-92 cluster induce cardiomyocyte proliferation in post-natal heart, and miR-19a and miR-19b were shown to be necessary and sufficient for the proliferation of isolated neonatal rat cardiomyocytes (Chen et al., 2013). Gao et al. observed a significant increase in the level of miR-19a and miR-19b post-MI, which was specific for MI, since no increase in miR-19a/19b expression was observed in a cardiac hypertrophy mouse model. In early stages post-MI, miR-19a/19b infer cardiac protection by repressing the immune response in infarcted hearts, which suggests that miR-19a/19b (and possibly other members of the miR-17-92 cluster) may serve as therapeutic targets in early MI treatment, preventing post-MI heart failure. The most probable mechanism of miR-19a/19b is by the repression of Pten (a direct target of miR-19a/19b), which is probably a direct mediator of MI-induced cardiomyocyte proliferation (Oudit et al., 2004).

One of the most promising results were obtained in a porcine left anterior coronary artery occlusion MI model (Gabisonia et al., 2019). MI pigs after an adeno-associated virus (AAV)-facilitated delivery of miR-199a into their left ventricles (LV) showed significant cardiac function improvement, recovery of LV ejection fraction (LVEF), and LV stroke volume at day 28; while LV end-systolic volume showed partial recovery and LV end-diastolic volume was not
changed. The reduced infarct size, diminished cardiac fibrosis, and improvement of contractile function in pigs treated with intracardiac miR-199a was effected by stimulating cardiomyocyte de-differentiation and proliferation. However, long-term expression of miR-199a led to sudden cardiac death of most treated animals. It underscores, as in multiple other cases, the need for a very precise and controlled dosing and delivery of any potential miRNA-based agents in clinical settings.

Delivery of miRNA-based therapeutics in MI

One of the hurdles limiting clinical applications of miRNA-based therapeutics is the targeted, specific delivery (Roberts et al., 2020; Dasgupta and Chatterjee, 2021). In MI, specifically, microvascular obstruction has to be overcome in order to deliver therapeutic agents to the infarct site. Hong et al. (Hong et al., 2020) developed an anti-coagulative nanocomplex to deliver miR-1 inhibitor loaded with dendrigraft poly-l-lysine (DGL). The nanocomplex was able to reduce microthrombus formation in microvessels and inhibit blood-coagulation factor Xa, thereby overcoming microvascular obstruction in the infarct area. The additional effect of miR-1 inhibitor lead to decreased cardiomyocyte apoptosis and reduced fibrosis, thus improving cardiac function.

A similar approach has been described by Bejerano et al. (Bejerano et al., 2018). They investigated a nanoparticle-based delivery of a miR-21 mimic to cardiac macrophages at the infarct site in a mouse ligation model. Cardiac macrophages in mice treated with miR-21 mimic showed a switch from pro-inflammatory toward anti-inflammatory state, leading to the increased angiogenesis, and reduction in apoptosis and pathological remodeling in the infarct area. However, these positive effects did not lead to an improvement in systolic function. Additionally, the level of miR-21 has been shown to be elevated in some cancers and other
pathologies (Feng and Tsao, 2016), raising concerns about potential dangers of its mimic’s systemic effects. The very precise nature of the regulation by miRNA and their often multi-pronged effects present serious challenges when devising miRNA-based clinical applications. Thus any potential miRNA-based therapeutics would have to be used locally rather than systemically and in a very controlled manner.

miRNAs in Heart Failure

A recent study provided an evidence in a large animal (pig) model that miRNA-based agents could be effectively used to treat HF (Hinkel et al., 2020). In the study one-hour-long LAD occlusion and subsequent reperfusion lead to HF following an MI affecting large heart area. Those pathological changes were associated with increased levels of miR-21, which is an important regulator of cardiac fibrosis through the activation of mitogen-activated protein (MAP) kinase signaling (Thum et al., 2008). Pigs treated with the locked nucleic acid (LNA)-antimiR-21, applied through an over-the-wire balloon catheter, showed significantly improved cardiac function and preserved liver and kidney function compared with control animals, up to a month post-MI (Hinkel et al., 2020). The beneficial effects of antimiR-21 are due to its role in inhibiting pro-fibrotic and pro-inflammatory transcriptional reprogramming triggered by miR-21 post-MI. Interestingly, heart-specific miRNA levels were not increased in the plasma, suggesting their local, not systemic, mechanism of action (Vegter et al., 2017b).

miR-222 in general seems to have cardioprotective effects in the heart. It is induced by physical exercise and mediates the cardioprotective benefits of exercise in healthy and HF-affected individuals (Liu et al., 2015). Transforming growth factor (TGF)-β-mediated downregulation of two microRNAs from the miRNA-221/222 family, miR-221-3p and miR-222-
3p, observed in pressure-overload-induced HF model and in HF patients, may activate profibrotic signaling, leading to fibroblast activation and fibrosis development (Verjans et al., 2018). Some potential targets of miRNA-221/222, such as c-Jun N-terminal kinase 1 (JNK1), TGF-β receptor 1 and 2, and ETS proto-oncogene 1 (ETS-1), are involved in TGF-β/SMAD signaling. Thus, a miRNA-221/222 mimic delivered to cardiac fibroblasts could potentially be used for HF treatment. However, its delivery to cardiomyocytes could be detrimental, as miRNA-221/222 overexpression in cardiomyocytes in mice led to cardiac fibrosis, dysfunction, and death (Su et al., 2015). As always, miRNAs prove to be promising, yet elusive, therapeutic targets and we are far from full understanding of their role in CV physiology and pathophysiology.

