

Insights Into the Role of microRNAs in Cardiac Diseases: From Biological Signalling to Therapeutic Targets

E. Zorio^{*,1a}, P. Medina^{2a}, J. Rueda^{1a}, J.M. Millán^{3b}, M.A. Arnau^{1a}, M. Beneyto³, F. Marín^{4a}, J.R. Gimeno^{4a}, J. Osca^{1a}, A. Salvador¹, F. España^{2a} and A. Estellés^{2a}

¹Cardiology Department, ²Research Centre and ³Unit of Genetics of the Hospital Universitario La Fe (Valencia) and ⁴Cardiology Department of the Hospital Universitario Virgen de La Arrixaca (Murcia), Spain

Abstract: microRNAs have recently opened new pathways to explain gene expression and disease biology in many scenarios, including cardiac diseases. microRNAs are endogenous small non-coding RNAs that mediate post-transcriptional repression or messenger RNA degradation. By annealing to inexact complementary sequences in the 3' untranslated region of the target messenger RNA, protein level is down-regulated. Several microRNAs appear to act cooperatively through multiple target sites in one gene and, conversely, most microRNAs can target several genes. miR-133 and miR-1 are specifically expressed in cardiac and skeletal muscle and control myogenesis, cardiac development, cardiac performance and cardiomyocyte hypertrophy (mainly by tuning transcription factors and other growth-related targets). They also modulate the expression of certain cardiac ion channels and related proteins with proarrhythmic effect. Besides them, other microRNAs have been shown to exert influence on the myocardial growth, the electrical balance and the angiogenesis processes that take place in the heart. Bioinformatics is a useful tool to identify potential targets of a given microRNA, although there is still substantial concern about their reliability. Experimental manipulation of microRNAs has provided a tantalizing basis to speculate that future research on microRNAs may yield important progress in the prevention of sudden cardiac death and in the treatment of cardiac heart failure. However, the final effect of the blockage of microRNAs *in vivo* remains unclear, since each of them can target hundreds of genes with different intensity. The era of the microRNAs in cardiovascular diseases has just started.

Key Words: microRNA, gene expression, differentiation, cardiac hypertrophy, ion channel, repolarization, connexin, angiogenesis.

GENERAL ASPECTS OF GENE SILENCING: RNA SILENCING

Small RNAs derive from double-stranded DNA and constitute a family of regulatory non-coding RNAs of 19-22 nt in length. This group comprises repeat-associated short interfering RNA (rasiRNAs), small scanRNA (scnRNA), microRNA (miRNA) and short interfering RNA (siRNA). rasiRNAs and scnRNAs can produce DNA silencing through histone and/or DNA methylation control, whereas miRNAs and siRNAs induce RNA silencing [1-3]. Initially perceived differences in siRNA and miRNA action have recently vanished [4-7]. In fact, both can cleave perfectly complementary messenger RNA (mRNA) targets and diminish the translation of partially complementary targets [8-10]. However, some differences can still be pointed out: siRNAs start out as folded double-stranded RNAs, whereas miRNAs start out as folded single-stranded molecules [4, 8]. Not surprisingly, RNA silencing pathways have fascinated researchers. Above all, interest has been focused on miRNAs because of their potential use in studying gene function, validating diagnostic biomarkers, confirming candidate drug targets, and perhaps

even treating certain diseases [5, 11]. As a result, a considerable number of miRNAs has already been identified and a never-ending list can be found in an updated miRNA Registry at <http://microrna.sanger.ac.uk/sequences/> [12, 13].

miRNA

Ever since miRNAs were discovered in 1993 by Lee *et al.* [14], research endeavours have documented that many miRNAs involved in their machinery are highly conserved across species [1, 10, 15]. Importantly, miRNA's transcriptional and post-transcriptional maturation processes take place in a tissue-specific manner. Therefore, miRNA transcription can be regulated by its own specific transcription units, but also by its host gene tissue-specific promoters [1, 17]. miRNAs are transcribed from different genomic locations, such as introns or exons of protein-coding mRNA or noncoding RNA [16]. Those miRNAs encoded by more than one locus are differentiated by numerical suffixes (for instance, miR-1-1 and miR-1-2), miRNAs which only differ in a reduced number of bases by alphabetical suffixes (for instance, miR-133a and miR-133b) and miRNAs deriving from the same hairpin precursor, but with different tissue-specific post-transcriptional maturation, by adding the arm from which they are originated in the suffix (for instance, miR-126-5p and miR-126-3p, derived from the 5' arm and the 3' arm, respectively) [5].

