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Review Article

MicroRNAs: a new piece in the paediatric cardiovascular disease puzzle

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Abstract Cardiovascular diseases in children comprise a large public health problem. The major goals of paediatric cardiologists and paediatric cardiovascular researchers are to identify the cause(s) of these diseases to improve treatment and preventive protocols. Recent studies show the involvement of microRNAs (miRs) in different aspects of heart development, function, and disease. Therefore, miR-based research in paediatric cardiovascular disorders is crucial for a better understanding of the underlying pathogenesis of the disease, and unravelling novel, efficient, preventive, and therapeutic means. The ultimate goal of such research is to secure normal cardiac development and hence decrease disabilities, improve clinical outcomes, and decrease the morbidity and mortality among children. This review focuses on the role of miRs in different paediatric cardiovascular conditions in an effort to encourage miR-based research in paediatric cardiovascular disorders.

Keywords: MicroRNAs; paediatric cardiovascular diseases; biomarker; therapeutic target; cardiac stem cells

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Cardiovascular disease claims 2300 lives each day in the United States, averaging one death every 39 s and consumes 17% of the national health budget in America. Without any change in preventive efforts or treatment practices, it is projected that the number of people with one or more forms of heart disease will increase from 36.9% to 40.5%, to a total 116 million American adults by the year 2030.[1] Many of these disorders have childhood origins and are therefore important to diagnose early and administer treatment in a timely manner. Efforts towards prevention are essential to decrease the prevalence of congenital heart defects in both young and ageing populations. This necessitates improvement and development of novel therapeutic modalities based on a better understanding of the underlying mechanism leading to disease.

The discovery of miRs has provided new insights into disease mechanisms. These small non-coding RNA molecules regulate the stability and/or the translational efficiency of target messenger RNAs.[2] Since their initial discovery in 1993, more than 1400 miRs have been identified in mammals, and have revolutionised our approach to understanding gene regulation.[3] MiRs add an entirely novel layer of post-transcriptional regulation[4] and are predicted to influence the activity of $\geq$50% of all protein-encoding genes in mammals.[5] MiRs have been shown to be important not only for heart and vascular development, but also as prerequisites for normal cardiac function. They play essential roles in cardiac pathophysiology, including hypertrophy, arrhythmia, and ischaemia.[6] Increasing evidence demonstrates that miRNAs are dysregulated in several cardiovascular

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disorders and that miRNA expression plays an important role in the pathogenesis of paediatric cardiovascular disorders (Table 1, Fig 1).

MiRs and congenital heart diseases

Congenital heart defects account for ~40% of prenatal deaths and more than 20% of deaths in the first month of life.[7] A complete cure of a congenital heart defect in childhood is exceptional, and with increasing life expectancy the population of adults with clinical manifestation of congenital heart diseases continues to expand, reaching up to 90% of children born with congenital heart diseases.[8] Among adults in the year 2000, the median age of the population with congenital heart diseases was 40 years, with a median age of 29 years in those with severe disease versus 42 years in those with other congenital heart diseases.[9]

MiRs are known now to play central roles as governors of gene expression during cardiovascular development,[10] involving the integration of multiple cell lineages into the three-dimensional organ and its connection to the vascular system.[11] The important roles of miRs in cardiogenesis and early embryonic patterning processes are evidenced by the rapid increase in detectable miRs in tissues derived from all three germ layers.[12] Such roles are further confirmed by gain and loss of function experiments in mice showing that aberrant expression of selective miR produce defects.[13]

MiR-1 was the first miR shown to regulate fundamental aspects of heart development.[14] Overexpression of miR-1 in the embryonic heart inhibits cardiomyocyte proliferation and prevents expansion of the ventricular myocardium, causing lethality due to deficiency of cardiomyocytes and insufficient muscle mass.[14] Consistent with this, development of Xenopus hearts is also blocked by injecting embryos with miR-1.[15] Targeted deletion of miR-1-2 in mice resulted in 50% embryonic lethality, largely due to ventricular septal defects, whereas the surviving mutant mice also died at a later stage because of conduction system defects.[16]

Conditional deletion of Dicer, the enzyme required for miR processing, causes mouse embryos to die from cardiac failure by embryonic day 12.5 (E12.5) because of underdeveloped ventricular myocardium.[17] Disturbance of neural crest cell migration into the derivatives of the pharyngeal arches and pouches can account for many of the developmental defects. Intriguingly, phenotypic overlap between genetic disorders in cardiovascular and neuronal-craniofacial defects, including DiGeorge Syndrome, Noonan syndrome, LEOPARD syndrome, cardio-facio-cutaneous syndrome, and Costello syndrome, has been described.[18,19] The targeted deletion of Dicer in neural crest cells led to severe craniofacial and cardiovascular defects, which are reminiscent of features of human congenital neuro-craniofacial-cardiac defects.[20]

MiR-133a-1/miR-1-2 and miR-133a-2/miR-1-1 genes are expressed throughout the ventricular myocardium and interventricular septum from E8.5 until adulthood.[14,21] Mice lacking either miR-133a-1 or miR-133a-2 do not display obvious cardiac abnormalities, whereas deletion of both miRs results in late embryonic and neonatal lethality due to ventricular septal defects and chamber dilatation.[22] Similar cardiac abnormalities are observed by targeted deletion of the miR-17~92 cluster.[23] MiR-196a was found in foetal human heart samples at a gestational age of 12–14 weeks.[24] This miR regulates HOXB8-Shh signalling, which is required throughout cardiac septation, outflow tract morphogenesis, and valve formation.[25]

Just recently, miRs have been explored in one of the congenital cyanotic heart diseases.[26] Expression studies of miRs in the right ventricular myocardium of children with non-syndromic tetralogy of Fallot showed a significantly altered expression of 61 miRs. Potential targets of the altered miRs are gene networks important for cardiac development.[26]

Comprehensive miR profiling in human foetal single-ventricle cardiac tissues revealed 48 differentially expressed miRs of which 38 were downregulated and 10 were upregulated in comparison to control cardiac tissue.[27]

Dysregulation of miRs has also been reported with syndromic congenital heart diseases such as trisomy 21 (Down syndrome), the most common genetic cause of congenital heart defects.[28] A total of five miRs – miR-99a, let-7c, miR-125b-2, miR-155, and miR-802 – were identified on human chromosome 21, and were found to be overexpressed in the heart of afflicted patients who contained the extra Hsa21 chromosome.[29] DiGeorge syndrome, which in many patients leads to congenital heart disease, is caused by a deletion of the DiGeorge syndrome critical region 8 on chromosome 22 (22q11.2), which encodes a component of the RNA-induced silencing complex. The fact that this syndrome results from haploinsufficiency of this locus raises the possibility that perturbation of miR expression could contribute to the gene dosage sensitivity of this disease by impacting numerous miR targets.[30] In conclusion, it is possible that lethal cardiovascular diseases related to both of the major genetic backgrounds of syndromic congenital heart diseases, Down and DiGeorge Syndrome, are caused by dysregulated expression of specific miRs including miR-99a, let-7c, miR-125b-2, miR-155, miR-802, and RNA-induced silencing complex.

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Table 1. An overview of miRNAs in different paediatric cardiovascular disorders.