Interestingly, miRNAs may be at least partially responsible for the differential prevalence of HF with preserved ejection fraction (HFpEF) between men and women (Florijn et al., 2018). HFpEF is diagnosed in approximately 50% of all HF cases (Oktay et al., 2013), but in twice as many women as men. It is often associated with multiple comorbidities, both CV, such as hypertension, coronary artery disease (CAD), and atrial fibrillation (AF); as well as non-CV, such as obesity, chronic kidney disease, and obstructive pulmonary disease (Bhatia et al., 2006). The prognosis for the HFpEF is poor, and its prevalence is increasing – both due to the population aging and to the shifting diagnostic criteria.

Florijn et al. have found that estrogen regulates the expression of multiple miRNAs through different mechanisms, e.g. recruitment of specific transcription factors, regulating RNA polymerase II activity and the expression of proteins directly involved in miRNA processing and function: Dicer and Argonaute-2 (Florijn et al., 2018). Differential miRNA expression between men and women and the resulting discrepancy in the inflammatory and cardiac remodeling can
underlie sex-specific differences in CV pathophysiology. These differences can be attributed to miRNAs activated by estrogen and those expressed from the X chromosome. While the former exhibit mostly protective effect, the latter are mostly deleterious to CV function. Therefore, estrogen deficiency in post-menopausal women leads to the loss of protective miRNAs and increased expression of the X-chromosome-associated deleterious ones, which brings about pathophysiological effects leading to HFpEF and, conceivably, to other sex-specific CV etiologies.

miRNA-based HF therapeutics in clinical trials

One of the most promising therapeutic targets for HF treatment is miR-132, which is a master regulator of a slew of pathophysiological processes leading to CVD, especially HF (Xu et al., 2021). Mice overexpressing miR-132 developed cardiac hypertrophy and HF, leading to death. These effects were prevented in mice treated with miR-132 antagoniR (Ucar et al., 2012). The rescue effect was confirmed in a pig HF model, in which antimiR-132 treatment prevented the development of post-MI HF in a dose-dependent manner (Foinquinos et al., 2020). Some confirmed and potential targets of miR-132 are FOXO3, SERCA2A (sarco/endoplasmic reticulum Ca2+-ATPase), TEK (TEK receptor tyrosine kinase), NOS3 (endothelial nitric oxide synthase 3), and STIL (SCL/TAL1 interrupting locus), whose dysregulation is associated with different aspects of maladaptive growth and remodeling leading to HF, such as fibrosis, apoptosis, redox regulation, and calcium handling. Actually, a miR-132 antigomiR, CDR132L, is the only miRNA-based CVD therapeutic currently tested in clinical trials (ClinicalTrials.gov). It proved to be safe, well-tolerated, and to selectively inhibit miR-132 level in a dose-dependent manner, mirroring the pre-clinical results, thus offering a hope of a similar effectiveness in preventing HF in humans (Taubel et al., 2021).
miRNAs as biomarkers in HF

Some miRNAs have been shown to associate with different clinical outcomes for HF patients, thus could potentially serve as biomarkers guiding prognosis evaluations and treatment decisions. E.g. low levels of circulating miR-199a-3p (hypoxia-related) and miR-27a-3p (angiogenesis-related) and a few other established HF-associated miRNAs have been shown to significantly associate with CV-related rehospitalizations within 18 months for patients initially admitted for HF (Vegter et al., 2017b). The best predictor of rehospitalization was actually let-7i-5p. All miRNAs shown to associate with rehospitalizations were involved in processes related to atherosclerosis, such as angiogenesis, inflammation, and endothelial dysfunction.

Circulating miR-122 level, specific for the liver, where it regulates cholesterol and fatty acid metabolism, has been reported to be an independent predictor of clinical outcomes in chronic systolic HF (Stojkovic et al., 2020). It could serve as an additional biomarker, to complement the established N-terminal pro B-type natriuretic peptide (NT-proBNP), for improved risk stratification.

