REVIEW

Noncoding RNAs in Cardiovascular Disease: Pathological Relevance and Emerging Role as Biomarkers and Therapeutics

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Noncoding RNAs (ncRNA) include a diverse range of functional RNA species—microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) being most studied in pathophysiology. Cardiovascular morbidity is associated with differential expression of myriad miRNAs; miR-21, miR-155, miR-126, miR-146a/b, miR-143/145, miR-223, and miR-221 are the top 9 most reported miRNAs in hypertension and atherosclerotic disease. A single miRNA may have hundreds of messenger RNA targets, which makes a full appreciation of the physiologic ramifications of such broad-ranging effects a challenge. miR-21 is the most prominent ncRNA associated with hypertension and atherosclerotic disease due to its role as a "mechano-miR", responding to arterial shear stresses. "Immuno-miRs", such as miR-155 and miR-223, affect cardiovascular disease (CVD) *via* regulation of hematopoietic cell differentiation, chemotaxis, and activation in response to many pro-atherogenic stimuli. "Myo-miRs", such as miR-1 and miR-133, affect cardiac muscle plasticity and remodeling in response to mechanical overload. This in-depth review analyzes observational and experimental reports of ncRNAs in CVD, including future applications of ncRNA-based strategies in diagnosis, prediction (e.g., survival and response to small molecule therapy), and biologic therapy.

Keywords: atherosclerosis; biomarker; blood pressure; hypertension; microRNA; miRNA; myocardial infarction; ncRNA; noncoding RNA.

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Hypertension and cardiovascular disease (CVD) have well-documented genetic, epigenetic, and environmental effectors. While approximately 25 mutations and 53 single nucleotide polymorphisms have been associated with hypertension,[1,2] environmental factors such as diet and the increased prevalence of obesity are predominant drivers of disease.[3] Obesity is most prevalent in countries where food-related expenditures represent a low percentage of per capita gross domestic product, such as the United States. Of the 85 countries analyzed, the United States has the lowest percentage (6.4%) of total consumer spending on food and 23.5% of deaths from CVD (CDC data), compared to Nigeria at 56.4%,[4] with 7% of mortality attributed to CVD (WHO data). Fittingly, bariatric surgery (e.g., Roux-en-Y gastric bypass) showed durable remittance of hypertension and dyslipidemia extending 12 years post intervention.[5] In attempts to better understand CVD etiology, noncoding RNAs (ncRNAs) have been widely assayed and shown to have differential expression in cardiovascular and metabolic disease, when examined in a variety of samples. *In vivo* interventions blocking microRNA (miRNA) activity have been shown to remediate CVD disease in animal models. This review will focus on the main ncRNAs involved in CVD, based on current literature.

NONCODING RNAS: BIOGENESIS AND FUNCTION

The human genome is 98.5% non–protein-coding DNA. Some of this non–protein-coding DNA is transcribed to a heterogeneous group of functional RNA species broadly called ncRNA.[6,7] ncRNAs are categorized into 3 classes based on size: small (~19–25 nt.), intermediate-sized (~20–200 nt.), and long (>200 nt.); Please see Table 1. miRNAs are small (21–25 nucleotides) single-stranded RNAs, which can be highly conserved across species[8] and are the mostly widely studied functional ncRNA. miRNAs may occur in the genome in a polycistronic organization [1 primary transcriptional event yields multiple mature miRNAs from a single primary miRNA strand, this primary strand can also function as an long ncRNAs (lncRNAs) with repressor functions, and may be further processed to miRNAs. These polycistronic miRNA genes are often referred to as a "cluster" of microRNAs, however, clusters may also—simply—refer to miRNA genes with <10 kb between them. The primary miRNA (product of RNA polymerase II) may then form multiple stem loop structures that are spliced by Drosha, DGCR8 RNase II complex into multiple pre-miRNAs within the nucleus before being exported (exportin 5) to the cytoplasm where they are cleaved (Dicer-TRBP complex) and associate with argonaute 2 (Ago2) protein to become part

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Table 1. Noncoding RNA classifications

Class	Size in bases	Location	Functional role	Example
Small[106–108]	miRNAs (~19–24)	Intracellular, extracellular	Post-transcriptional regulation of gene expression by degradation or translation repression of target mRNAs	miRNA-21
	siRNA (~21–23)	Exogenous	Silencing gene expression by degradation of target mRNAs	TTR-siRNA[109]
	piRNAs (~26–31)		Repression of reproductive cell-specific gene expression	piRNAs targeting LINE1 elements
Intermediate[107,110,111]	snoRNAs (~20–200)	Intra-nucleolus	Modification and processing of rRNA precursors	SNORD
	snRNAs	Intra-nuclear	Nuclear maturation of primary transcripts of mRNAs, RNA splicing	U snRNA
Long[106,112]	>200	Nucleus, cytoplasm, mitochondria	Maintaining inactive chromatin as scaffold by repressing genes including HOXD genes	HOTAIR (>200)
			X-chromosome inactivation	XIST (~17 kb)
			Repression of XIST (antisense transcript of XIST)	TSIX (>200)

Abbreviations: HOTAIR, (HOX) transcript antisense RNA; HOX, a subset of homeotic genes; miRNA, microRNA; mRNA, messenger RNA; piRNA, piwi-interacting RNA; rRNA, ribosomal RNA; siRNA, small interfering RNA; snoRNA, small nucleolar RNA; snRNA, small nuclear RNA; TSIX, XIST antisense RNA; XIST, X-inactive specific transcript.

of the microRNA-induced silencing complex (miRISC), referred to from now as a "mature" microRNA. mRNA knockdown with siRNAs essentially utilize this endogenous miRISC pathway to knockdown targets (Figure 1). It is important to note, however, that a family of miRNAs may be coded by different chromosomes but have the same or very similar seed sequences and similar targets and physiologic roles. The miRNA expression atlas by Landgraf *et al.* is a seminal contribution to our understanding of the variable abundance of miRNA species across tissue compartments *in vivo*.[9]