MiRs	Function	Dysregulation	Target	Associated disorder	Reference
Congenital heart defects					
MiR-1	Inhibits CM proliferation[14]	Upregulation		VSDs[16]	14,16
MiR-133a-1/miR-1-2 miR-133a-2/miR-1-1		Deletion		Embryonic and neonatal lethality due to VSDs and chamber dilatation in mice[14,21,22]	14,21,22
MiR-17∼92 cluster[23]		Deletion		VSDs and chamber dilatation	23
MiR-196a[24]	Required for cardiac septation, outflow tract morphogenesis, and valve formation[25]		Regulates HOXB8-Shh signalling		24
MiR-99a, let-7c, miR-125b-2, miR-155, and miR-802	Identified on human chromosome[21]	Upregulation		Down Syndrome	28,29
Cardiometabolic disorders					
Lipid metabolism and obesity					
Adipogenesis					
MiR-21	Increased during early adipogenic differentiation in the human multi-potent MSCs[44]				44
MiR-20	Upregulated in mature differentiated adipocytes[46]				46
MiR-103	Upregulated during differentiation of human pre-adipocytes	Responsible for adipogenic gene expression?			50
	Overexpressed in response to increased triglyceride accumulation	Targets cellular acetyl-CoA pathways			
MiR-15a	Reduce pre-adipocyte size		Fine-tuning of Dlk1[48]		48
MiR-210	Stimulates lipid droplet formation and adipocyte hypertrophy in 3T3-L1 cells[42]	Upregulated			42
MiR-27b	Stimulates lipid droplet formation and adipocyte hypertrophy[47]	Downregulated			47
Adipogenic inhibition					
MiR-27a	In pre-adipocytes suppresses adipocyte differentiation[45]	Overexpressed	Repressing expression of *PPARγ* in human MSCs[44,52]		44,52
MiR-448			Represses KLF5[53]		53
Glucose metabolism and hyperglycaemia					
Pancreatic islet-specific miR-375, miR-124a and let-7b	Regulates blood glucose homoeostasis through regulation of β-cell function, particularly exocytosis of insulin-containing vesicles				
MiR-30d	Influences insulin transcription and protects β-cell functions impaired by proinflammatory cytokines		Map4k4 in pancreatic β-cells and influences insulin transcription[56]		56

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Table 1. *Continued*

MiRs	Function	Dysregulation	Target	Associated disorder	Reference
MiR-33a and -b	Regulate cholesterol metabolism, fatty acid oxidation, and insulin signalling[57]			Metabolic syndrome	57
Heart failure					
MiR-1, miR-29, miR-30, miR-133 and miR-150		Downregulated			
MiR-21, miR-23a, miR-125, miR-146, miR-195, miR-199 and miR-214[61–63]		Upregulated			61–63
MiR-17 ~ 92	Adverse structural remodelling during heart failure[65,66]		Targets CTGF		65,66
Macrophage-derived miR-155		Increased expression[67]			67
Arrhythmia					
MiR-1	Shortens the terminal phase of atrial electrical remodelling[90]	Decreased in AF patients	Upregulation of Kir2.1 subunits maintains	AF	90
MiR-328	Adverse electrical remodelling		Partially through targeting L-type Ca^{2+} channel genes[91]	AF	91
MiR-1202		Downregulated miR in patients with AF + mitral stenosis[92]		AF + mitral stenosis	92
MiRs-1, miR -133a and their target mRNAs	Encoding ion channels	Were downregulated[94]		AF	94
MiR-1	Exacerbates arrhythmogenesis Enhances cardiac excitation–contraction coupling Promotes arrhythmogenic sarcoplasmic reticulum Ca^{2+} release[95] Exacerbates arrhythmogenesis in normal and infarcted hearts[96,97]	Overexpressed	Direct repression of KCNJ2 and GJA1[95]		95–97
Biomarkers					
MiRNA SNP rs11614913 in miR-196a2	Predictor of CHD[100]				100
Increased plasma miR-1	Biomarkers in heart failure[101,102]				101,102
Reduced levels of miR-126, the miR-17/92 cluster (miR-17, miR-20a, and miR-92a), miR-130a, miR-221, miR-21 and members of the let-7 family	Biomarker for CAD[106]				106
SNPs in pre-miRNAs has-miR-196a2 and hsa-miR-499	Biomarker for increased risk of DCM[111]				111

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Table 1. *Continued*

MiRs	Function	Dysregulation	Target	Associated disorder	Reference
Therapeutic targets					
Anti-miR-33	Treating metabolic syndrome through regulation of fatty acid metabolism and insulin signalling[108]				108
Anti-miR-122	Potential therapeutic target for the treatment of hypercholesterolaemia[109]				109
MiR-27a mimics	To regulate pre-adipocyte proliferation and may evolve into useful anti-adipogenic drugs[110]				110
MiR-1 and miR-133	Have an arrhythmogenic action[97]				97
Cardiac stem cells					
MiR-99b and miR-181a	Component of an endothelial miRNA signature and play important roles in the differentiation of pluripotent hESCs to vascular endothelial cells[123]				123
MiR-181b	Component of an endothelial miRNA signature and plays important roles in the differentiation of pluripotent hESCs to vascular endothelial cells[123]				123
MiR-125b	hESC differentiation into myocardial precursors and CMs Plays a regulatory role in the early stages of hESC differentiation through targeting Lin28? Induces the formation cardiac mesoderm Upregulates of GATA4 and Nkx2-5 Accelerates progression of hESC-derived myocardial precursors to embryonic CM phenotype				124
MiR-1	Cardiac differentiation of hESCs[125] Facilitates electrophysiological maturation[125]				125
MiR-499	Cardiac differentiation of hESCs[125] Promotes ventricular specification of hESCs and miR-1 Facilitates electrophysiological maturation[125] Promotes differentiation of hESCs into mechanically integrated CMs[126]				125,126
Mix of miR-21, miR-24, and miR-221	Improves engraftment of transplanted cardiac progenitor cells[121]				121

AF = atrial fibrillation; CAD = coronary artery disease; CHD = coronary heart disease; CMs = cardiomyocytes; CTGF = connective tissue growth factor; DCM = dilated cardiomyopathy; hESCs = human embryonic stem cells; Map4k4 = mitogen-activated protein 4 kinase 4; miRs = microRNAs; MSCs = mesenchymal stem cells; SNP = single-nucleotide polymorphism

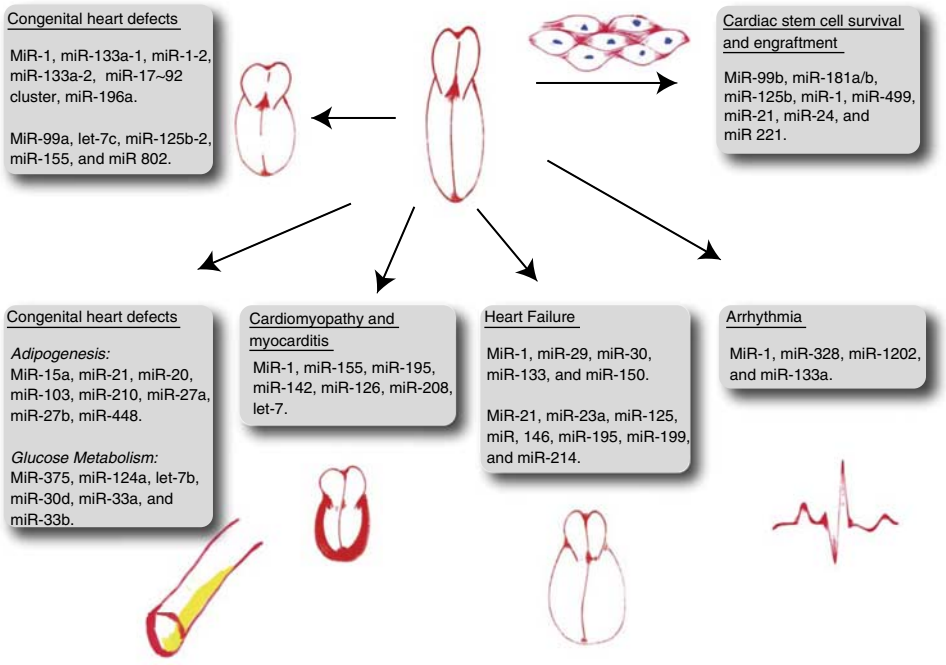

Figure 1.
Role of miRNAs in cardiovascular diseases.