Bruno et al. have identified a set of circulating miRNAs differentially expressed in HF patients who developed early renal failure (Bruno et al., 2016). These miRNAs could serve as diagnostic and prognostic biomarkers helpful in selecting best treatment options for patients with acute HF. Increased serum creatinine and neutrophil gelatinase associated lipocalin (NGAL) levels (current standard indicators of worsening renal function (WRF)) were significantly associated with lower levels of miR-27a-3p, miR-199a-3p, miR-423-5p, miR-652-3p, and miR-let-7i-5p for creatinine and with lower levels of miR-18a-5p, miR-106a-5p, miR-199a-3p, miR-223-3p, and miR-423-3p for NGAL, with the strongest predictor of WRF being miR-199a-3p (Bruno et al., 2016).
miRNAs have also been indicated as potential useful early and non-invasive predictors of heart transplant rejection. Patients treated with heart allografts for end-stage HF are currently monitored with invasive heart biopsies to detect early signs of transplant rejection. Tissue and circulating levels of miR-10a, miR-31, miR-92a, and miR-155 were significantly different between normal and rejecting grafts, with miR-10a decreased, and the other 3 miRNAs increased, in rejecting transplants (Duong Van Huyen et al., 2014). Thus, the miRNAs might serve as a promising non-invasive alternative for heart transplant monitoring.

However, Vegter et al. advise caution in extrapolating any results obtained in rodent models to humans, as circulating miRNA levels observed in HF patients didn’t correlate with those found in established mouse and rat HF models: Ren2 transgenic rat, angiotensin II-infused mice, and LAD ligation mouse model of ischemic HF (Vegter et al., 2017a). They did not observe any significant changes in the levels of miR-16-5p, miR-27a-3p, miR-199a-3p, miR-208a-3p, miR-223-3p, miR-499-5p, and let-7i-5p, in the plasma, kidneys, and hearts of HF animals compared with controls, even though these miRNAs were reported to be differentially expressed in corresponding human HF samples. One possible explanation of the discrepancy can be that the rodent models don’t mirror the clinical development and phenotype of human HF.

miRNAs in Atherosclerosis and Coronary Artery Disease

CAD, developing as a consequence of atherosclerosis, e.i. the formation of plaques inside of cardiac arteries, is a major mortality and morbidity cause in the United States (Benjamin et al., 2017).

miR-216a, whose level is significantly increased in CAD patients, especially older ones, has been found to contribute to atherosclerosis and CAD by inducing vascular endothelial
senescence and inflammation through SMAD family member 3/NF-κB inhibitor alpha (Smad3/IκBα) pathway, which is the main downstream mediator of TGF-β1 and underlies its anti-inflammatory effect (Yang et al., 2018). Smad3 is a potential target of miR-216a, which could potentially serve as biomarker and target for endothelial dysfunction and development of atherosclerosis.

miR-29a downregulates Collagens I and III, which are known factors in development of atherosclerosis, and the Quaking protein, which subsequently regulates scavenger receptor A and lipid uptake (Liu et al., 2017). All of these downstream factors are involved in atherosclerosis development and progression. Other potential mechanisms through which miR-29a may contribute to atherosclerosis are increased angiogenesis through the PTEN/AKT pathway and HMG box transcription factor 1 (HBP1), a regulator of cell cycle; and/or through phosphatase gene (Wang et al., 2013). Thus, miR-29a antagomiR could potentially be used as a treatment of atherosclerosis and the level of miR-29a, as a biomarker for its development.

miRNAs as biomarkers in atherosclerosis and CAD

Early detection of CAD, before it causes any symptoms, would save thousands of lives every year. Resting ECG and resting echocardiography cannot detect asymptomatic CAD and an early detection necessitates a stress test, which is cost-ineffective and impractical in the clinical setting (Cassar et al., 2009). There is an urgent need for a better diagnostic tool, which would detect CAD early, reliably, and in a cost-effective way. There are some indications that miRNAs could prove to be such tools.

Liu et al. have found increased plasma levels of miR-29a in patients with atherosclerosis – specifically, it correlated well with the carotid intima-media thickness (Liu et al., 2017), which is supported by its role in regulating collagens. Level of combined miR-483-5p with miR-451a
or with miR-155-5p 0.5 h or 1 h, correspondingly, after a percutaneous coronary intervention (PCI)-induced plaque rupture, were an accurate indicator of plaque rupture detection and timing (Li et al., 2017). However, as with other CVD, more research is required to confirm the promising preliminary results.

microRNAs in Atrial Fibrillation

AF, a highly prevalent arrhythmia, can cause stroke, HF, and sudden death, especially in older patients (Lip et al., 2017). Its mechanism is not fully understood. There are some indications that microRNAs may play a role in its development.