miRNA genes may also be present in the introns between exons of a coding gene and are often referred to as intronic miRNAs. Intronic primary miRNAs are transcribed at approximately a 1:1 ratio with pre-mRNAs, with 1 transactivation of the "gene" potentially resulting in a mature miRNA and a mature mRNA; a context-appropriate example of this is miR-21 (MIRN21 gene) which is intronic within the mRNA coding TMEM49/VMP1 (vacuole membrane protein-1) gene.[10] Intronic ncRNAs (lnc or mi-RNA) may have a negative feedback loop with the host mRNA directly, or the pathway in which the host gene ("host RNA") plays a role. Exemplary intronic miRNA-mRNA feedback relationships include miR-33a/b (intronic) with SREBF2/SREBF1 pathways. miR-33b represses ABCA1 expression, the main cholesterol efflux protein within cells, while the host RNA SREBP2 functions to increase transcription of cholesterol synthetic and uptake genes HMGCR, LDLR, and HMGCS. miR-33a represses fatty acid degradation genes CPT1A, CROT, and HADHB, while SREBP1 increases fatty acid synthetic genes FASN, SCD, and ACC.[11,12] Other reports of intronic miRNA-host mRNA pathway feedback loop includes but are not limited to miR-579-ZFR, miR-26b-CTDSP2, miR-301-SKA2, and miR-338-AATK pathway,[13–18] making first-line investigation of the immediate pathways around the host RNA reasonable for an intronic ncRNA in question. While the TMEM49 protein product is putatively involved with autophagy mechanisms, no clear link has been verified between miR-21 and TMEM49. Data support a transcriptional schema in which miR-21 is semi-independent of TMEM49-transcription through an extra-ordinary mechanism.[19,20] An example of an intergenic miRNA gene (between protein coding genes) pertinent to CVD is miR-223 (MIRN223). A pri-miR-223 strand produces no coding mRNA and MIRN223 is present once in the human (and mouse) genome. Also, the pri-miR-223 transcript may function as a lncRNA—linc-223—with effects on interferon regulatory factor 4 (IRF4) mRNA, as studied in the context of leukemogenesis by Mangiavacchi *et al.*[21] The role of miR-223 in CVD is discussed further in the text.

Alternative host mRNA adenylation is a mechanism by which a ncRNA-host mRNA feedback loop can be differentially regulated.[13,22] More specifically, the length of a mRNA 3′UTR may be changed due to alternative polyadenylation (APA; shortened vs. the maximal length variant) removing miRNA binding sites effectively producing mature mRNA transcript variants that may not be targeted by an miRNA or ncRNA. This is obviously case specific, though >50% of human genes yield APA-derived transcript variants,[23] and may account for some of the inaccuracy in miRNA-mRNA target prediction. Currently, links between APA, ncRNAs, and CVD are not numerious,[24–26] though reports will likely increase as RNA-Seq-based analysis required for such studies becomes more commonplace.

NONCODING RNAS: LOCALIZATION AND DETECTION STRATEGIES

miRNA species may be present in bodily fluids at exceedingly low levels, that require quantitative polymerase chain reaction (qPCR)-based methods for their

detection. Those found in serum or plasma require qPCR detection and are commonly called "circulating" miRNAs and can be associated: with albumin, as a part of lipoprotein particles (high-density lipoprotein cholesterol, low-density lipoprotein cholesterol), or within submicroscopic vesicles [microvesicles (100–1000 nm), exosome (30–100 nm)] of varying sizes.[27–29] Due to the complex and not fully understood compartmental enrichment of miRNAs, it is important to reference the primary work to fully understand the methodology used to isolate miRNA, as findings will vary due to sample processing and the association of the mature miRNA species to other complexes. These caveats are especially relevant to the development of an extracellular miRNA or signature of miRNAs as a clinical biomarker.[30] Processing can have dramatic effects on miRNA levels, including contamination with red blood cell–derived ncRNAs due to hemolysis. Additionally, platelet contamination can affect plasma-derived miRNA levels. Anticoagulated blood should be spun at sufficient speed to remove platelets, and care should be taken when separating the plasma following centrifugation. It should be noted that heparin is not compatible with qPCR-based methods used for biofluid (e.g., plasma, urine, tears, etc.) miRNA detection, and whole blood should be collected in EDTA containing vacutainers. Plasma miRNAs are quite stable, and RNA preservation compounds added to the vacutainer are not needed at the time of blood collection, making retrospective or freezer-studies acceptable upon peer scrutiny. Small RNA-Seq and array-based detection methods can be used when miRNAs and lncRNAs are isolated from sources of abundant levels, such as cells or tissue.

A default PubMed search at the time of writing for "((microRNA) AND hypertension)" resulted in 528 publications—7 clinical trials including work by our group on renin-sensitive microRNAs in humans.[31] All publications are for primary literature not indexed within PubMed as a review article. A "((microRNA) AND atherosclerosis)" search returned 617 publications with 4 indexed as clinical trials. "((lncRNA) AND atherosclerosis)" yielded only 49 publications (2 clinical trials) and "((lncRNA) AND hypertension)" only 44 (0 clinical trials). Thus, this review will focus primarily on microRNA (miRNA) species. Table 2 contains a miRNA species-specific search, which dictated which miRNAs were focused on in the text. Tables 3 and 4 are not limited by the number of reports on a miRNA and are intended to be broad and inclusive.

NCRNAS ASSOCIATED WITH MYOCARDIAL INFARCTION, FOCUS ON MIR-150

ncRNAs reported in relation to myocardial infraction (MI) will be addressed briefly before the roles of individual miRNAs are reviewed. A 4-plasma-miRNA signature [miR-16, miR-27a, miR-101, and miR-150 (odds ratio = 0.08)] was associated with left ventricular (LV) dysfunction from a robust cohort of AMI patients (N = 150).[32] Decreased