MiRs and cardiometabolic clusters

Childhood obesity is a major concern because of its close association with hypertension, dyslipidaemia, and type 2 diabetes mellitus. The worldwide prevalence of childhood obesity increased from 4.2% in 1990 to 6.7% in 2010. If this trend is to continue, the prevalence is expected to reach 9.1% by 2020.[31] With the rise in obesity rates, metabolic syndrome has become prevalent among children worldwide,[32] which is one of the most serious challenges to global health in the modern world. Obesity and type 2 diabetes mellitus are major risk factors for coronary heart disease, the leading cause of death in the West.[33] It is estimated that the prevalence of coronary heart disease in the United States will increase by 16% by the year 2035 – a significant component attributable to increased type 2 diabetes mellitus and adolescent obesity.[34] This will place a major and perhaps insurmountable burden on our healthcare systems, and preventive measures, early diagnosis, and early intervention are urgently needed. Obese children develop early-onset atherosclerotic lesions and clustering of metabolic abnormalities that persist during adulthood.[35–38] This renders childhood obesity as a consistent predictor of adult heart disease and hypertension in both young and old.[39] Much evidence now indicates aberrant genetic components including miRs that cause enhanced predisposition of some individuals to obesity and type 2 diabetes, and treatment strategies based on these are under development.

MiRs have been shown to have regulatory roles in glucose and lipid metabolism and many of the steps leading to obesity, including adipocyte development, proliferation, differentiation, insulin action, and fat metabolism.[40–44]

In vitro cell studies showing that different miRs increase at different stages of adipocyte development suggest key roles for miRs in the stage-specific regulation of adipogenesis. MiR-21 increased transiently during early adipogenic differentiation of human multi-potent mesenchymal stem cells,[45] while increased miR-20 levels were reported in mature adipocytes.[46] Overexpression of miR-210 promotes lipid droplet formation and adipocyte hypertrophy in 3T3-L1 cells.[42] Similar effects were observed by downregulation of miR-27b during adipocyte differentiation.[47] Adipocyte proliferation is further promoted by miR-15 through fine-tuning of Dlk1, whereas miR-15a inhibition appears to reduce pre-adipocyte size.[48]

MiR-103 and the closely related miR-107 are upregulated in the liver of obese mice, and treatment of mice with miR-103/107 antagomiRs was shown to reduce obesity.[49] MiR-103 is also upregulated during differentiation of human pre-adipocytes, and its levels are enhanced during adipogenic stimuli, for example, in response to increased triglycerides. One of the targets of miR-103/107 is caveolin-1,

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and downregulation of caveolin-1 expression by miR-103/107 results in decreased insulin sensitivity by inhibition of the insulin receptor, depressed AKT activity, and decreased glucose uptake. Other targets pathways of miR-103/107 include enzymes of acetyl-CoA and lipid metabolism.[50] These miRs are attractive candidates for the treatment of hyperlipidaemia and obesity possibly through the use of liver-directed anatgomiRs. Similar to all such approaches, there may be off-target consequences of such treatments, which may cause adverse side effects; such safety issues will need to be thoroughly tested in appropriate preclinical models.

MiRs have been identified that enhance or inhibit growth and differentiation of adipose. For example, overexpression of miR-27a in pre-adipocytes suppresses adipocyte differentiation[51] possibly by repression of *PPARγ*, an established transcriptional factor for adipogenic genes.[44,52] MiR-27a expression is depressed in mature adipocytes from obese mice compared with lean mice, suggesting that miR-27a downregulation is required for adipocyte hypertrophy.[45] Another potential inhibitor of adipogenesis is miR-448, which targets and represses transcription factor Krueppel-like factor 5, a nuclear protein that binds the epidermal growth factor response element.[53]

In addition to obesity, hyperglycaemia is another major component of the metabolic syndrome that is also under significant regulation by miRs (reviewed in).[54] MiRs significantly regulate the production and secretion of insulin, while simultaneously affecting the sensitivity of its target tissues.[55] Specifically, pancreatic islet-specific miR-375 along with miR-124a and let-7b play key roles in blood glucose homeostasis through regulation of β-cell function, particularly exocytosis of insulin-containing vesicles. MiR-124a and let-7b are both abundantly expressed in pancreatic islet β-cells. MiR-30d influences insulin transcription and protects β-cell functions from impairment by proinflammatory cytokines by targeting mitogen-activated protein 4 kinase 4.[56] The resultant hyperinsulinaemia causes an increased rate of fat storage and deposition in organs and tissues. MiR-33a and -b regulate three of the major metabolic pathways involved in the risk for metabolic syndrome. In concert with their host genes, the sterol-regulatory element-binding protein transcription factors, miR-33a and -b, balance cholesterol metabolism, fatty acid oxidation, and insulin signalling.[57] Treatment of metabolic syndrome and decreasing its prevalence is the ultimate intermediate goal in the process of preventing coronary heart disease, which renders miR-33a and -b of special interest for further research.

Metabolic derangements including insulin resistance, hyperlipidaemia, and hyperglycaemia are accompanied by dysregulation of specific sets of miRs, and these conditions in turn trigger dysregulation of secondary miRs with targets that lead to obesity, metabolic syndrome and ultimately increased risk of cardiovascular disease. These miRs work by positive and negative regulation of multiple genes and are becoming attractive targets for global suppression of metabolic syndrome.

MiRs and heart failure

Heart failure is a major public health problem, affecting nearly 23 million people, and accounts for 5% of all medical hospital admissions and 2% of global health spending worldwide. Heart failure among accounts for at least 50% of referrals for paediatric heart transplantation.[58] The largest heart failure burden comes from children with congenital malformations. It has been estimated that 15% to 25% of children who have structural heart disease develop heart failure.[59] The involvement of miRs in the pathogenesis and progression of heart failure is further supported by a recent review,[60] explaining the role of miRs in myocyte hypertrophy, cardiomyocyte apoptosis, interstitial fibrosis, reduced capillary density, and activation of the immune system. This review documents the growing evidence that miRs contribute to pathological remodelling of the heart by regulating the expression of target genes that are involved in fibrosis, endothelial cell function, angiogenesis, and inflammation. Although some miRs have very specific functions in one cell type – for example, miR-126 in endothelial cells, other miRs are more ubiquitously expressed and regulate gene expression in multiple cell types.[60]

Recent studies comparing miR expression profiles from failing, non-failing, and foetal human hearts found that reactivation of a foetal miR program may be a feature of gene expression defects in the failing human heart.[24] Specific miRs are consistently found to be aberrantly expressed in the myocardium of heart failure patients and reveal a signature pattern of expression. Among these are miR-1, miR-29, miR-30, miR-133, and miR-150 that are downregulated in heart failure patients, whereas miR-21, miR-23a, miR-125, miR-146, miR-195, miR-199, and miR-214 were upregulated.[61–63] Interestingly, most of the constitutively down- and upregulated miRs during heart failure are similarly down- and upregulated in cardiomyocyte-specific Dicer knockouts, suggesting a normal high-level expression in cardiomyocytes. Dicer knockout hearts have severely depressed amounts of contractile proteins and consequent contractile insufficiency. Therefore, miRs have clear and essential roles in myocardial development and maturation, and

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defective expression of these miRs may contribute significantly to the origin and progression of congestive heart failure.[64] In terms of mechanism of action, there is emerging evidence that many of the identified miRs regulate the expression levels of genes that govern the process of adaptive and maladaptive cardiac remodelling.[64] For example, miR-17~92 has been reported to target connective tissue growth factor that, in the heart, is associated with adverse remodelling during heart failure.[65,66] Connective tissue growth factor is a matricellular protein with roles in many biological processes, including cell adhesion, migration, and proliferation, and with a critical role in regulating inflammation and fibrosis, disease suggests a further role of inflammation-associated miRs in the pathogenesis of heart failure. This suggestion is further supported by increased macrophage-derived miR-155 expression during heart failure in mice, indicating a role for non-cardiomyocyte-derived miR-155 in the immune pathogenesis of heart failure as well.[67] Low let-7i levels was also associated with poor clinical outcome.[68]