Inositol-3-phosphate receptors (IP₃Rs) are important regulators of cardiac function, strategically localized in cytoplasmic compartments and in the nucleus of cardiomyocytes, especially in the atria (Kockskamper et al., 2008). Qi et al. have recently found miR-26a downregulated in AF cardiomyocytes in a canine AF model (Qi et al., 2021). One of miR-26a’s targets, ITPRI (gene coding for IP₃R1), is overexpressed in dogs with AF. Silencing of miR-26a by its antagomiR lead to increased IP₃R1 expression, while an overexpression of miR-26a mimic caused downregulation of IP₃R1. There were no significant corresponding changes in the IP₃R1 mRNA levels, further supporting the evidence of a posttranscriptional regulation by miR-26a. Those changes in IP₃R1 expression levels were consistent with corresponding changes in Ca²⁺ handling in isolated cardiomyocytes, thus confirming a potential role of miR-26a in dysregulation of nuclear Ca²⁺ handling in AF. In AF there is an increase in nuclear diastolic Ca²⁺ concentration, which leads to dysregulation of calmodulin-dependent protein kinase II (CaMKII) and, consequently, histone deacetylase 4 (HDAC4), which leads to dysregulation of diverse
transcription factors and, ultimately, to AF-associated cardiac dysfunction (Wang et al., 2021), (Rahm et al., 2021).

miR-155 level was increased, while the level of its predicted target, α1c subunit of L-type calcium channel (CACNA1C), was decreased, in cardiomvocytes isolated from AF patients (Wang et al., 2021). Human induced pluripotent stem cell derived atrial cardiomvocytes (hiPSC-aCMs) transfected with miR-155 showed reduction in L-type Ca$^{2+}$ current ($I_{Ca,L}$) density, a feature also observed in AF patients. Similar changes were observed in transgenic mice overexpressing miR-155, while miR-155 knock-out mice and miR-155 transgenic mice treated with a miR-155 inhibitor did not show any changes in $I_{Ca,L}$ characteristics. L-type Ca$^{2+}$ channels are known to regulate such signaling pathways as Ca$^{2+}$/calcineurin/MEF2. Activation of the latter may lead to an adaptive fetal reprogramming, but which ultimately may contribute to fibrosis and atrial dysfunction. Thus, the data indicate miR-155 as a potential therapeutic target in AF treatment.

miR-328 is one of miRNA found to be significantly increased in AF, along with the cardiac-enriched miR-1. Intracardiac levels of miR-328 have increased more than its plasma or pulmonary vein levels (which didn’t reach significance), indicating that it’s produced mostly in the left atrium (LA) (Soeki et al., 2016). Its levels positively correlated with the LA voltage zone index (surrogate markers for an arrhythmogenic substrate underlying tachyarrhythmias (van Schie et al., 2021)) and LA volume index. Some of potential targets of miR-328 are CACNA1C and CACNB1, coding for L-type calcium channel α1C and β1 subunits, respectively. Thus, the local production of miR-328 may contribute to pathophysiological atrial remodeling in AF, by regulating genes involved in electrophysiological modulation via an autocrine or paracrine mechanism (Soeki et al., 2016).
In general, multiple miRNAs have been found to contribute to different aspects of AF pathogenesis (van den Berg et al., 2017; Komal et al., 2019; Ravelli and Mase, 2021): electrical remodeling: miR-1, miR-26, miR-133, miR-499 (deregulated potassium channels); miR-1, miR-21, miR-29a, miR-34a, miR-106b-25 cluster, miR-208b, miR-328, and miR-499 (impaired calcium currents and calcium handling); miR-1, miR-208a (abnormal impulse propagation); as well as structural remodeling: miR-1, miR-21, miR-133, miR-208a (hypertrophy and apoptosis); miR-1, miR-10a, miR-21, miR-26, miR-133, miR-590 (fibrosis). Other mechanisms underlying the electrical and structural remodeling in AF include impaired oxidation processes, regulated by such miRNAs as miR-24, miR-31, and miR-155 (NOS production); miR-1, miR-133, miR-210, miR-499 (mitochondrial function); and inflammation, regulated by miR-125a, miR-150, miR-155, and miR-302a (cytokine regulation) (Ravelli and Mase, 2021).

miRNAs as biomarkers in AF

The duration of AF is an important factor in predicting clinical outcomes, as long-term AF leads to deterioration of atrial and cardiac functions. Serum levels of miR-483-5p were increased in patients with AF, regardless of the arrhythmia duration, at baseline, 12 months, and 24 months after AF diagnosis (Wang et al., 2020). However, miR-200b-3p was upregulated in serum only at baseline and at 12 months. Targets of miR-200b-3p are involved in Wnt signaling pathway, whose dysregulation is known to contribute to the development of AF (Wolke et al., 2021). On the other hand, miR-34a-5p was upregulated at 12 and 24 months, while miR-125b-5p was downregulated at baseline. One of miR-125b-5p target genes is E3F transcription factor 3 (E2F3), which is crucial for normal cardiac development. miR-34a has been implicated in development and electrophysiological remodeling associated with AF through its target gene, ankyrin B (Ank-B) (Zhu et al., 2018). The differential miRNA levels could serve as indicators of
AF duration and as therapeutic targets in stage-dependent AF treatment. Circulating levels of miR-328 might serve as another potential sensitive biomarker for evaluating the severity of atrial remodeling (Mase et al., 2019).