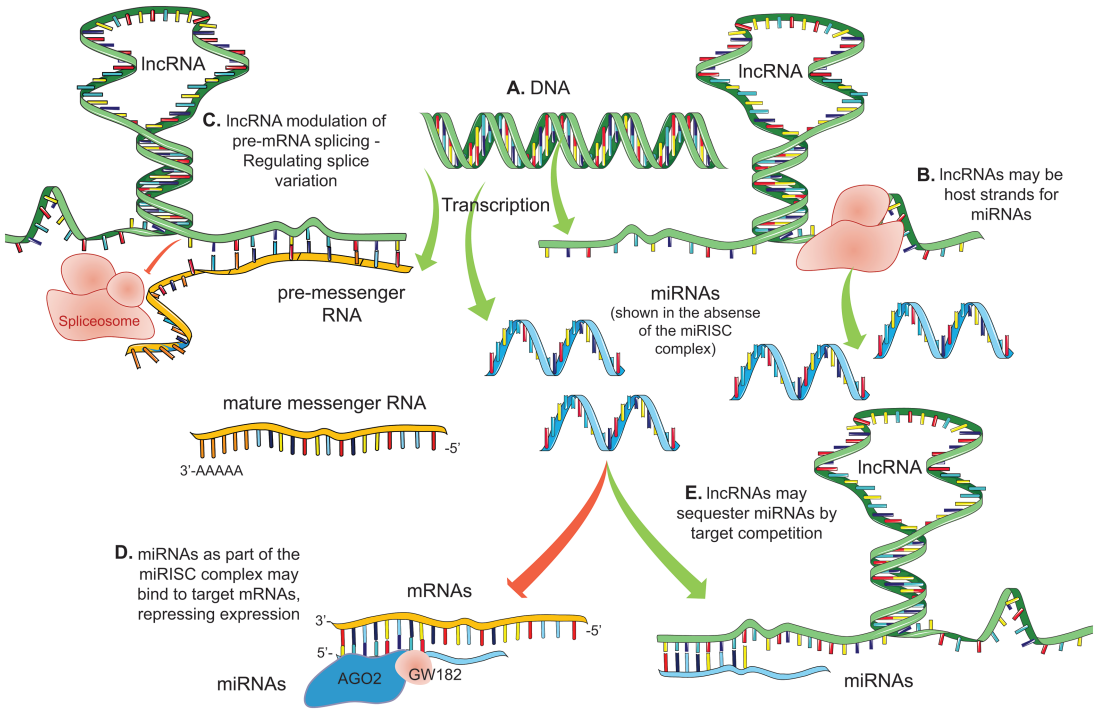

Figure 1. Dysregulation of miRNA levels in human CVD. Venn diagram depicts how miRNAs change in 3 main categories, hypertension, atherosclerosis, and MI. The arrow that precedes the miRNA represents the authors summary of the literature and is not conclusive; there may be conflicts in reports but the authors have attempted to generalize the findings to the best of their abilities. miR-21 is one of the only miRNAs that has a reasonable history of being reported in human hypertension, atherosclerosis, and MI. miR-150 is the species most associated with acute MI in humans. Many miRNAs are associated with hypertension and atherosclerosis and these will be reviewed in the text. Abbreviations: CVD, cardiovascular disease; MI, myocardial infraction; miRNA, microRNA.

Table 2. PUBMED database search returns by miRNA species

RNA name	AND (hypertension)	AND (atherosclerosis)	Combined	Family/cluster
miR-21	44	49	93	
miR-155	17	48	65	
miR-126	17	46	63	
miR-146a/b	19	37	50	
miR-145	16	23	39	A
miR-27a/b	19	15	34	
miR-143	12	19	31	A
miR-223	12	19	31	
miR-221	8	22	30	
miR-27a/b	18[a]	12[a]	30	
miR-17	18	11	29	B
miR-210	21	8	29	
miR-125	6	15	21	
miR-29	14	4	18	
let-7	9	8	17	
miR-222	6	11	17	
miR-130a	10	4	14	
miR-122	5	8	13	
miR-1	9	3	12	C
miR-34a/b	4	7	11	
miR-106a/b	3	7	10	B
miR-16	8	2	10	
miR-499	8	2	10	
miR-133	5	2	7	C
miR-200a/b	3	3	6	
miR-206	4	2	6	C
miR-10a/b	1	4	5	
miR-217	2	2	4	
miR-361	2	2	4	
miR-365	0	4	4	
miR-101	1	3	4	
miR-362	2	1	3	
miR-1185	0	2	2	
miR-15	2	0	2	
miR-483	1	1	2	
miR-188	0[b]	0[b]	0	

[a]miRNAs with a/b variants were searched in the following manner: (miR-XXa AND hypertension) OR (miR-XXb AND hypertension); (miR-XXa AND atherosclerosis) OR (miR-XXb AND atherosclerosis).

[b]Mishra[113]– does not return with search parameters in pubmed.org.

miR-150 in heart tissue itself had been previously reported in MI vs. normal heart tissue.[33] Patients (n = 90) with the lowest levels of plasma miR-150 had increased rates of cardiac remodeling at follow-up compared to patients with higher miR-150 levels,[34] and miR-150 outperformed Nt-proBNP for predicting LV remodeling in the patient cohort. The authors go on to hypothesize that this is due to miR-150-mediated repression of adrenoceptor beta 1 (ADRB1), C-reactive protein, and tumor necrosis factor (TNF) receptor-associated factor 2,[34] genes associated with heart remodeling.

Qin *et al.* found that the expression of miR-150 was significantly upregulated in human umbilical cord vein endothelial cells (HUVECs) during oxidized low-density lipoprotein (ox-LDL)-induced apoptosis, and inhibition of miR-150 partially reduced cell death, suggesting potential therapeutic function of antagomiR-150 in hyperlipidemia-induced endothelial dysfunction.[35]

Table 3. Differential microRNA expression in human hypertension and atherosclerosis

miRNA	Patient population	Tissue source	Findings	Author
miR-17 miR-15	Hypertensives with CKD *vs.* hypertensive controls (w/o CKD); mean age 77 years, African Americans	Whole blood (PAXgene Blood RNA tubes, BD, USA)	↓ miR-17 ↓ miR-15 in CKD	Nandakumar (2017)[114]
let-7	Hypertensives, carotid intima-thickness (CIT)	Plasma RNA	let-7 positively correlated with CIT	Huang (2017)[115]
miR-361-5p miR-362-5p	Salt-sensitive hypertensives vs. salt-resistant normotensive, *N* = 91	Plasma RNA	↓ in both miRNAs	Qi (2017)[116]
miR-221-3p	PAH patients (*n* = 10) vs. control (*n* = 10)	Human lung tissue Pulmonary artery smooth muscle cells	↑ miR-221-3p in lung tissues	Nie (2017)[117]
miR-1185	Northern Chinese population, *N* = 406	Plasma RNA	miR-1185 positively correlated with arterial stiffening, VCAM1, and E-Selectin	Deng (2017)[118]
miR-143 miR-145	Hyperhomocysteinaemia (Hhcy), Hhcy + atherosclerosis, atherosclerosis alone *vs.* healthy controls, *N* = 100	Plasma RNA (total)	↑ miR-143 ↑ miR-145 in disease cohort vs. control	Liu (2017)[73]
miR-221, miR-130a, and miR-155	Coronary atherosclerosis patients of different ethnicities, (*N* = 932, 681 M, 251 F), 587 Han, 146 Uygur, 91 Kazakh, 67 Hui, and 41 "other"	Plasma RNA	↓ Plasma miR-221, miR-130a, and miR-155 These miRNAs are potential risk factors for CHD	Jia (2017)[63]
miR-155	Eplerenone (MR antagonism) in older adults (*N* = 16)	Serum RNA	↑ miR-155 positively correlated with decrease in SBP	DuPont (2016)[42]
miR-365	Atherosclerosis (n = 21 M, 17 F) patients *vs.* healthy (n = 18 M, 8 F) control	Arterial plaque tissue and adjacent endometrial tissue; peripheral blood monocytes, serum	↓ miR-365 ↑ IL-6 in coronary plaque and blood monocytes	Lin (2016)[119]
miR-143 let7f miR-1 miR-29b	Atherosclerosis, *N* = 39 (*n* = 14 asymptomatic, *n* = 10 symptomatic carotid) vs. control (*n* = 15 nonatherosclerotic mammary artery)	Carotid endarterectomy	↑ miR-143 ↓ let7f ↓ miR-1 ↓ miR-29b	Markus (2016)[74]
miR-143-3p miR-222-3p	Primary human coronary artery smooth muscle cells (HCASMC, normal donors) exposed to plasma from familial hypercholesterolemic vs. normolipidemic donors (*n* = 24)	HCASMC-derived microparticle-associated RNA	↓ miR-143-3p ↓ miR-222-3p in microparticles, no change in HCASMC cells	de Gonzalo-Calvo (2016)[120]
miR-21	Hypertensives vs. normotensives (*N* = 56), with measures for carotid intima media thickness (CIMT)	Plasma RNA	↑miR-21, miR-21 positively correlated with hypertension and negatively correlated with eNOS and NOx	Cengiz (2015)[121]
miR-143, miR-145, miR-133 miR-21, miR-1	Hypertension (*n* = 60) vs. healthy (*n* = 29)	PBMC RNA	↓ miR-143, miR-145, and miR-133 ↑ miR-21, miR-1	Kontaraki (2014)[75]
miR-223	Acute ischemic stroke (*n* = 79) vs. nonstroke age matched controls (*n* = 75)	PBMC RNA	↑ miR-223 post stroke	Wang (2014)[87]
↑miR-16, miR-27a; ↓miR-101, miR-150	Post acute MI (*N* = 150)	Plasma total RNA	Changes in these miRNAs helped predict impaired LV contractility	Devaux (2013)[32]
miR-146a, miR-499	Hypertension (*n* = 340) vs. healthy (*n* = 515)	Unclear	miR-146a C>G polymorphism and allelic combinations, affect susceptibility to hypertension	Jeon (2013)[69]