Very recently, new data have indicated that miR-22 acts as an integrator of Ca (+2) homeostasis and myofibrillar protein content during stress in the heart, and therefore shed light on the mechanisms that enhance propensity towards heart failure.[69] Alterations in miR expression have been observed during the process of right ventricular remodelling and in the gene regulatory pathways, leading to right ventricular hypertrophy and right ventricular failure.[70] Interesting observations made in this study include differential regulation of miRs between the right and left ventricles. MiR-34a, miR-28, miR-148a, and miR-93 were upregulated in right ventricular hypertrophy/right ventricular failure, but remained downregulated or unchanged in left ventricular hypertrophy/left ventricular failure. Therefore, dysregulation of these miRs may contribute to the increased susceptibility of right ventricular hypertrophy to heart failure.[70] MiR-21 regulates gene expression in multiple cell types in the heart. MiR-21 is profibrotic in fibroblasts, anti-apoptotic in cardiomyocytes, anti-angiogenic in endothelial cells, and anti-inflammatory in immune cells. Direct targets of miR-21 responsible for these effects include PDCD4 (phosphatase and tensin homologue, anti-inflammatory), SMAD7, Spry1 (sprouty homologue 1), PTEN, RhoB (ras homologue gene family member B), and FasL (fas ligand).[60]

Characterisation of miRs in heart failure may lead to new therapies including miRs/antagomiRs perhaps combined with gene therapy to treat heart failure. SERCA2a gene therapy for failing hearts was shown to restore miR-1 expression by a pathway involving Akt/FoxO3A that normalised the expression of the sodium–calcium exchanger-1 (NCX1) and improved cardiac function.[71] These findings are supported by several studies that indicate that miR-1 plays a protective role against decompensated cardiac hypertrophy and heart failure.[72–74] Although gene therapy for adult heart failure is still in the trial stage, whether miR-mediated therapy could be applied usefully to paediatric heart failure patients needs to be elaborated.

MiRs in myocarditis and cardiomyopathy

Myocarditis represents a serious cause of cardiac dysfunction in children,[75] which results in chronic dilated cardiomyopathy and death in up to 20% of the affected children.[76] The pathogenesis of the disease is poorly understood, morbidity and mortality are high, and currently employed treatment strategies have little impact on improving the outcome. Only very recently, miRs were identified to be involved in viral myocarditis pathogenesis and susceptibility. It has been suggested that miRs possibly play a role in the pathogenesis of viral myocarditis through regulation of ion channel protein expression and adverse immune response to cardiotropic viruses.

MiR-1 may play a major role in myocarditis. MiR-1 is characteristically upregulated in viral myocarditis and causes suppression of Cx43,[77] the main protein forming gap-junction channels in ventricular myocardium that allows electrical coupling and communication between adjacent cardiomyocytes,[77] revealing the ability of a miR to regulate the expression of an ion channel protein in viral myocarditis. An antiviral activity for anti-miR-1 (AmiR-1) and AmiR-2 has been detected in Coxsackie virus B3 myocarditis.[78] The application of pRNA technology in the treatment of Coxsackie virus B3 infection and viral myocarditis in this study may be further developed as a system for RNAi-based drug design and delivery. The inflammatory miR-155 is also upregulated during acute myocarditis. It contributes to the adverse inflammatory response to viral infection of the heart and has been identified as a potential therapeutic target.[79]

Hypertrophic cardiomyopathy and dilated cardiomyopathy

Hypertrophic cardiomyopathy and dilated cardiomyopathy constitute a group of primary myocardial disorders that are associated with miR dysregulation and lead to childhood death. Cardiomyopathies are typified by repeated re-hospitalisation and/or require cardiac transplantation within 1 year of the

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first admission.[80] Metabolic or syndromic causes are identified in >35% of children with hypertrophic cardiomyopathy and dilated cardiomyopathy.[81]

It is believed that miRs play key roles in maintaining cardiomyocyte integrity and that their dysregulation contributes to the pathogenesis of decompensated hypertrophic cardiomyopathy and progression to dilated cardiomyopathy.[82] Hypertrophic growth and myocyte disarray resulting in dilated cardiomyopathy and heart failure have been observed in mouse models with cardiac overexpression of miR-195[83] and knockout mice for miR-133.[22] An additional study showed dysregulation of cardiac contractile proteins and profound sarcomeric disarray leading to rapidly progressive dilated cardiomyopathy in Dicer mutant mice.[17]

MiRs-142-3p and -5p are repressed by serum-derived growth factors in cultured cardiac myocytes and this may reflect similar changes in cardiac hypertrophy in vivo. Downregulation of miR-142 is a critical element of adaptive hypertrophy and mediates cytokine-induced survival signalling during cardiac growth in response to haemodynamic stress. Furthermore, miR-142 was found to be a global inhibitor of cytokine signalling and function in the myocardium, in part through its ability to target gp130 and downregulate the expression of α-Actinin.[84] miR-142 also represses multiple components of the Nuclear Factor-Kappa B pathway, acting as a critical regulator of immune response in myocardial tissue.[84]

Dilated cardiomyopathy patients show decreased levels of let-7i, miR-126, and miR-155 in endomyocardial tissues relative to controls.[68] A decreased level of let-7i specifically has been associated with poor clinical outcome in patients with dilated cardiomyopathy. Similarly, miR-208 was found to be increased and shown to be a strong predictor of clinical outcomes for patients with dilated cardiomyopathy.[85] A different panel of miRs is expressed in patients with hypertrophic cardiomyopathy.[86] Patients with Friedreich ataxia cardiac hypertrophy[87] have polymorphism of the miR-155 binding sites in the angiotensin II receptor, type 1 gene promoter that may contribute to the hypertrophy phenotype.

Further identification of the post-transcriptional basis for myocarditis and associated cardiomyopathies through unravelling the roles of miRs, their regulation, and targets is urgently required to provide new treatment strategies and disease outcomes for these poorly understood conditions.

MiRs and arrhythmias

MiRs regulate all properties of cardiac excitability including conduction, repolarisation, automaticity, Ca^{2+} handling, spatial heterogeneity, apoptosis, and fibrosis. In addition to the wide range of actions on the myocardium, miRs are involved in the regulation of expression of a variety of proteins associated with the maintenance of the electrical properties of the heart.[88] Symptomatic arrhythmias are responsible for 5% of all emergency hospital admissions in paediatrics. Although mostly benign in nature, arrhythmias can be life threatening. Childhood arrhythmias are unlikely to resolve spontaneously and may need long-term anti-arrhythmic treatment or catheter ablation.[89]

Atrial fibrillation is rare in children, but studies of the role of miRs have been quite extensive. MiR-1 levels are greatly decreased in atrial fibrillation patients, causing upregulation of Kir2.1 subunits with consequent shortening of the terminal phase of atrial electrical remodelling and sustained atrial fibrillation.[90] MiR-328 is increased in atrial fibrillation and contributes to adverse electrical remodelling, partially through targeting L-type Ca^{2+} channel genes.[91] MiRs are also found to be differentially expressed in mitral stenosis patients with atrial fibrillation compared with those without atrial fibrillation. MiR-1202 was the most downregulated miR in these patients.[92] To our knowledge, no target or function of this miR has been reported. The prevalence of ventricular arrhythmia increases in children and adolescents with structural cardiac disease or cardiac surgery, putting them at significant risk for cardiac syncope and sudden cardiac death.[93] Alterations in cardiac miRs including miR-1, miR-133 and ion channel expression predispose patients to ventricular tachycardia. In patients with advanced non-ischaemic cardiomyopathy with ventricular tachycardia, both miRs-1 and miR-133a and their target mRNAs encoding ion channels were downregulated.[94] Consistent with this regulation, miR-1 overexpression was found to exacerbate arrhythmogenesis by direct repression of KCNJ2 and GJA1. GJA1 encodes connexin 43, the main cardiac gap-junction channel responsible for intercellular conductance in the ventricle.[95] KCNJ2 encodes the potassium inwardly rectifying channel.

MiR-1 enhances cardiac excitation–contraction coupling by selectively increasing phosphorylation of the L-type Ca^{2+} channels and ryanodine receptors (RyR2). It does this by disrupting the localisation of the protein phosphatase PP2A to these channels. Through translational inhibition of the PP2A regulatory subunit B56, miR-1 causes CaMKII-dependent hyperphosphorylation of RyR2, enhances RyR2 activity, and promotes arrhythmogenic sarcoplasmic reticulum Ca^{2+} release.[95] Muscle-specific miR-1 has also been identified as a cardiac arrhythmia-related miR in human and rat hearts after ischaemia.