AF ablation (pulmonary vein isolation, PVI) an effective treatment for AF, is associated with relatively high rate (around 40%) of AF recurrence. Some microRNAs have been suggested as promising potential predictors of successful AF ablation (Tsiachris et al., 2019). However, a thorough further verification by Kiliszek et al. (Kiliszek et al., 2020), using next generation RNA sequencing and digital PCR, have not corroborated this proposition, as none of the tested miRNAs’ serum levels showed significant differences that could potentially predict AF ablation outcomes. As in many other cases involving miRNAs, more definitive research, with more enrolled participants, is necessary to establish miRNA applicability in the clinic.

miRNAs in Other Arrhythmias

miRNAs are also emerging as important regulators in the development of other arrhythmias. Exome analysis of multigenerational family members with Wolff-Parkinson-White (WPW) syndrome found a mutation in \textit{MYH6} (\textit{MYH6} p.E1885K) segregating with the phenotype (Bowles et al., 2015). \textit{MYH6} codes the cardiac α-myosin heavy chain. The variant is localized in a highly conservative residue in the myosin tail and is predicted to elicit deleterious effects by \textit{in silico} analyses. One of \textit{MYH6} introns encompasses miR-208a, which has been involved in cardiac arrhythmias, AMI, CAD, and diabetic cardiomyopathy (DCM). miR-208a transgenic mice showed impaired expression of connexin 4 and of diverse cardiac transcription factors (Callis et al., 2009). Since the expression of miR-208a is directly correlated with the expression of \textit{MYH6}, it is conceivable that the mutation found in WPW patients could destabilize the \textit{MYH6}
mRNA and, consequently, the expression of miR-208a, which, in turn, could affect all downstream targets of miR-208a, thus exacerbating the effects of the MYH6 variant.

miRNAs in Ischemic Stroke (IS)

Some miRNAs, specifically miR-16 and miR-126, have been shown to modulate pathophysiological processes leading to and following IS (Badacz et al., 2018) (Venkat et al., 2019). Treatment with miR-126-enriched exosomes derived from mouse brain endothelial cells induced neurorestorative effect in diabetic mice after stroke (Venkat et al., 2019).

miRNAs as biomarkers in IS

Early diagnosis of IS is crucial for saving lives and limiting the resulting neurological complications (Herpich and Rincon, 2020). A few miRNAs have been shown to correlate with early stages of ischemic stroke (Bejleri et al., 2021). Of these, miR-335 proves to be the most promising candidate to serve as early biomarker for IS. It was decreased in a rat model of acute IS. One of its targets genes codes for NOTCH1 – the dysregulation of NOTCH signaling is associated with aortic valve calcification and stenosis, which may link it to acute IS (Bejleri et al., 2021). However, research on the clinical applicability of miRNAs for the detection and prognosis of stroke is promising, but very preliminary, currently without any workable solutions.

miRNAs in CV Complications of Diabetes and Metabolic Syndrome

Diabetes often coexists with HF. DCM develops through 3 stages: the asymptomatic (early), diastolic dysfunctional, and systolic dysfunctional stages (Evangelista et al., 2019). Hyperglycemia, insulin resistance, and hyperinsulinemia are known independent risk factors in the development of DCM, mainly mediated through diverse vascular effects (Ouwens and
miRNAs can contribute to the development of DCM through the enhancement of systemic inflammation, myocyte hypertrophy, and fibrosis.

Even diabetic patients with intensive glycemic control are at high risk of developing HF (Luscher, 2015). It is believed to be due to so called ‘hyperglycemic’ or ‘metabolic’ memory, as the deleterious effects of increased level of reactive oxygen species (ROS) unleashed by initial hyperglycemia do not subside even after glucose level is normalized (Ceriello, 2009). Costantino et al. have shown that miRNAs could be responsible for this phenomenon, as diabetes induces a persistent change in specific miRNA levels in the heart, which don’t revert to pre-diabetic levels even after intensive glucose level control (Costantino et al., 2016). Many miRNAs involved in the process are associated with apoptosis (miR-34a, miR-320b, miR-378), myocardial fibrosis (miR-29b, miR-30a, miR-125b, miR-150, miR-199a), hypertrophy (miR-1, miR-29a, miR-125b, miR-133a, miR-150, miR-199a, miR-212, miR-214, miR-221), autophagy (miR-30a, miR-133a, miR-212, miR-221), redox signaling (miR-19b, miR-27a, miR-34a, miR-125b, miR-146a, miR-155, miR-210, miR-221), and HF (miR-199a, miR-423, miR-499). miR-212 and miR-221, associated with autophagy and myocyte hypertrophy, through calcineurin/NFAT and p27/mTOR signaling, respectively (Ucar et al., 2012; Su et al., 2015), were increased multi-fold in hyperglycemic mice, even after 3 weeks of insulin treatment (Costantino et al., 2016).