Table 3. *Continued*

miRNA	Patient population	Tissue source	Findings	Author
miR-122-3p miR-519e-5p miR-200b-5p	HF-REF vs. healthy, ~70% hypertension in both groups	Whole blood (PAXgene Blood RNA tubes, BD)	miR-519e-5p negatively correlated with survival, 8 miRNA signature biomarker for SHF	Vogel (2013)[122]
miR-145	Hypertension (n = 15) vs. control (n = 7)	Atherosclerotic plaques from carotid artery	↑ miR-145	Santovito (2013)[76]
miR-21 miR-210 miR-34a miR-146a/b	Atheroma samples from 3 locations vs. left internal thoracic nonatherosclerotic artery (N = 50)	Carotid plaque Aortic plaque Femoral plaque Control arteries	Ten miRNAs differentially expressed: ↑ miR-21, -34a, -146a, -146b-5p, and -210	Raitoharju (2011)[56]

Abbreviations: CHD, coronary heart disease; CKD, chronic kidney disease; eNOS, endothelial nitric oxide synthase; HF-REF, non-ischemic heart failure with reduced ejection fraction; PAH, pulmonary arterial hypertension; F, female; M, male; MR, mineralocorticoid receptor; N, total study sample size; n, group-specific sample size; NOx, nitric oxide; PBMCs, peripheral blood mononuclear cells; SHF, systolic heart failure.

Knockdown of a cardio-related lncRNAs, ZFAs1 reduced infarct size in a rat model of acute MI.[36] miR-150 and ZFAs1 bind each other, with ZFAs1 acting as a miRNA-sponge reducing the net efficacy of the miR-150 pool within a cell to repress mRNA targets. Overexpression of miR-150 had a similar infarct-reducing effects as knock-down of ZFAs1.[36]

MIRNA REGULATION OF THE RENIN–ANGIOTENSIN–ALDOSTERONE SYSTEM

Angiotensin II (AngII) signaling through AGTR1 contributes etiologically to the development and progression of hypertension, vascular hypertrophy, and atherosclerosis *via* increased mineralocorticoid secretion by the adrenal cortex and *via* direct, deleterious effects on vascular smooth muscle cells (*VSMCs*).[37] miR-31was upregulated in rats post-MI and antagomiR-31 treatment reduced infarct size and improved post-MI left ejection fraction, while rescuing miR-31-mediated suppression of NR3C2 (mineralocorticoid receptor gene).[38] miRNA-488-3p directly targets multiple components of the renin–angiotensin–aldosterone system when examined in human and rat cells, including smooth muscle and kidney.[39] Also, AngII treatment of rat aortic SMCs increased miR-21 expression.[39] Angiotensin II receptor blocker (ARB) and angiotensin converting enzyme (ACE) inhibition in humans has been reported to alter circulating miRNA levels; however, the physiological significance of this is uncertain.[40] miR-146a/b was significantly higher in CAD patients before randomization to ARB/ACEI (plus statin therapy) for 12 months, after which ARB + statin decreased miR-146a/b levels more than ACEI + statin, though miR-146a/b was decreased from baseline in both groups. Furthermore, miR-146a/b levels at baseline predicted cardiac events in the top tertile (third) over the 12-month study.[41] We remind the reader that miR-155 upregulation correlated with the response (decrease in blood pressure) after eplerenone.[42] Overexpression of miR-155 or miR-221/222 abrogated AngII-stimulated adhesion of T cells to HUVECs.[43] Clearly, miRNA regulation of renin–angiotensin–aldosterone system is complex and understanding of the intricacies may lead to novel therapeutics for hypertension.

LONG NCRNAS AND CVD

LncRNAs are longer than 200 nucleotides and lack functional open reading frames. They are involved in cellular differentiation, proliferation, DNA damage response, and chromosomal imprinting.[44] There are an estimated 30,000 lncRNAs, with the large majority lacking functional delineation. LncRNAs function post-transcriptionally to regulate RNA splicing, mRNA degradation, and protein translation (Figure 1).[45] The first publication reporting a role for lncRNAs in CVD appeared in 1996, with the identification of H19 expression in human atherosclerosis.[46] H19 is a developmental gene that is most highly expressed during muscle during development, with undetectable levels in healthy,