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As discussed above, miR-1 targets the genes GJA1 and KCNJ2, thereby causing slowing conduction and depolarising the cytoplasmic membrane.[51] In normal or infarcted hearts, overexpression of miR-1 exacerbated arrhythmogenesis, whereas elimination of miR-1 by an antisense inhibitor in infarcted hearts relieved arrhythmogenesis.[96,97]

MiRs as a paediatric cardiovascular biomarker and therapeutic targets

The identification of distinct circulating miRs may impact the development of specific miRs as biomarkers in paediatric cardiovascular diseases, especially for foetal congenital heart defects.[98] Placental-expressed miRs have been detected in maternal plasma and can be associated with congenital heart diseases[99] and may be useful molecular markers for monitoring pregnancy-associated diseases. This discovery will open new possibilities for non-invasive and early prenatal diagnosis, allowing for early interventional and/or surgical treatment that are important to improve the prognosis of neonates with congenital heart diseases. A novel functional miR SNP rs11614913 in miR-196a2 was found to be a predictor of congenital heart diseases.[100] Circulating miRs are also emerging as biomarkers in heart failure,[101] with increased plasma miR-1 being the front leader.[102]

The identification of miRs that are dysregulated during the development of obesity could provide obesity biomarkers for early clinical diagnosis.[103] Several miRs are dysregulated in coronary artery disease patients and are detectable in peripheral blood mononuclear cells.[104] These miRs are expressed in endothelial cells and show significantly reduced levels of miR-126, the miR-17/92 cluster (miR-17, miR-20a, and miR-92a), miR-130a, miR-221, miR-21, and members of the let-7 family.[105] This can help in early and non-invasive detection of asymptomatic coronary artery disease in obese children, especially those with other risk factors of metabolic syndrome, allowing for very early therapeutic interventions to slow or even reverse the atherosclerotic lesions.[106]

MiRs are not only serving as potential biomarkers for early detection and diagnosis of disease, but also as therapeutic targets. Altering expression levels of disease-causing miRs by either their overexpression or inhibition are considered to be of tremendous therapeutic potential for the treatment of cardiovascular disease.[107] Antagonising endogenous miR-33 has been suggested as a therapeutic strategy for treating metabolic syndrome through regulation of fatty acid metabolism and insulin signalling.[108] Observational and functional studies of miR-122 have highlighted it as a potential therapeutic target for the treatment of hypercholesterolaemia.[109] Potentially, miR-27a mimics could be used to regulate pre-adipocyte proliferation and may evolve into useful anti-adipogenic drugs.[110] MiR-1 and miR-133 target pacemaker channels such as HCN2 and HCN466, with miR-1 confirmed to have an arrhythmogenic action[97] that could be reversed by knocking down miR-1 using its specific inhibitor antisense oligonucleotides.[96] The development and optimisation of AmiRs has great therapeutic potential. The safe, effective, and targeted delivery of in vivo RNA therapeutics remains an important challenge for clinical development. Another potential use of miRs to consider is their use for the diagnosis of individuals at risk for a specific disorder. Based on the observation that common single-nucleotide polymorphisms in pre-miRshas-miR-196a2 and hsa-miR-499 are associated with a significant increase in the risk of dilated cardiomyopathy,[111] suggests a possible benefit to use miRs for screening.

MiRs and cardiac stem cells

Cardiovascular regenerative medicine aims to restore damaged myocardium, both vasculature and muscle. Successful bone marrow stem cell therapy for myocardial regeneration in an infant with hypoplastic left heart syndrome has been reported.[112] The regenerative capacity of human cardiac progenitor cells in young patients with non-ischaemic congenital heart defects showed that human cardiac progenitor cells are functional and also have potential in congenital cardiac repair.[113]

MiRs have been shown to regulate multiple steps of stem cell growth, self-renewal, and differentiation including stem cell pluripotency[114] lineage specification,[12] embryonic stem cell differentiation,[115] stem cell self-renewal,[116] regulating embryonic stem cells identity,[117] reprogramming of somatic cells to pluripotent cells,[118] and allogeneic stem cell transplantation.[119] A human myocardial precursor derived from human cardiac progenitor cells that gives origin to atrial, ventricular, and specialised conduction cardiomyocytes has been recently identified.[120]

MiRs have been identified to play roles in endothelial progenitor and cardiac myocyte specification and differentiation.[121,122] MiR-99b, miR-181a, and miR-181b are considered to comprise an endothelial miR signature. These miRs play essential roles in the differentiation of pluripotent human cardiac progenitor cells to endothelial progenitor and endothelial cells.[123] MiR-125b is important during human embryonic stem cell differentiation into myocardial precursors and

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cardiomyocytes. MiR-125b also plays a regulatory role in the early stages of human embryonic stem cells differentiation, possibly by targeting Lin28, a miR-binding protein that binds to and enhances the translation of the insulin-like growth factor 2 mRNA. Lin28 has also been shown to bind the let-7 pre-miR and block production of mature let-7 in endothelial progenitor cells. MiR-125b appears to induce the formation of mesoderm, and cardiac mesoderm from human embryonic stem cells. Overexpression of miR-125b was shown to mediate upregulation of the early cardiac transcription factors GATA4 and Nkx2-5 and accelerate progression of human embryonic stem cell-derived myocardial precursors to an embryonic cardiomyocytes phenotype. These findings suggest that manipulation of miR-125b-mediated pathways could provide a novel approach to directing the differentiation of human embryonic stem cell-derived cardiomyocytes for cell therapy applications.[124] These studies shed insights into the suitability of human embryonic stem cell-derived cardiomyocytes for therapies not only to heart attack victims, but also to those bearing the burden of other diseases where the child's heart is damaged or not functioning properly.

MiR-1 and miR-499 play differential roles in cardiac differentiation of human embryonic stem cells in a context-dependent manner; miR-499 promotes ventricular specification of human embryonic stem cells and miR-1 facilitates electrophysiological maturation.[125] MiR-499 further promotes the differentiation of human cardiac stem cells into mechanically integrated cardiomyocytes, a function that offers great hope for the treatment of human heart failure.[126] An miR pro-survival cocktail of miR-21, miR-24, and miR-221 is expected to improve the engraftment of transplanted cardiac progenitor cells and therapeutic efficacy for treatment of ischaemic heart disease through overcoming the low survival of the transplanted cell.[127]

It is noteworthy that miR-1 is involved in cardiac development, function, pathology, and treatment. The development of human embryonic stem cells lines and/or induced pluripotent stem cell with enhanced ability to differentiate into cardiomyocyte tissue holds great promise for several paediatric cardiovascular researches, especially if immunological rejection issues can be resolved.[113] The elucidation of the role of miRs in this area will aid in the development of new approaches for paediatric cardiovascular disease profiling and cell therapy.

Conclusion

Despite the inherent limitations, much progress has been made towards developing effective treatments for paediatric cardiovascular diseases, offering hope for millions of children with these diseases. The role of miRs in heart development and different cardiovascular diseases makes them especially attractive for study if our goal is to secure normal development. Research efforts directed towards a greater understanding of the mechanisms and functional significance of the aberrant expression of miRs in cardiovascular diseases will assist in the development of less toxic therapies, and provide better markers for disease classification. In short, the discovery of miRs will open new research avenues for paediatric cardiovascular disorders, which are expected to advance this area of research from the crawling stage to the walking stage.