Recent data indicate that anti-senescence effect of metformin, a popular glucose-lowering drug, is exerted through miRNAs (Giuliani et al., 2020). Metformin have been shown to modulate the expression of 27 miRNAs in human umbilical vein endothelial cells (HUVECs) undergoing replicative senescence. Many of them targeted genes associated with pathways related to cell proliferation, such as TGF-β, ErbB, Wnt, MAPK pathways; and, with the greatest
number of targets, PI3K-Akt-mTOR pathway, which corroborates with the known inhibitory effects of metformin on mTOR signaling.

miRNAs as biomarkers in CV complications of diabetes

miRNAs’ responsiveness to cardiac and vascular impairment and their stability in circulation makes them promising candidates for biomarkers and prognostics of different stages of DCM. Some of the possible candidates are miR-1, miR-1/206, miR-34a, miR-133a, miR-195, miR-212, miR-221, miR-320, miR-373, miR-378, and miR-451, as their levels have been shown to correlate with DCM onset and/or severity (Evangelista et al., 2019). Changes in miR-32 serum levels are currently being evaluated as a diagnostic and prognostic biomarker in the coronary artery calcification (CAC) progression, another CV complication of type 2 diabetes (ClinicalTrials.com).

Summary/Future Perspectives

In recent years our understanding of the role of miRNAs in the development of CVD have greatly expanded, but there is still a lot of unknowns. One of the aspects of miRNA activity appreciated in recent years has been an important role of exosomes in facilitating intercellular communication effected by miRNAs (Zara et al., 2020).

In spite of years of promising basic research, pre-clinical, and early (stage I and stage II) clinical trial results, so far miRNAs have not been approved for treatment of any CVD (Zhang et al., 2021). Many microRNAs affect multiple signaling pathways and fine-tune multiple physiological processes, which makes them enticing but also tricky therapeutic candidates. Such factors as dosing, potential cross-reactivity, and unwanted systemic effects need to be recognized and addressed through comprehensive basic and clinical research. Zhang et al. attribute the
difficulty of achieving a clean desired effect with miRNA-based therapeutics to the property intrinsic to miRNAs, which they call “too many targets for miRNA effect” (TMTME). Based on their analyses, miRNA-based drugs have 30 to up to 1000 targets (Zhang et al., 2021), making it especially difficult to prune out all undesirable effects. There are also practical hurdles that need to be overcome, such as effective delivery and stability of microRNA-based therapeutic agents.

Some of miRNAs currently in clinical trials for other indications could conceivably be repurposed for CVD treatment (Michell and Vickers, 2016). E.g. miravirsen (developed by Roche) (Janssen et al., 2013), the first antimiR to enter clinical trials, and RG-101 (developed by Regulus Therapeutics) (van der Ree et al., 2017), both target miR-122 for the treatment of chronic hepatitis C virus (HCV) infection. Both drugs initially showed promising results in reduction of HCV viral load, but were ultimately withdrawn due to their undesired immunological effects and hyperbilirubinemia (Pharmaceutical Business Review, 2017), respectively (Chakraborty et al., 2021). miR-122, targeted by these drugs, is an important regulator of lipid metabolism, and its inhibition in mice reduced plasma cholesterol levels, probably through the reduced expression of multiple genes involved in cholesterol synthesis. Thus, conceivably, drugs targeting miR-122 could be repurposed, after some tweaking to reduce their undesirable effects, to treat dyslipidemias and CVD (Esau et al., 2006).

The two main miRNA-based treatment strategies are miRNA inhibition with antagomiRs and replacement therapy with miRNA mimics. The former can be delivered as a single-stranded oligonucleotide, the latter usually require double-stranded molecules, which could be incorporated into miRISC complexes. The oligonucleotides are usually delivered as RNA derivatives, such as LNA, for increased stability (especially nuclease degradation resistance), cellular uptake, and improved pharmacokinetic properties (Duygu et al., 2019). Recent
improvements in the delivery of miRNA-based therapeutics are encouraging. Diverse delivery systems and vehicles have been proposed and tested, as reviewed in Figure 3, including systems that could potentially be coupled with established CVD treatments - stents and balloon catheters (Michell and Vickers, 2016; Dasgupta and Chatterjee, 2021). Each of these methods has its advantages and disadvantages and they all require robust testing and optimization. Conceivably, different treatments will require specific delivery systems and optimization to improve specificity, targeting, and efficacy, depending on their target cells, the amount of therapeutic agent required, duration of treatment, etc.