Table 4. microRNAs in animal and cell culture models

miRNA	Model	Intervention	Finding	Target/pathway	Author
			Animal Models		
Dicer KO (↓ all mature miRNA—AGO2 complexes)	Mouse, Dicer KO	Genetic ablation of Dicer (needed for maturation of pre-miRNAs / active mature miRNAs)	↓ Systemic blood pressure in Dicer-KO miR-30c targets 5-HT2A in primary mVSMCs	5-HT2A	Dahan (2017)[81]
miR-34a	Rat, Myocardial ischemia-reperfusion (IR) injury model, Proximal LAD Coronary ligation	miR-34a mimic	↑ Apoptosis	SIRT1	Fu (2017)[123]
miR-31	Rat, MI model LAD (left anterior descending) coronary ligation	AntagomiR-31 (LNA-based)	↓ Infarct size 10% improvement in LEF (left ejection fraction)	NR3C2 TIMP4 TNNT2 E2F6	Martinez (2017)[38]
miR-34b	Rat, spontaneously hypertensive (SHR) vs. Wister Kyoto	Mimetic si-CDK6	↑ miR-34b	CDK6	Yang (2017)[124]
miR-34a	Rat, chronic hypoxia model	Hypoxia, miR-34 mimic and inhibitor	↓ miR-34a in hypoxic lung tissue and pulmonary artery	PDGFá	Wang (2016)[125]
miR-155	Mouse, WT vs. smooth muscle cell—mineralocorticoid receptor (MR) KO mice	↑ Age-related blood pressure	↓ miR-155 with aging, MR-KO rescued miR-155 levels in mesenteric resistance vessels	Cav1.2 AgtR1	DuPont (2016)[42]
miR-222	Mouse (C57BL/6 and Tg-miR-222)	Coronary ligation and reperfusion Exercise models	miR-222 is protective ↓ Cardiac remodeling ↓ Ischemic injury ↑ Cardiomyocyte growth	HMBOX1 p27 HIPK1/2 Vinculin	Liu (2015)[126]
miR-21	C57BL6/J mice	4% NaCl-induced hypertension, antagomiR-21 (LNA-based) *vs.* control oligo	↓ Blood pressure with antagomiR-21	JAG1	Kriegel (2015)[127]
miR-143 miR-145	miR-143, miR-145, LDLR triple knockout (KO) vs. LDLR KO	Western diet, 16 weeks	↓ Plaque volume in triple KO mice ↓Total plasma cholesterol	↑ABCA1	Sala (2014)[78]
miR-126-5p	Mouse, miR-126$^{-/-}$ Apoe$^{-/-}$	Wire injury or partial carotid ligation and high-cholesterol diet	↓Atherosclerotic lesion formation	DLK1 (Notch1 inhibitor)	Schober (2014)[128]
miR-21	Rat, acute MI model	Left coronary artery ligation, adenoviral miR-21 overexpression	Differential expression of miR-21: ↓ Infarcted areas, ↑ Border areas; ↓ Ischemia-induced cell apoptosis	PDCD4	Dong (2009)[129]
miR-21	Rat, carotid artery balloon injury model	AntagomiR-21	↓ Cell proliferation; ↑ apoptosis	PTEN and Bcl-2	Ji (2007)[130]
			***In-vitro* Models**		
miR-222	Primary, rat pulmonary arterial smooth muscle cells	miR-222 mimic	↑ Proliferation of pulmonary arterial smooth muscle cells	TIMP3 and P27	Xu (2017)[131]
miR-34a	Chronic hypoxic rat model, human pulmonary artery smooth muscle cells (HPASMC), human embryonic kidney (HEK-293) cell line	Hypoxia, miR-34 mimic and inhibitor	Promotes proliferation of human pulmonary artery smooth muscle cells	PDGFα	Wang (2016)[125]

Table 4. *Continued*

miRNA	Model	Intervention	Finding	Target/pathway	Author
miR-483-3p	Human embryonic kidney (HEK-293) clonal cell lines, rat HA-AT1R, rat HA-AT2R cell lines; primary human aortic smooth muscle cells Rat aortic smooth muscle cell line	AT1R-specific blockers [Sar1] AngII or losartan/candesartan 24 hours	VSMC-specific 22 miRNAs modulated by AngII, and AT1R regulate 17 of these miRNA; miR-483-3p specifically modulate AT1R-regulated expression of ACE-1 in VSMCs	Renin–angiotensin system (RAS)	Kemp (2014)[39]
miR-155	Primary human endothelial cells (26 individuals)	miR-155 mimic and inhibitor	eNOS is a direct target of miR-155 TNFα-mediated ↓ eNOS *via* miR-155	eNOS TNFα-eNOS axis	Sun (2012)[65]
miR-21	HUVECs, HeLa, and THP-1 (human monocyte) cell lines	miR-21 mimic, oscillatory shear stress	↑ AP-1 activation ↑ expression of adhesion molecules, ↑ VCAM-1, and MCP1	↓ PPARα	Zhou (2011)[58]
miR-365	HUVEC cell line	oxLDL-induced endothelial cell apoptosis	miR-365 promotes endothelial cells apoptosis; ↑ Atherosclerosis	Bcl-2	Qin (2011)[132]
miR-663	HUVEC cell line	Oscillatory shear stress	↑ Atherosclerosis ↑ Inflammation	IL8, ATF3, and KLF4	Ni (2011)[133]
miR-21	HUVEC cell line Uni-directional shear stress	miR-21 mimic	↓ Apoptosis ↑ eNOS phosphorylation ↑ nitric oxide production	PTEN	Weber (2010)[57]
miR-125a-5p/125b-5p	H5V-murine cardiac microvascular endothelial cell line	anti-miR-125a and anti-miR-125b	Anti-atherosclerotic Inhibit endothelial dysfunction	ET-1	Li (2010)[134]
miR-10a	Primary endothelial cells harvested from swine arterial tissues; swine aortas and aorto-renal bifurcations from, the inner curvature of the aortic arch and nearby descending thoracic aorta, and the cranial wall and caudal wall of the aorto-renal branches and the distal renal artery	Differential endothelial miRNA expression in athero-susceptible and athero-protected regions of aorta and renal arteries	↓ miR-10a athero-susceptible regions ↓ Atherosclerosis ↓ Inflammation	MAP3K7 TRC	Fang (2010)[135]
miR-188	HL-1 (cardiac muscle) cell line	Homocysteine-induced cardiac remodeling	↓ miR-188	Dicer and miR-188 are involved in Hcy-induced cardiac remodeling	Mishra (2009)[113]
miR-217	HUVEC, HAEC, and HCAEC cell lines	Cell senescence	Pro-atherosclerotic Anti-angiogenic	SIRT1	Menghini(2009)[136]
miR-221 miR-222	HUVEC cell line, HEK 293T	Wound healing assay, transfection of miR-221 and miR-222	Inhibit angiogenesis Anti-atherosclerotic	c-Kit STAT5A	Poliseno (2006)[137]

Abbreviations: ATF3, cyclic AMP-dependent transcription factor ATF-3; Bcl-2, B-cell CLL/lymphoma2; DLK1, delta-like 1 homolog; ET-1, endothelin 1; HAEC, human aortic endothelial cells; HCAEC, human coronary artery endothelial cells; Hcy, homocysteine; HIPK1/2, homeodomain interacting protein kinase 1/2; HMBOX1, homeobox containing 1; HUVEC, Human umbilical vein endothelial cells; JAG1, jagged 1; LAD, left anterior descending; MAP3K7, mitogen-activated protein kinase kinase 7; MCP1, monocyte chemotactic protein-1; oxLDL, oxidized low-density lipoprotein; PDGFα, platelet-derived growth factor receptor alpha; PTEN, phosphatase and tensin homolog; KLF4, Kruppel-like factor 4; PDCD4, programmed cell death 4; PPARα, peroxisome proliferator-activated receptor alpha; SIRT1, silent information regulator 1; STAT5A, signal transducer and activator of transcription 5A; TIMP, tissue inhibitor of metalloproteinase; VSMCs, vascular smooth muscle cells.

adult vascular smooth muscle tissue. In the study, H19 became "re-expressed" in the thickened intima of human atheromas.