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References

1. Heidenreich PA, Trogdon JG, Khavjou OA, et al. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. Circulation 2011; 123: 933–944.
2. Flynt AS, Lai EC. Biological principles of microRNA-mediated regulation: shared themes amid diversity. Nat Rev Genet 2008; 9: 831–842.
3. Rota R, Ciarapica R, Giordano A, Miele L, Locatelli F. MicroRNAs in rhabdomyosarcoma: pathogenetic implications and translational potentiality. Mol Cancer 2011; 10: 120.
4. Krol J, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 2010; 11: 597–610.
5. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215–233.
6. Cai B, Pan Z, Lu Y. The roles of microRNAs in heart diseases: a novel important regulator. Curr Med Chem 2010; 17: 407–411.
7. Trojnarska O, Grajek S, Katarzynski S, Kramer L. Predictors of mortality in adult patients with congenital heart disease. Cardiol J 2009; 16: 341–347.
8. Khairy P, Ionescu-Ittu R, Mackie AS, Abrahamowicz M, Pilote L, Marelli AJ. Changing mortality in congenital heart disease. J Am Coll Cardiol 2010; 56: 1149–1157.
9. Marelli AJ, Mackie AS, Ionescu-Ittu R, Rahme E, Pilote L. Congenital heart disease in the general population: changing prevalence and age distribution. Circulation 2007; 115: 163–172.
10. Bruneau BG. The developmental genetics of congenital heart disease. Nature 2008; 451: 943–948.
11. Chen J, Wang DZ. MicroRNAs in cardiovascular development. J Mol Cell Cardiol 2012; 52: 949–957.
12. Ivey KN, Muth A, Arnold J, et al. MicroRNA regulation of cell lineages in mouse and human embryonic stem cells. Cell stem cell 2008; 2: 219–229.
13. Callis TE, Chen JF, Wang DZ. MicroRNAs in skeletal and cardiac muscle development. DNA Cell Biol 2007; 26: 219–225.

https://doi.org/10.1017/S1047951113000048 Published online by Cambridge University Press

14. Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets hand2 during cardiogenesis. Nature 2005; 436: 214–220.
15. Chen JF, Mandel EM, Thomson JM, et al. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nat Genet 2006; 38: 228–233.
16. Zhao Y, Ransom JF, Li A, et al. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miR-1-2. Cell 2007; 129: 303–317.
17. Chen JF, Murchison EP, Tang R, et al. Targeted deletion of dicer in the heart leads to dilated cardiomyopathy and heart failure. Proc Natl Acad Sci U S A 2008; 105: 2111–2116.
18. Roberts A, Allanson J, Jadico SK, et al. The cardiofaciocutaneous syndrome. J Med Genet 2006; 43: 833–842.
19. Perez E, Sullivan KE. Chromosome 22q11.2 deletion syndrome (DiGeorge and velocardiofacial syndromes). Curr Opin Pediatr 2002; 14: 678–683.
20. Huang ZP, Chen JF, Regan JN, et al. Loss of microRNAs in neural crest leads to cardiovascular syndromes resembling human congenital heart defects. Arterioscler Thromb Vasc Biol 2010; 30: 2575–2586.
21. Liu N, Williams AH, Kim Y, et al. An intragenic mef2-dependent enhancer directs muscle-specific expression of microRNAs 1 and 133. Proc Natl Acad Sci U S A 2007; 104: 20844–20849.
22. Liu N, Bezprozvannaya S, Williams AH, et al. MicroRNA-133a regulates cardiomyocyte proliferation and suppresses smooth muscle gene expression in the heart. Genes Dev 2008; 22: 3242–3254.
23. Ventura A, Young AG, Winslow MM, et al. Targeted deletion reveals essential and overlapping functions of the miR-17 through 92 family of miR clusters. Cell 2008; 132: 875–886.
24. Thum T, Galuppo P, Wolf C, et al. MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. Circulation 2007; 116: 258–267.
25. Goddeeris MM, Rho S, Petiet A, et al. Intracardiac septation requires hedgehog-dependent cellular contributions from outside the heart. Development 2008; 135: 1887–1895.
26. O'Brien JE Jr, Kibiryeva N, Zhou XG, et al. Noncoding RNA expression in myocardium from infants with tetralogy of fallot. Circ Cardiovasc Genet 2012; 5: 279–286.
27. Yu ZB, Han SP, Bai YF, Zhu C, Pan Y, Guo XR. MicroRNA expression profiling in fetal single ventricle malformation identified by deep sequencing. Int J Mol Med 2012; 29: 53–60.
28. Kuhn DE, Nuovo GJ, Martin MM, et al. Human chromosome 21-derived miRs are overexpressed in down syndrome brains and hearts. Biochem Biophys Res Commun 2008; 370: 473–477.
29. Latronico MV, Catalucci D, Condorelli G. MicroRNA and cardiac pathologies. Physiol Genomics 2008; 34: 239–242.
30. Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The drosha-dgcr8 complex in primary microRNA processing. Genes Dev 2004; 18: 3016–3027.
31. de Onis M, Blossner M, Borghi E. Global prevalence and trends of overweight and obesity among preschool children. Am J Clin Nutr 2010; 92: 1257–1264.
32. Ferreira AP, Oliveira CE, Franca NM. Metabolic syndrome and risk factors for cardiovascular disease in obese children: the relationship with insulin resistance (HOMA-IR). J Pediatr (Rio J) 2007; 83: 21–26.
33. Lloyd-Jones D, Adams R, Carnethon M, et al. Heart disease and stroke statistics–2009 update: heart disease and stroke statistics–2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation 2009; 119: 480–486.
34. Bibbins-Domingo K, Coxson P, Pletcher MJ, Lightwood J, Goldman L. Adolescent overweight and future adult coronary heart disease. N Engl J Med 2007; 357: 2371–2379.
35. Akgun C, Dogan M, Akbayram S, et al. The incidence of asymptomatic hypertension in school children. J Nihon Med Sch 2010; 77: 160–165.
36. Li Y, Yang X, Zhai F, et al. Prevalence of the metabolic syndrome in Chinese adolescents. Br J Nutr 2008; 99: 565–570.
37. Perichart-Perera O, Balas-Nakash M, Schiffman-Selechnik E, Barbato-Dosal A, Vadillo-Ortega F. Obesity increases metabolic syndrome risk factors in school-aged children from an urban school in Mexico city. J Am Diet Assoc 2007; 107: 81–91.
38. Hayman LL, Meininger JC, Daniels SR, et al. Primary prevention of cardiovascular disease in nursing practice: focus on children and youth: a scientific statement from the American Heart Association Committee on Atherosclerosis, Hypertension, and Obesity in youth of the Council on Cardiovascular Disease in the Young, Council on Cardiovascular Nursing, Council on Epidemiology and Prevention, and Council on Nutrition, Physical Activity, and Metabolism. Circulation 2007; 116: 344–357.
39. Baker JL, Olsen LW, Sorensen TI. Childhood body-mass index and the risk of coronary heart disease in adulthood. N Engl J Med 2007; 357: 2329–2337.
40. Heneghan HM, Miller N, Kerin MJ. Role of microRNAs in obesity and the metabolic syndrome. Obes Rev 2010; 11: 354–361.
41. Xie H, Sun L, Lodish HF. Targeting microRNAs in obesity. Expert Opin Ther Targets 2009; 13: 1227–1238.
42. Qin L, Chen Y, Niu Y, et al. A deep investigation into the adipogenesis mechanism: profile of microRNAs regulating adipogenesis by modulating the canonical wnt/beta-catenin signaling pathway. BMC genomics 2010; 11: 320.
43. Martinelli R, Nardelli C, Pilone V, et al. Mir-519d overexpression is associated with human obesity. Obesity (Silver Spring) 2010; 18: 2170–2176.
44. Kim SY, Kim AY, Lee HW, et al. Mir-27a is a negative regulator of adipocyte differentiation via suppressing ppargamma expression. Biochem Biophys Res Commun 2010; 392: 323–328.
45. Kim YJ, Hwang SJ, Bae YC, Jung JS. MiR-21 regulates adipogenic differentiation through the modulation of TGF-beta signaling in mesenchymal stem cells derived from human adipose tissue. Stem Cells 2009; 27: 3093–3102.
46. Esau C, Kang X, Peralta E, et al. MicroRNA-143 regulates adipocyte differentiation. J Biol Chem 2004; 279: 52361–52365.
47. Karbiener M, Fischer C, Nowitsch S, et al. MicroRNA miR-27b impairs human adipocyte differentiation and targets PPARgamma. Biochem Biophys Res Commun 2009; 390: 247–251.
48. Andersen DC, Jensen CH, Schneider M, et al. MicroRNA-15a fine-tunes the level of delta-like 1 homolog (DLK1) in proliferating 3T3-L1 preadipocytes. Exp Cell Res 2010; 316: 1681–1691.
49. Trajkovski M, Hausser J, Soutschek J, et al. MicroRNAs 103 and 107 regulate insulin sensitivity. Nature 2011; 474: 649–653.
50. Wilfred BR, Wang WX, Nelson PT. Energizing miRNA research: a review of the role of miRNAs in lipid metabolism, with a prediction that miR-103/107 regulates human metabolic pathways. Mol Genet Metab 2007; 91: 209–217.
51. Lin Q, Gao Z, Alarcon RM, Ye J, Yun Z. A role of miR-27 in the regulation of adipogenesis. FEBS J 2009; 276: 2348–2358.
52. Wang T, Li M, Guan J, et al. MicroRNAs miR-27a and miR-143 regulate porcine adipocyte lipid metabolism. Int J Mol Sci 2011; 12: 7950–7959.
53. Kinoshita M, Ono K, Horie T, et al. Regulation of adipocyte differentiation by activation of serotonin (5-ht) receptors 5-ht2ar and 5-ht2cr and involvement of microrna-448-mediated repression of klf5. Mol Endocrinol 2010; 24: 1978–1987.
54. Natarajan R, Putta S, Kato M. MicroRNAs and diabetic complications. J Cardiovasc Transl Res 2012; 5: 413–422.