*Address correspondence to this author at the Hospital Universitario La Fe, Servicio de Cardiología, Av. Campanar, 21, 46009 Valencia, Spain; Tel: 34 963862759; Fax: 34 961973018; E-mail: estherzorio@hotmail.com

^aRETICS (RECAVA) and ^bCIBERER both funded by the Instituto de Salud Carlos III

miRNA biosynthesis and functions are summarized in Fig. (1). Like protein-coding mRNAs, miRNAs are transcribed as long primary transcripts in the nucleus [9]. However, unlike protein-coding mRNAs, miRNAs are subsequently cleaved by nuclear RNase III enzyme Drosha [4] to produce stem-loop-structured precursor molecules of 70-90 nt in length (pre-miRNAs), which are exported to the cytoplasm by exportin 5 [18]. Both the nuclear transcription of miRNA genes and the cytoplasmatic maturation of the pre-miRNAs, represent important points in the biogenesis of miRNAs for they ensure tissue-specific protein expression [19]. Given the fact that the maturation machinery is usually restricted to certain tissues, inhibiting the processing step or differently editing a given pre-miRNA in a cell-selective manner may offer a unique opportunity to obtain a range of different miRNA isoforms with different mRNA targets and functions, depending on the cell type under study [19, 20]. RNase III enzyme Dicer further processes it into a miRNA: miRNA duplex [1]. One strand of this duplex is rapidly degraded and the remaining mature 22 nt miRNA sequence assembles into effector complexes, ready to anneal to its target mRNA through the *seed sequence*, which normally involves nucleotides 2-8 in the 5' end of the miRNA [21]. Although many proteins have been identified within these complexes, only one family is consistently found in all of them, the highly conserved Argonaute (Ago) proteins [22]. Among them, Ago2 acts as a 'slicer' enzyme, able to cleave target mRNA [23].

Currently, more than 400 human miRNAs have been cloned and sequenced among an estimated number of 1000 miRNAs. Given that miRNAs are only partially complementary to their mRNAs targets, a single miRNA can regulate the expression of a wide variety of genes (up to 200) with different intensities. Conversely, many miRNAs appear to act cooperatively through multiple target sites in one gene. It is widely believed that as many as 30% of all genes could be miRNA targets [24] and that miRNA genes may represent 2-3% of the total number of genes in humans [25], some of them with a well characterized tissue and developmental stage-specific expression [9, 26-28]. Therefore, any misregulation of the miRNA pathways may break the physiological homeostasis and lead to pathological situations and diseases, such as uncontrolled growth or cell proliferation (cardiac hypertrophy or cancer) or electrical dysbalance (sudden cardiac death).

Gene regulation by miRNAs is achieved by three mechanisms, namely translational repression, mRNA degradation, and transcriptional control, not only in the host cell, but also in adjacent cells, given that this genetic material can be exchanged through cell-cell interactions as *exosomal shuttle RNA* [29]. Low complementarity between a miRNA and its target mRNA results in translational repression, though the exact point of repression is still unknown (inhibition of eIF4E-dependent initiation, elongation, and/or cotranslational nascent protein degradation) [5, 30]. On the other hand, almost perfect base-pairing between the miRNA and its target leads to mRNA degradation, which can be conducted by either direct (Ago2 mediated [23, 31]) or indirect (increased mRNA digestion in the *specialized cytoplasmic processing bodies* [8, 9, 32, 33]) mechanisms. Recently, translation repression and mRNA degradation mechanisms have been harmonized in a two-step model, which establishes that, upon binding to the 3' untranslated region (UTR) of the target mRNA, the effector complex inhibits translation initiation, and then moves to the processing bodies for storage (and possibly reuse) or digestion [9]. Although the final effect of a given miRNA on the expression of a given protein is variable, it normally hovers around 50% of protein level decrease. This modulation is mainly achieved through translational repression, without significant modification of mRNA levels [34-37]. However, synthetic RNAs, mostly acting as siRNAs, can robustly and specifically lower protein levels as much as 12-fold [38]. Besides these post-transcriptional mechanisms, miRNAs might also down-regulate host gene transcription by targeting transcription factors mRNAs, therefore acting as negative feedback regulators, for instance [17]. Moreover, newly released data support that certain mature miRNAs might also be imported back to the nucleus to exert some sort of transcriptional control (such as regulation of transcription or splicing processes of target transcripts). This transfer is related to the presence of a distinctive hexanucleotide terminal motif at the 3' terminus, which may be crucial for designing stable siRNAs or miRNAs useful for the manipulation of nuclear steps in gene expression [39]. Occasionally, through a competitive action, certain miRNAs can repress the target protein, so that the target protein level paradoxically increase [37].