Vascular remodeling is an important pathological feature of hypertension.[47] The Natarajan group was the first to identify 24 novel, AngII-modulated lncRNAs in rat VSMCs, including lnc-Ang32 which promotes VSMC proliferation.[48] Lnc-Ang362-mediates VSMC proliferation in part by encoding pre-miR-221/222.[48] LincRNA-p21, a p53-induced lncRNA, represses smooth muscle cell proliferation and is increased in human CVD.[49] Inhibition of lincRNA-p21 resulted in intimal hyperplasia following carotid wire injury in mice. LncRNA-GAS5 (growth arrest–specific 5) is another novel regulator of hypertension-induced vascular remodeling.[50] LncRNA-GAS5 knockdown (KD) in SHR rats increased both systolic and diastolic pressures. KD rats had increased intimal thickness measures with evident worsening of vascular remodeling. The findings were attributed to GAS5 regulation of the β-catenin pathway.[50] Gopalakrishnan *et al.* reported 273 differentially expressed lncRNAs in the kidney of Dahl salt-sensitive vs. salt-resistant rats.[51] The group has not yet published mechanistic pathways by which these lncRNAs alter blood pressure. Myosin heavy chain-associated RNA transcript (Mhrt) is a cardiac-specific lncRNA that is intergenic between the genes Myh6 and Myh7 (myosin heavy chain).[52] LncRNA-Mhrt is cardioprotective, preventing activity of the chromatin-binding protein, Brg1. Brg1 is part of a chromatin suppressor complex (Brg1-Hdac-Parp) that mediates cardiac remodeling and hypertrophy. The endothelium-expressed and hypoxia-induced lncRNAs-MALAT1 promoted endothelial proliferation. Furthermore silencing of MALAT1 promoted endothelial cell migration.[53]

Interestingly, miR-150 (its protective role in post-MI LV remodeling discussed previously) is known to target lncRNA-MIAT. MIAT which is positively associated with diabetes-related microvascular dysfunction and endothelial dysfunction.[54] MIAT competes with other mRNA targets for miR-150 binding, effectively reducing funtional miR-150 levels. Thus, increased MIAT may mediate its deleterious effects *via* a sponge-effect on miR-150.

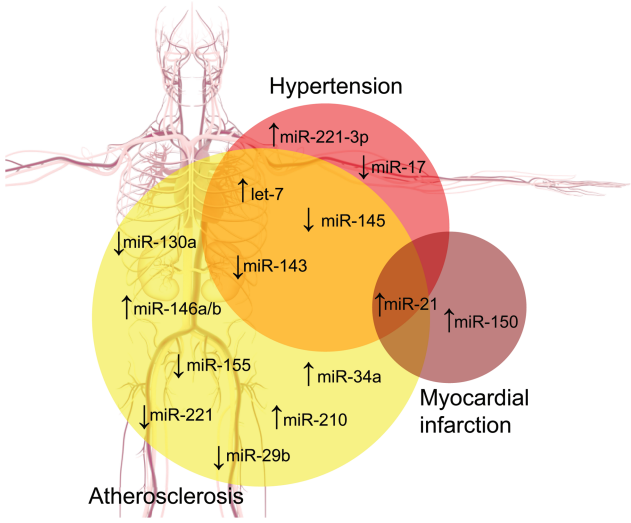

Figure 2. Summary of ncRNA biogenesis and functions within the gene expression machinery. This figure focuses on how miRNAs and lncRNAs affect each other and messenger RNA expression, but it is not exhaustive. mRNAs, lncRNAs, and miRNAs arise from the transcription of DNA by RNA polymerase II (**a**). (**b**) LncRNAs may be the host strand of miRNAs—lncRNAs may be processed into active miRNAs. Lnc and miRNAs may both be transcribed as an intronic segment of a premessenger RNA or as a product of their own transcription events, independent of mRNA synthesis. (**c**) LncRNAs may regulated the splicing of pre-mRNAs, effecting abundance of mature mRNAs as wells as the relative levels of mRNA splice variants within the cell. (**d**) The "seed sequence" at the 5′ end of the miRNA binds to complementary sequences of a target mRNA, often (but not exclusively) in the 3′-UTR. (**e**) LncRNAs may complementarily bind with miRNAs resulting in miRNA sequestration. This competitive inhibition of miRNA function reduces the number of mature miRNAs that are free to bind target mRNAs, potentially resulting in a net increase in gene expression. ncRNAs are also capable of reducing mRNA translation rates *via* interactions with ribosomes (not depicted). Abbreviations: lncRNA, long noncoding RNA; mRNA, messenger RNA; miRNA, microRNA; ncRNA, noncoding RNA.

MIR-21 AND CVD

miR-21 is one of the first mammalian microRNAs identified and is one of the most studied miRNAs in CVD (Table 1, position 1, Figure 2). miR-21 is considered by some to be a "mechano-miR"—an miRNA that is abundant in the vessel wall and responds with differential expression upon shear or mechanical stress to the vessel.[10] In humans, miR-21 is expressed in most cell types and is highly expressed in podocytes, dendritic cells, and $CD14^+$ monocytes but is nearly undetectable in kidney cells (Supplementary Table S5 from Landgraf *et al.*).[9] miRNA-21 is widely expressed in multiple types of cancers and in CVD. It is one of the most dynamically regulated miRNA in various pathophysiological process (cellular survival, apoptosis, and cell invasiveness).[55] It was found to be upregulated in human atherosclerotic plaque.[56] Weber *et al.* reported that miRNA-21 expression on endothelial cells is significantly upregulated by shear stress treatment and that it also causes an anti-apoptotic effect by directly targeting PTEN tumor suppressor gene.[57] Another study using similar methods in endothelial cells showed that miRNA-21 overexpression inhibits the expression of peroxisome proliferator-activated receptor-alpha (PPARα), resulting in upregulation in expressions of VCAM-1 and MCP-1.[58] Recently, it was reported that hydrogen peroxide and lipopolysaccharide (LPS) differentially affect the expression of several microRNAs, including miR-21, in endothelial cells before and after co-culture with monocytes.[59] These studies demonstrate an important role of miR-21 in the development of atherosclerosis (athero); however, the effect of increased miR-21 expression in endothelial cells in the context of atherosclerosisneeds further examination.

MIR-155 AND CVD

miR-155 is the prototypical "immuno-miR," with a primary role in almost all immune cells (innate and adaptive), and is the second most-studied miRNA in respect to atherosclerosis and hypertension. miR-155 promotes B cell-related immunoglobulin production, T cell proliferation in response to antigen, cytokine production, and is significantly expressed

in CD34+ cells (hematopoietic stem cells).[60] Substantial evidence supports a role for mineralocorticoid stimulation in hypertension; Dupont *et al.* found that those patients who upregulated serum miR-155 in response to MR blockage (eplerenone) had a significantly greater reduction in systolic blood pressure vs. those who did not upregulate miR-155.[42] Further, miR-155 levels were found to be dramatically downregulated in the aortas of aged WT mice, whereas SMC-MR knockout mice had a modest increase in miR-155 with age. SMC-MR-KO mice are resistant to age- and aldosterone-induced hypertension *via* dilatory effects in resistance vessels (renal and mesenteric etc).[61] Plasma miR-155 was negatively correlated with severity of coronary atherosclerosis(Gensini score[62]) in a large, multiethnicity study of 932 patients in China.[63] In mice, lack of miR-155 in bone marrow-derived cells increased atherosclerosisin LDLR-KO mice.[64] In regards to mRNA targets, miR-155 partially mediated the effect of inflammatory stimuli (TNFα)-induced endothelial nitric oxide synthase downregulation in HUVEC cells.[65] Simvastatin pretreatment ameliorated TNFα (20 ng/ml)-induced miR-155 expression in HUVECs, further clarifying how statin modulation of the mevalonate-geranylgeranyl-pyrophosphate-RhoA signaling pathway has anti-atherosclerotic effects.[65] Abundant *in vitro* studies clearly demonstrate that miR-155 is proinflammatory; however, observational human studies measuring plasma miR-155 show that low circulating levels may be predictive of adverse outcomes. Furthermore, miR-155-KO mice have increased atherosclerosis. The precise reason for this disparity, in the context of the inflammatory theory of CVD, is unclear.