https://doi.org/10.1017/S1047951113000048 Published online by Cambridge University Press

55. Poy MN, Spranger M, Stoffel M. MicroRNAs and the regulation of glucose and lipid metabolism. Diabetes Obes Metab 2007; 12: 67–73.
56. Tang X, Muniappan L, Tang G, Ozcan S. Identification of glucose-regulated miRs from pancreatic {beta} cells reveals a role for miR-30d in insulin transcription. RNA 2009; 15: 287–293.
57. Terán-García M, Bouchard C. Genetics of the metabolic syndrome. Appl Physiol Nutr Metab 2007; 32: 89–114.
58. Boucek MM, Edwards LB, Keck BM, Trulock EP, Taylor DO, Hertz MI. Registry for the international society for heart and lung transplantation: seventh official pediatric report–2004. J Heart Lung Transplant 2004; 23: 933–947.
59. Kay JD, Colan SD, Graham TP Jr. Congestive heart failure in pediatric patients. Am Heart J 2001; 142: 923–928.
60. Tijsen AJ, Pinto YM, Creemers EE. Non-cardiomyocyte microRNAs in heart failure. Cardiovasc Res 2012; 93: 573–582.
61. Martinez J, Patkaniowska A, Urlaub H, Luhrmann R, Tuschl T. Single-stranded antisense siRNAs guide target RNA cleavage in RNAi. Cell 2002; 110: 563–574.
62. Mathonnet G, Fabian MR, Svitkin YV, et al. MicroRNA inhibition of translation initiation in vitro by targeting the cap-binding complex eIF4F. Science 2007; 317: 1764–1767.
63. Matkovich SJ, Van Booven DJ, Youker KA, et al. Reciprocal regulation of myocardial microRNAs and messenger RNA in human cardiomyopathy and reversal of the microRNA signature by biomechanical support. Circulation 2009; 119: 1263–1271.
64. Topkara VK, Mann DL. Clinical applications of miRNAs in cardiac remodeling and heart failure. Per Med 2010; 7: 531–548.
65. Ernst A, Campos B, Meier J, et al. De-repression of CTGF via the miR-17-92 cluster upon differentiation of human glioblastoma spheroid cultures. Oncogene 2010; 29: 3411–3422.
66. Schellings MW, Vanhoutte D, van Almen GC, et al. Syndecan-1 amplifies angiotensin ii-induced cardiac fibrosis. Hypertension 2010; 55: 249–256.
67. van de Vrie M, Heymans S, Schroen B. MicroRNA involvement in immune activation during heart failure. Cardiovasc Drugs Ther 2011; 25: 161–170.
68. Satoh M, Minami Y, Takahashi Y, Tabuchi T, Nakamura M. A cellular microRNA, let-7i, is a novel biomarker for clinical outcome in patients with dilated cardiomyopathy. J Card Fail 2011; 17: 923–929.
69. Gurha P, Abreu-Goodger C, Wang T, et al. Targeted deletion of microRNA-22 promotes stress-induced cardiac dilation and contractile dysfunction. Circulation 2012; 125: 2751–2761.
70. Reddy S, Zhao M, Hu DQ, et al. Dynamic microRNA expression during the transition from right ventricular hypertrophy to failure. Physiol Genomics 2012; 44: 562–575.
71. Kumarswamy R, Lyon AR, Volkmann I, et al. SERCA2a gene therapy restores microRNA-1 expression in heart failure via an Akt/FoxO3A-dependent pathway. Eur Heart J 2012; 33: 1067–1075.
72. Ikeda S, He A, Kong SW, et al. MicroRNA-1 negatively regulates expression of the hypertrophy-associated calmodulin and Mef2a genes. Mol Cell Biol 2009; 29: 2193–2204.
73. Luo X, Lin H, Pan Z, et al. Down-regulation of miR-1/miR-133 contributes to re-expression of pacemaker channel genes HCN2 and HCN4 in hypertrophic heart. J Biol Chem 2008; 283: 20045–20052.
74. Care A, Catalucci D, Felicetti F, et al. MicroRNA-133 controls cardiac hypertrophy. Nat Med 2007; 13: 613–618.
75. Saji T, Matsuura H, Hasegawa K, et al. Comparison of the clinical presentation, treatment, and outcome of fulminant and acute myocarditis in children. Circ J 2012; 76: 1222–1228.
76. Feldman AM, McNamara D. Myocarditis. N Engl J Med 2000; 343: 1388–1398.
77. Xu HF, Ding YJ, Shen YW, et al. MicroRNA-1 represses Cx43 expression in viral myocarditis. Mol Cell Biochem 2012; 362: 141–148.
78. Ye X, Liu Z, Hemida MG, Yang D. Targeted delivery of mutant tolerant anti-coxsackievirus artificial microRNAs using folate conjugated bacteriophage Phi29 pRNA. PLoS One 2011; 6: e21215.
79. Corsten MF, Papageorgiou A, Verhesen W, et al. MicroRNA profiling identifies microRNA-155 as an adverse mediator of cardiac injury and dysfunction during acute viral myocarditis. Circ Res 2012; 111: 415–425.
80. Jansen JA, van Veen TA, de Bakker JM, van Rijen HV. Cardiac connexins and impulse propagation. J Mol Cell Cardiol 2010; 48: 76–82.
81. Kindel SJ, Miller EM, Gupta R, et al. Pediatric cardiomyopathy: importance of genetic and metabolic evaluation. J Card Fail 2012; 18: 396–403.
82. Rao PK, Toyama Y, Chiang HR, et al. Loss of cardiac microRNA-mediated regulation leads to dilated cardiomyopathy and heart failure. Circ Res 2009; 105: 585–594.
83. van Rooij E, Sutherland LB, Liu N, et al. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. Proc Natl Acad Sci U S A 2006; 103: 18255–18260.
84. Sharma S, Liu J, Wei J, Yuan H, Zhang T, Bishopric NH. Repression of miR-142 by p300 and mapk is required for survival signalling via gp130 during adaptive hypertrophy. EMBO Mol Med 2012; 4: 617–632.
85. Satoh M, Minami Y, Takahashi Y, Tabuchi T, Nakamura M. Expression of microRNA-208 is associated with adverse clinical outcomes in human dilated cardiomyopathy. J Card Fail 2010; 16: 404–410.
86. Palacin M, Reguero JR, Martin M, et al. Profile of microRNAs differentially produced in hearts from patients with hypertrophic cardiomyopathy and sarcomeric mutations. Clin Chem 2011; 57: 1614–1616.
87. Kelly M, Bagnall RD, Peverill RE, et al. A polymorphic miR-155 binding site in AGTR1 is associated with cardiac hypertrophy in Friedreich ataxia. J Mol Cell Cardiol 2011; 51: 848–854.
88. Wang Z. The role of microRNA in cardiac excitability. J Cardiovasc Pharmacol 2010; 56: 460–470.
89. Massin MM, Benatar A, Rondia G. Epidemiology and outcome of tachyarrhythmias in tertiary pediatric cardiac centers. Cardiology 2008; 111: 191–196.
90. Girmatsion Z, Biliczki P, Bonauer A, et al. Changes in microRNA-1 expression and IK1 up-regulation in human atrial fibrillation. Heart Rhythm 2009; 6: 1802–1809.
91. Lu Y, Zhang Y, Wang N, et al. MicroRNA-328 contributes to adverse electrical remodeling in atrial fibrillation. Circulation 2010; 122: 2378–2387.
92. Xiao J, Liang D, Zhang Y, et al. MicroRNA expression signature in atrial fibrillation with mitral stenosis. Physiol Genomics 2011; 43: 655–664.
93. Serwer G. Ventricular arrhythmia in children: diagnosis and management. Curr Treat Options Cardiovasc Med 2008; 10: 442–4427.
94. Amin AS, Giudicessi JR, Tijsen AJ, et al. Variants in the 3′ untranslated region of the KCNQ1-encoded Kv7.1 potassium channel modify disease severity in patients with type 1 long QT syndrome in an allele-specific manner. Eur Heart J 2012; 33: 714–723.
95. Jongsma HJ, Wilders R. Gap junctions in cardiovascular disease. Circ Res 2000; 86: 1193–1197.
96. Yang B, Lin H, Xiao J, et al. The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. Nat Med 2007; 13: 486–491.
97. Terentyev D, Belevych AE, Terentyeva R, et al. miR-1 overexpression enhances Ca(2+) release and promotes cardiac arrhythmogenesis by targeting PP2A regulatory subunit B56alpha and causing CaMKII-dependent hyperphosphorylation of RyR2. Circ Res 2009; 104: 514–521.