Among the hundreds of miRNAs discovered, only a small fraction has assigned target mRNAs or an established role. A reliable identification of the targets for a given

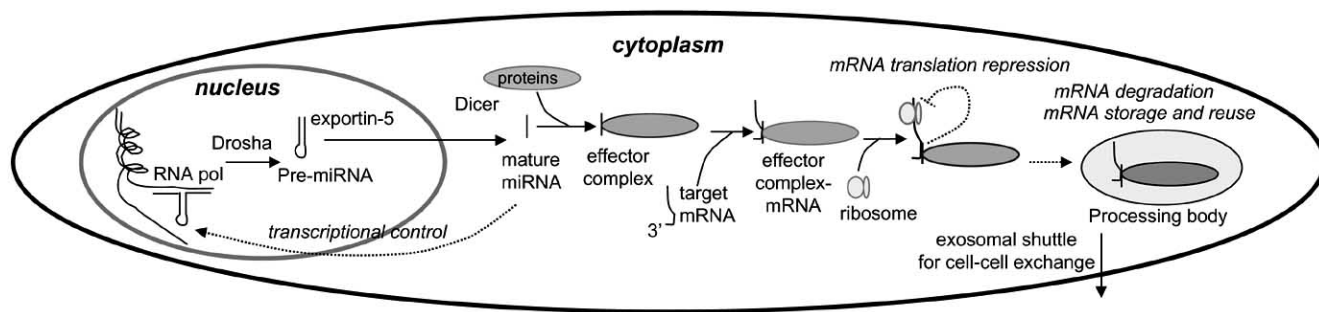


Fig. (1). miRNA synthesis and RNA silencing mechanisms. See explanation in the text. miRNA functions are highlighted with italics. RNA, ribonucleic acid. RNA pol, RNA polymerase. miRNA, microRNA. mRNA, messenger RNA.

miRNA deserves further investigation, since bioinformatics algorithms leading to target prediction identify too many putative targets among which some are strongly repressed, while others are only weakly affected, even though they share the same *seed sites* [27, 40, 41]. In order to explain this phenomenon, Zhao *et al.* introduced the concept of *target selectivity* by which a stable environment (low free energy) might be less accessible for miRNA silencing complexes, whereas miRNAs target 3'UTR regions with a less complex secondary structure would be more accessible and prone to bind miRNAs [28]. Some available bioinformatics algorithms are Diana-MicroT (http://www.diana.pcbi.upenn.edu/cgi-bin/micro_t.cgi), PicTar (<http://pictar.bio.nyu.edu>), miRanda (http://microrna.org/miranda_new.html), and TargetScan (<http://www.targetscan.org>), reviewed elsewhere [5]. Functional studies of miRNAs require development of appropriate tools. To date, these studies have been conducted in knockout animals, in which further gene profile expression analyses with microarrays have been performed. This strategy is unfortunately hampered by the fact that there might be more than one copy of a miRNA coding sequence within the genome, some of them embedded in sequences encoding mRNAs of other genes, whose expression might also be affected after deleting the miRNA coding sequence. Another approach to study miRNAs is to block miRNA function with antisense oligonucleotides, *antagomirs*, *decoys*, and *miRNAs sponges* [5]. Antisense oligonucleotides compete with a mRNA to bind a given miRNA and when they are cholesterol-linked single-stranded RNAs, they are termed *antagomirs* [42]. *Decoy* oligonucleotides placed at the 3'UTR of a reporter gene act as miRNAs traps when this gene is expressed [43]. Similarly, *miRNAs sponges* are transcripts expressed from strong promoters, containing multiple, tandem binding sites to a miRNA of interest [44].

miRNA AND THE HEART

The heart reacts to cardiac injury or burden by activating a range of intracellular signalling pathways which result in the so-called heart-failure phenotype by switching the pattern of expressed genes to an embryonic profile, and by favouring the remodelling of the extracellular matrix and myocardial fibrosis [45]. Despite the high expression of miRNAs in the heart, their roles still remain unclear. Some of them, such as miR-1 and miR-133, are preferentially expressed in cardiac and skeletal muscles [26-28], but others show a more ubiquitous profile (miR-21, miR-195, miR-206 and miR-208). This updated review of the literature summarizes the current knowledge about their physiologic and pathologic functions (Table 1).