Circulating miRNAs are relatively stable, due to their association with diverse proteins and encapsulation in exosomes, and their levels change early and quickly in response to many pathophysiological changes accompanying the development of CVD, which makes them enticing candidates for biomarkers facilitating diagnostic and treatment decisions. So far, no miRNA has been established as a reliable diagnostic and/or prognostic biomarker in CVD, but a lot of research suggest miRNAs might eventually fill some existing gaps or even replace currently used diagnostic standards.

There is a consensus that miRNA-based treatments and miRNAs as biomarkers are worth developing, in spite of the roadblocks encountered on the way. A better understanding of miRNAs, their mode of action, specificity, etc. would allow for a more targeted, localized or even microlocalized approach and better control over miRNA-based therapeutics, providing healthcare providers with new solutions to diagnose and treat CVD patients and thus lessen the burden of CVD.
Authorship Contributions

Wrote or contributed to the writing of the manuscript: Wronska, A.
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Chistiakov DA, Orekhov AN and Bobryshev YV (2016) Cardiac-specific miRNA in cardiogenesis, heart function, and cardiac pathology (with focus on myocardial infarction). J Mol Cell Cardiol 94:107-121.


Table 1. miRNAs as potential therapeutic targets and biomarkers in CVD, and pathways and processes through which they are involved in the development of CVD.

<table>
<thead>
<tr>
<th>CVD</th>
<th>miRNAs as: potential therapeutic targets (in bold), biomarkers (in italic), or both (in bold italic), in clinical trials (underlined)</th>
<th>Pathways/processes implicated in the development of CVD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MI</td>
<td>miR-1, miR-19a/19b, miR-21, miR-34a-5p, miR-92a, <strong>miR-132</strong>, miR-144, miR-146a, miR-155, miR-181a/181b, miR-199a, miR-210, miR-212, miR-363, miR-381, miR-449a</td>
<td>PKCδ, AQ9/PI3K/AKT, PTEN, Notch; inflammation, fibrosis, oxidation, apoptosis, angiogenesis, cardiomyocyte proliferation</td>
</tr>
<tr>
<td>HF</td>
<td>miR-10a, miR-16p, miR-18a-5p, <strong>miR-21</strong>, miR-25; miR-27a-3p, miR-31, <strong>miR-34a</strong>, miR-92a, <strong>miR-99a</strong>, miR-106a-5p, miR-122, <strong>miR-132</strong>, miR-155, miR-195-5p, <strong>miR-199a-3p</strong>, miR-208a-3p, miR-212/132, miRNA-221-3p, miR-222, miR-223-3p, miR-423-3p, miR-423-5p, miR-499-5p, miR-652-3p, let-7i-5p</td>
<td>MAP kinase, TGF-β/SMAD, Notch; fibrosis, inflammation, angiogenesis, lipid metabolism, endothelial dysfunction</td>
</tr>
<tr>
<td>Atherosclerosis/CAD</td>
<td><strong>miR-29a</strong>, miR-121, <strong>miR-126-5p</strong>, miR-129a, <strong>miR-133</strong> <strong>miR-143/145, miR-155</strong>, <strong>miR-216a</strong>, miR-451a, miR-483-5p</td>
<td>PTEN/AKT, Smad3/IκBα, Notch; inflammation, angiogenesis,</td>
</tr>
<tr>
<td>Category</td>
<td>MiRNAs</td>
<td>Pathways/Signatures</td>
</tr>
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<td>--------------------------------</td>
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<td>-------------------------------------------------------------------------------------</td>
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<tr>
<td>AF</td>
<td>miR-1, miR-10a, miR-21, miR-24, miR-26a, miR-29a, miR-31, <strong>miR-34a-5p</strong>, miR-106b-25 cluster, miR-125a, <strong>miR-125b-5p</strong>, miR-133, miR-150, <strong>miR-155</strong>, <strong>miR-200b-3p</strong>, miR-208a/208b, miR-210, miR-302a, <strong>miR-328</strong>, <strong>miR-483-5p</strong>, miR-499, miR-590</td>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;/CaMKII/HDAC4, Ca&lt;sup&gt;2+&lt;/sup&gt;/calcineurin/MEF2, Wnt, E2F3; ICa,L density, fibrosis, hypertrophy, apoptosis, electrical remodeling, oxidation, inflammation, mitochondrial function, cytokine regulation</td>
</tr>
<tr>
<td>Other arrhythmias</td>
<td><strong>miR-1</strong>, <strong>miR-34a</strong>, <strong>miR-208a</strong></td>
<td>connexin 4, Notch, cardiac transcription factors</td>
</tr>
<tr>
<td>IS</td>
<td>miR-16, <strong>miR-126</strong>, <strong>miR-335</strong></td>
<td>NOTCH</td>
</tr>
<tr>
<td>Diabetes/Metabolic syndrome</td>
<td><strong>miR-1</strong>, <strong>miR-1/206</strong>, miR-19b, miR-27a, miR-29a, miR-29b, miR-30a, <strong>miR-32</strong>, <strong>miR-34a</strong>, miR-125b, <strong>miR-133a</strong>, miR-146a, miR-150, miR-155, <strong>miR-195</strong>, miR-199a, miR-210, <strong>miR-212</strong>, miR-214, <strong>miR-221</strong>, <strong>miR-320</strong>, miR-320b, <strong>miR-373</strong>, <strong>miR-378</strong>, miR-423, <strong>miR-451</strong>, miR-499</td>
<td>PI3K-Akt-mTOR, TGFβ, ErbB, Wnt, MAPK, calcineurin/NFAT, p27/mTOR; oxidation, apoptosis, fibrosis, hypertrophy, autophagy</td>
</tr>
</tbody>
</table>
Footnotes