https://doi.org/10.1017/S1047951113000048 Published online by Cambridge University Press

98. Yu Z, Han S, Hu P, et al. Potential role of maternal serum microRNAs as a biomarker for fetal congenital heart defects. Med Hypotheses 2011; 76: 424–426.
99. Kotlabova K, Doucha J, Hromadnikova I. Placental-specific microRNA in maternal circulation – identification of appropriate pregnancy-associated microRNAs with diagnostic potential. J Reprod Immunol 2011; 89: 185–191.
100. Xu J, Hu Z, Xu Z, et al. Functional variant in microRNA-196a2 contributes to the susceptibility of congenital heart disease in a Chinese population. Hum Mutat 2009; 30: 1231–1236.
101. Tijsen AJ, Creemers EE, Moerland PD, et al. MiR423-5p as a circulating biomarker for heart failure. Circ Res 2010; 106: 1035–1039.
102. Cheng Y, Tan N, Yang J, et al. A translational study of circulating cell-free microRNA-1 in acute myocardial infarction. Clin Sci (Lond) 2010; 119: 87–95.
103. McGregor RA, Choi MS. MicroRNAs in the regulation of adipogenesis and obesity. Curr Mol Med 2011; 11: 304–316.
104. Hoekstra M, van der Lans CA, Halvorsen B, et al. The peripheral blood mononuclear cell microRNA signature of coronary artery disease. Biochem Biophys Res Commun 2010; 394: 792–797.
105. Fichtlscherer S, De Rosa S, Fox H, et al. Circulating microRNAs in patients with coronary artery disease. Circ Res 2010; 107: 677–684.
106. Omran A, Elimam D, He F, Peng J, Yin F. Potential role of blood microRNAs as non-invasive biomarkers for early detection of asymptomatic coronary atherosclerosis in obese children with metabolic syndrome. Med Hypotheses 2012; 79: 889–893.
107. Fang J, Song XW, Tian J, et al. Overexpression of microRNA-378 attenuates ischemia-induced apoptosis by inhibiting caspase-3 expression in cardiac myocytes. Apoptosis 2012; 17: 410–423.
108. Davalos A, Goedeke L, Smibert P, et al. miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. Proc Natl Acad Sci U S A 2011; 108: 9232–9237.
109. Esau C, Davis S, Murray SF, et al. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 2006; 3: 87–98.
110. Rosen ED, MacDougald OA. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol 2006; 7: 885–896.
111. Zhou B, Rao L, Peng Y, et al. Common genetic polymorphisms in pre-microRNAs were associated with increased risk of dilated cardiomyopathy. Clin Chim Acta 2010; 411: 1287–1290.
112. Rupp S, Zeiher AM, Dimmeler S, et al. A regenerative strategy for heart failure in hypoplastic left heart syndrome: intracoronary administration of autologous bone marrow-derived progenitor cells. J Heart Lung Transplant 2010; 29: 574–577.
113. Mishra R, Vijayan K, Colletti EJ, et al. Characterization and functionality of cardiac progenitor cells in congenital heart patients. Circulation 2011; 123: 364–373.
114. Mallanna SK, Rizzino A. Emerging roles of micrornas in the control of embryonic stem cells and the generation of induced pluripotent stem cells. Dev Biol 2010; 344: 16–25.
115. Gan L, Schwengberg S, Denecke B. MicroRNA profiling during cardiomyocyte-specific differentiation of murine embryonic stem cells based on two different miR array platforms. PLoS One 2011; 6: e25809.
116. Shekar PC, Naim A, Sarathi DP, Kumar S. Argonaute-2-null embryonic stem cells are retarded in self-renewal and differentiation. J Biosci 2011; 36: 649–657.
117. Laurent LC, Chen J, Ulitsky I, et al. Comprehensive microRNA profiling reveals a unique human embryonic stem cell signature dominated by a single seed sequence. Stem Cells 2008; 26: 1506–1516.
118. Lakshmipathy U, Davila J, Hart RP. miRNA in pluripotent stem cells. Regen Med 2010; 5: 545–555.
119. Wei L, Wang M, Qu X, et al. Differential expression of microRNAs during allograft rejection. Am J Transplant 2012; 12: 1113–1123.
120. Ritner C, Wong SS, King FW, et al. An engineered cardiac reporter cell line identifies human embryonic stem cell-derived myocardial precursors. PLoS One 2011; 6: e16004.
121. Small EM, Olson EN. Pervasive roles of microRNAs in cardiovascular biology. Nature 2011; 469: 336–342.
122. Evans SM, Moretti A, Laugwitz KL. MicroRNAs in a cardiac loop: progenitor or myocyte? Dev Cell 2010; 19: 787–788.
123. Kane NM, Howard L, Descamps B, et al. Role of microRNAs 99b, 181a, and 181b in the differentiation of human embryonic stem cells to vascular endothelial cells. Stem Cells 2012; 30: 643–654.
124. Wong SS, Ritner C, Ramachandran S, et al. miR-125b promotes early germ layer specification through lin28/let-7d and preferential differentiation of mesoderm in human embryonic stem cells. PLoS One 2012; 7: e36121.
125. Fu JD, Rushing SN, Lieu DK, et al. Distinct roles of microRNA-1 and -499 in ventricular specification and functional maturation of human embryonic stem cell-derived cardiomyocytes. PLoS One 2011; 6: e27417.
126. Hosoda T, Zheng H, Cabral-da-Silva M, et al. Human cardiac stem cell differentiation is regulated by a mircrine mechanism. Circulation 2011; 123: 1287–1296.
127. Hu S, Huang M, Nguyen PK, et al. Novel microRNA prosurvival cocktail for improving engraftment and function of cardiac progenitor cell transplantation. Circulation 2011; 124: S27–S34.

https://doi.org/10.1017/S1047951113000048 Published online by Cambridge University Press