MIR-146 AND CVD

Another "immuno-miR," miR-146 functions primarily in innate immune cells to negatively regulate the production of proinflammatory cytokines.[66] miR-146a is on chr5 in humans, and miR-146b is on chr 10, and the 5p sequence of mature hsa-miR-146a/b is the most abundant.[67,68] miR-146a is also one of the few instances of allelic variance in humans, with a C>G polymorphism called miR-146aG is positively correlated with incidence of ischemic stroke.[69] Severe preeclampsia, requiring termination of pregnancy, was associated with a decrease in maternal whole blood miR-146a.[70] Raitoharju *et al.* (n = 50) examined miRNA expression in the arterial wall of atheroma-containing and normal vessels and found that miR-146a, miR-146b, and miR-21 levels were elevated in plaque-burdened arteries.[56] miR-146a has been shown to be the mediator by which ApoE suppresses myeloid cell inflammation (NF- κB activation).[71] ApoE functions to enhance normophysiologic removal of circulating triglyceride-rich particles (e.g., VLDL); allelic variance (e4) or mutations that impair apoE function result in a pro-atherogenic environment with enhanced myeloid cell and vessel wall lipid accumulation. Reports favor a strong anti-inflammatory and atheroprotective role for the miR-146 family.

MIR-143/145 AND CVD

miR-143 and miR-145 are found in close proximity on human chr5, forming a classical "cluster" (149428918-149429023 [+] and 149430646-149430733 [+], respectively on GRCh38). Though part of the same cluster, miR-143 levels tend to be higher than miR-145 due to an unknown mechanism(s).[72] Human clinical data suggesting differential expression of miR-143/145 in CVD is substantial (see Table 2–3).[73–77] A large study (N = 100) by Liu *et al.* examining plasma miRNA levels found that miR-143/145 were both significantly higher in subjects with hyperhomocysteinemia (Hhcy) vs. healthy.[73] Cohorts examined included Hhcy without carotid atherosclerosis, Hhcy with carotid atherosclerosis, and carotid atherosclerosis without Hhcy. Plasma miR-143/145 negatively correlated most to total plasma cholesterol and LDL levels, but did not correlate well with measures of plaque volume.[73] Similarly, peripheral blood mononuclear cell miR-143/145 was significantly lower in hypertensives vs. healthy subjects, with miR-143/145 negatively correlating with blood pressure. miRNA levels were measured in endarterectomy samples from 2 cohorts of subjects: carotid stenosis with a history of ischemia or stroke, or asymptomatic stenosis. Data from both of these endarterectomy samples were compared to each other and to data from nonatherosclerotic control (mammary) arteries from the same endarterectomy patients.[74] The authors found that miR-143 was elevated in the asymptomatic cohort vs. mammary artery miR-143 levels. Surprisingly, this study did not assay miR-145 levels in samples. In contrast, miR-145 was found to be elevated in carotid atheromas (endarterectomy) from hypertensive patients vs. carotid atherosclerosis-without hypertension.[76] *Ex vivo* studies of human coronary smooth muscle cells found that treatment with plasma from subjects with familial hypercholesterolemia decreased miR-143 levels vs. plasma from normolipidemic individuals.[77] miR-143/145/LDLR triple KO mice were protected from plaque vs. single (LDLR) KO mice.[78]

Endothelial cells increased miR-143/145 in response to increasing laminar flow, and it is believed that endothelial-derived vesicles enriched in miR-143/145 may be exported to and taken up by smooth muscle cells potentiating vasorelaxation.[79] Vesicles collected *in vitro* were shown to decrease atherosclerosisprogression in an ApoE-KO model, which conflicts with the miR-143/145/LDLR-KO mouse findings. miR-143/145 are highly expressed in smooth muscle cells and SMC lineage cells.[80,81] Bottega *et al.* clearly demonstrated that miR-143/145-KO mice had deregulated blood pressure, due to a shift in smooth muscle cells from a contractile phenotype to a synthetic phenotype (61% decrease in potassium-induced contraction vs. WT). VSMC of KO mice have increased membrane-bound ACE-1, resulting in angiotensin resistance (miR-145 represses ACE-1 *via* 3′-UTR binding) and lower blood pressure when challenged with AngII-infusion. Neointimal lesions were found in the femoral arteries of 18-month-old miR-143/145 KO mice, while WT controls lacked lesions. These findings support a role for the miR-143/145 cluster in the phenotypic change of VSMC toward a synthetic, pro-atherosclerotic phenotype.

MIR-223 AND CVD

Similar to miR-155, miR-223 is a prototypical immuno-miR, with highest expression in myeloid cells and

downregulation required from monocyte-to-macrophage differentiation.[82,83] Thus, a role in inflammation-related diseases is not unexpected; our group found that miR-223 was elevated in the visceral adipose of obese humans in the absence of hyperlipidemia and hypertension.[84] Increased miR-223 is positively correlated with incidence of acute ischemic stroke.[85] Upregulation of miR-223 maybe be due, in part, to hypomethylation of the miR-223 promoter. miR-223 promoter hypomethylation was found to be elevated in atherosclerotic cerebral infarction patients vs. healthy controls ($N = 55$) and peripheral blood mononuclear cell miR-223 levels were found to be higher in the atherosclerotic cerebral infarction cohort, as expected.[86] In a Han-Chinese population of stroke patients ($n = 79$), miR-223 was elevated in total PMBC RNA from acute ischemic stroke victims (<72 hours after stroke) compared to blood leukocytes from older age- and sex-matched nonstroke controls ($n = 75$).[87] Interestingly, an increase in miR-223 has been shown to have an anti-inflammatory effect *in vitro*,[88] and MIR223-KO predisposed mice to a proinflammatory state with high-fat diet feeding.[89] It should be noted, however, that there are a number of human studies showing a decrease in tissue and plasma miR-223 levels in diabetes and CVD.[90] The authors are of the opinion that an increase in miR-223 in inflammatory disease is a protective, compensatory response, and that miR-223 mimetics would have a beneficial effect on CVD and inflammation *in vivo,* though this has not been reported.