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No author has an actual or perceived conflict of interest with the contents of this article.
Figure Legends

**Figure 1.** Some of the signaling pathways regulated by miRNAs in the development of CVD.

MicroRNAs (blue boxes) inhibit the expression of their direct targets (▼), leading to specific pathway regulation (black arrows) and, consequently (wide arrows), pathophysiological processes associated with CVD (red boxes).

**Figure 2.** Exosomes as miRNAs’ intra/intercellular communication facilitators.

Microsomes enriched with specific miRNAs communicate with neighboring and/or distant cells via autocrine, paracrine, and/or endocrine modes.

**Figure 3.** Delivery systems & vehicles for miRNA-based therapeutics.
miR-1
\[ \downarrow \]
miR-29a
\[ \downarrow \]
PTEN
\[ \downarrow \]
PI3K/AKT
\[ \downarrow \]
Angiogenesis, apoptosis
\[ \downarrow \]
Cardiac development, inflammation

miR-132
\[ \downarrow \]
FOXO1, SERCA2A, NOS3
\[ \downarrow \]
Fibrosis, apoptosis, redox regulation, calcium handling

miR-155
\[ \downarrow \]
CACNA1C
\[ \downarrow \] L-type Ca\(^{2+}\) current
\[ \downarrow \] Ca\(^{2+}/\)calcineurin/MEF2
\[ \downarrow \] Fibrosis

miR-200b-3p
\[ \downarrow \]
Wnt1
\[ \downarrow \]
Inflammation, lipid metabolism

miR-212
\[ \downarrow \]
AQ9
\[ \downarrow \]
PI3K/AKT
\[ \downarrow \] Apoptosis

miR-216a
\[ \downarrow \]
Smad3
\[ \downarrow \]
Smad3/\(\text{I\kappa}\beta\alpha)
\[ \downarrow \] Endothelial senescence, inflammation

miR-181b
\[ \downarrow \]
PKC\(\delta\)
\[ \downarrow \] Inflammation

miR-132
\[ \downarrow \]
FOXO1, SERCA2A, NOS3
\[ \downarrow \]
Fibrosis, apoptosis, redox regulation, calcium handling

miR-155
\[ \downarrow \]
CACNA1C
\[ \downarrow \] L-type Ca\(^{2+}\) current
\[ \downarrow \] Ca\(^{2+}/\)calcineurin/MEF2
\[ \downarrow \] Fibrosis

miR-200b-3p
\[ \downarrow \]
Wnt1
\[ \downarrow \]
Inflammation, lipid metabolism

miR-212
\[ \downarrow \]
AQ9
\[ \downarrow \]
PI3K/AKT
\[ \downarrow \] Apoptosis

miR-216a
\[ \downarrow \]
Smad3
\[ \downarrow \]
Smad3/\(\text{I\kappa}\beta\alpha)
\[ \downarrow \] Endothelial senescence, inflammation

miR-335
\[ \downarrow \]
Jag1
\[ \downarrow \] NOTCH
\[ \downarrow \] Atherosclerosis, aortic valve calcification/stenosis

Figure 1
Figure 2
**Figure 3.** Delivery systems & vehicles for miRNA-based therapeutics.

<table>
<thead>
<tr>
<th>DNA vectors</th>
<th>retroviral vectors</th>
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<tr>
<td>Viral systems</td>
<td>AAV 3,5,9</td>
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<tr>
<td></td>
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<td>bacteriophage-based VLP vectors</td>
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<td>Liposomes</td>
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<td>lipid nanoparticles</td>
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<td>ionizable cationic lipid nanoparticles</td>
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<td>Polymeric systems</td>
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<td>PEI-PEG (polyethylene glycol)</td>
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<td>poly(amine-co-ester) (PACE)</td>
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<td>polymer micelles</td>
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<td>Inorganic compound-based systems</td>
<td>carbonate apatite</td>
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<td>Exosome-based systems</td>
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<td>exosome-GE11 peptide</td>
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<td>Ultrasound-targeted microbubbles destruction (UTMD)</td>
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<td>Polyelectrolyte complex micelles</td>
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<td>Self-assembled RNA-triple-helix hydrogel scaffolds</td>
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<td>Stents and catheters</td>
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