MIR-1, MIR-133, AND CVD

In humans, there are 2 distinct microRNA genes, miR-1-1 (chr20) and miR-1–2 (chr18), which produce an identical mature sequence collectively referred to as miR-1. This genomic repetitiveness is not unique and there are instances of 3 genomic locations for a single miRNA (i.e., miR-133 on chr 18, 20, and 6 in humans). Both miR-1 and miR-133a/b, often called "myo-miRs", are transcribed in a bicistronic fashion and have pivotal roles in heart development; whole genome miR-133 deletion results in murine embryonic lethality.[91,92] Similarly, whole body Dicer deletion is embryonically lethal in mice and conditional dicer deletion in the myocardium resulted in massive cardiac remodeling.[93] miR-1 is an important regulator of heart adaptation after ischemia or ischemic stress, and it is upregulated in the myocardium of patients after MI, along with miR-150 and miR-133a/b.[94] miR-1, like all miRNAs, has a -3p and a -5p strand. The miR-1-3p strand is more abundant based on RNA-Seq data (reads per million) than the -5p strand in human samples.[94] Most studies do not distinguish the chromosomal origin of mature miR-1. Elevated peripheral blood mononuclear cell -derived miR-1, along with miR-21 and miR-133, were found in hypertensives and correlated with 24-hour ambulatory blood pressure.[75] A follow-up study by the authors again reported a negative correlation between miR-1 and miR-133 levels and LV mass in hypertensives.[95] A combination of miRNA-mimics (miR-1, miR-133, miR-208, and miR-499) changed the epigenetic status in cultured fibroblasts, reduced promoter methylation, and upregulated the expression of cardiogenic genes. This has implications for cardiac muscle regeneration after ischemia-reperfusion injury.[96] Finally, plasma miRNAs from 444 acute MI patients were examined and analyzed against outcome and plasma high sensitivity troponin T (hsTnt) levels.[97] The authors found that miR-133a levels were able to discriminate survivors from nonsurvivors but did not enhance the discriminatory ability of hsTnt, at 6 months post event. Thus, hsTnt remains the definitive myonecrosis marker, superior to plasma miR-133 or miR-1 in this study. Plasma miR-1 was not associated with 6-month post-event mortality, but was significantly elevated vs. an angina control cohort.[97]

NCRNA-BASED THERAPEUTICS

It has been estimated that the elimination of hypertension would reduce CVD mortality by 30.4% to 38.0%.[98] Accomplishing this will likely require next generation biologics including oligonucleotide-based approaches targeting pathologic ncRNAs, mRNAs, and proteins. Antagonizing pathologic ncRNAs is an approach that has been used with success in animal models, including nonhuman primates (as outlined in Table 4).[99,100] However, effective *in vivo* mimetics are not available due in large part to immunologic-reactivity. Targeting proteins using aptamer technology (RNA or DNA-aptamers that bind proteins) is a promising approach which has to-date focused on eye-related disease such as macular degeneration (NCT02686658—complement (C5) binding aptamer, Zimura; aptamer to vascular endothelial growth factor (VEGF)—Macugen/Pegaptanib, FDA approval 2004), though potential uses are vast. The authors believe that aptameric approaches have great potential for the future of biologics as these complex conjugates allow for a large array of modifications and are better adapted to high throughput screening than minimally modified oligonucleotides. Aptamers perform a similar function to monoclonal antibody-based therapies, without requiring animal products/production of immunoglobulins resulting in a reduced risk of toxin contamination and a lower potential for immunogenicity. Aptamers can be synthesized entirely using chemistry-based modifications, increasing purity and stability beyond that of mAbs, at a lower production cost. Aptamer-based screening of human plasma offers a high throughput and highly precise means of proteomic analysis of clinical samples, with great relevance to CVD.[101]

Stability was the primary barrier to the use of nucleotide-based drugs, as naked RNA and DNAs with a serum half-life of minutes for RNAs[102]; however, great strides have been made to improve stability, making them suitable therapeutics. Modifications can be made in order to improve or modify binding properties, many of which were developed during the production of aptamers over the last 3 decades. Aptamer technology is maturing and is based on a DNA or RNA backbone, with the aptamer targeting a protein (e.g., IL-6, VEGF, Factor IXa). Development of an aptamer involves screening a target (protein) with an aptamer library; similar to small molecule drug screening. New methods (e.g., SELEX) have identified many useful aptamer-protein target interactions.[103] To date, aptamers have had limited success many due to immune-reactivity, as was found with the aptamer to factox IXa. A significate benefit of an aptamer-based approach is that aptamers can have antidotes allowing for near-immediate cessation of aptameric effects,

something that is not available for mAb-based therapies. Aptamer pursuits helped lead to the development of modifiers (sugar backbone and phosphate backbone modifications) that stabilize RNA- and DNA-based injectable. Newly developed mutant polymerases (Y639L, H784a, Y639F, etc.) allow for the addition of "pre-modified" (e.g., 2'OMe bases) reactants to the polymer (as opposed to post-synthesis, polymer modification) allowing for more efficient production of stable oligos. Also, high throughput of 50 base long oligos using proprietary solid phase phosphoramidite chemistry allows for synthesis of gram quantities at GMP+ purity needed for human therapeutics.[104]

siRNAs/shRNA to mRNAs are particularly effective at targeting liver gene expression. Localization of oligonucleotide-based therapeutics to the liver is similar to other drugs and is a limitation that needs improvement for potency in peripheral tissues. One clinical use that takes advantages of hepatic accumulation is for the treatment of familial transthyretin amyloidosis leading to cardiomyopathy or neuropathy. siRNAs, as previously mentioned, utilize the RISC assembly (native to miRNAs) to prevent the translation and production of mutated TTR, reducing accumulation of the mutant protein. RNAi-based approaches are currently being tested for use in various conditions including cancer and for further information, we suggest the review article by Sullenger *et al.*[103]

The least investigated but most exciting potential use is in RNA-guided therapies using endoncucleases (e.g., CRISPR-Cas9) for *in vivo* gene editing, essentially ncRNA-mediated genetic engineering.[103,105] Clinical applications are still far off but are in development.

ncRNAS AS BIOMARKERS IN CLINICAL MEDICINE

Blood tests for circulating ncRNA levels measured or other bodily fluids will become part of standard of care in the clinic for diagnosis as well as to measure response to therapy. The main hurdle to this is standardization of sample processing, identification of the most relevant component of the blood or biofluid to assay (Blood-lipoprotein-associated ncRNAs, albumin, peripheral blood mononuclear cells, platelets, etc), streamlining of ncRNA assays, and sufficient prospective clinical testing. Plasma miR-133a/b was examined as a putative marker of MI and was found to be sensitive, though inferior to hsTnT measures.[97] Furthermore, combining miR-133 and hsTnt data did not increase the ability to discriminate between MI survivors and nonsurvivors vs. hsTnT alone.

The future of ncRNA-based biomarkers and therapeutics is bright and has been expanding as opposed to contracting. Thus, the authors believe that we will see more such approaches to human disease reach the clinic.

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DISCLOSURE

The authors declared no conflict of interest.

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