Influence on the Myocardium and the Developing Heart

miRNAs, such as bicistronic miR-1 and the miR-133 cluster (including miR-1-1 and miR-1-2 in the first family and miR-133a-1, miR-133a-2, and miR-133b in the second) and miR-206, are transcriptionally regulated by the myogenic differentiating factors, such as the myogenic differentiation 1 (MyoD), the myocyte enhancer factor 2 (Mef2), and the serum response factor (SRF) [27]. This regulation can be tissue-specific, as in the case of miR-1, which is controlled by SRF in the heart and by MyoD and Mef2 in skeletal muscles [5]. Furthermore, inhibitors of these factors, the inhibitor

of the differentiation-3 protein (Id1-3) and the myogenic repressor (MyoR), decrease upon miR-206 introduction, suggesting the presence of additional mechanisms by which miRNAs enforce the differentiation program [41]. miR-1 can be detected in the mouse heart in early embryonic stages and increases with progression of differentiation [27, 41, 46, 47]. The role of the miR-1 family in embryonic and post-embryonic development is undoubted since the targeted deletion of the locus coding for miR-1-2 in mice yielded a striking incidence of heart morphogenesis abnormalities with high in utero and post partem mortality, even in the presence of structurally normal hearts [28, 48]. Its recognized actions include promotion of differentiation and maintenance of the differentiated state [49], induction of maturation into myotubes [50], favouring of apoptotic mechanisms [51] and inhibition of cardiac development [46]. However, according to different authors, opposite effects of miR-1 have been suggested on muscle cell proliferation, which increases myogenesis by targeting the transcriptional repressor of the muscle gene expression histone deacetylase 4 [52], or inhibiting proliferation by down-regulating growth-related targets, such as the Ras GTPase-activating protein (RasGAP), the cyclin-dependent kinase 9 (Cdk9), fibronectin and the Ras homolog enriched in the brain (Rheb) [5, 28, 50]. Probably the final effect on proliferation may depend on the balance between both mechanisms. The promotion of differentiation exhibited by miR-206 seems to be related to the observed down-regulation of the p180 subunit of DNA polymerase α , which is necessary for DNA synthesis, by cleavage of its mRNA. Accordingly, the opposite effect (inhibition of muscle differentiation and cell quiescence) can be observed when miR-206 is inhibited with antisense oligonucleotides [41]. However, upon regulation of SRF, miR-133 exhibits a totally different pattern of effects on muscle cells since it prevents skeletal differentiation [52], enhances myoblast proliferation by repressing SRF [52], exerts effects as a regulator of cardiac hypertrophy and cardiogenesis [43], and also as an inhibitor of apoptotic mechanisms [51]. Several targets for miR-133 have been identified, such as RhoA (a GDP-GTP exchange protein regulating cardiac hypertrophy), Cdc42 (a signal transduction kinase implicated in hypertrophy), and Nelf-A/WHSC2 (a nuclear factor involved in cardiogenesis) [43].

The complex mechanisms by which miRNAs control the protein expression sometimes intersect hormone signalling. An example to illustrate this complex collaborative network is the repression of the thyroid hormone receptor-associated protein (THRAP) 1 by miR-208 through a mechanism that involves the regulation of the α - and β -myosin heavy chain (MHC) expression. Indeed stress stimuli, responsible for the reduction of α -MHC transcription, also reduce the expression of miR-208, which is embedded in one of its introns. In turn, the previous repression exerted by miR-208 on its target mRNA, THRAP1, is relieved. The resulting increase in THRAP1 differentially fosters the thyroid receptor-regulated expression of α - and β -MHC, which are finally up- and down-regulated, respectively. This network seems to play an important role in cardiac remodelling, hypertrophic growth and β -MHC up-regulation under stress and hypothyroidism [54].

Table 1. List of miRNA with Recognized Effects on the Heart

miRNA	Regulated Protein	Phenotype	Ref
miR-1	Downregulated: transcription factor Hand2*, RasGAP, Cdk9, fibronectin, Rheb, HSP60 and HSP70, histoneacetylase 4	Cardiac proliferation, cardiac morphogenesis (VSD), myoblast differentiation, maintenance of the differentiated state, induction of maturation into myotubes, proapoptosis	28,47-52
	Downregulated: Connexin 43	Prolongs repolarization, slows conduction velocity, promotes ADs and PVBs	34
	Downregulated: Kir2.1 subunit of the IK1 current	Prolongs action potential and promotes ADs	34
	Downregulated: beta Mink of the IKs current	Prolongs action potential	61
	Downregulated: HCN2 or cAMP-sensitive subunit of the If	Decreases heart rate	62
	Downregulated: transcription factor Irx5*	Ventricular repolarization abnormalities (bundle branch block)	28
miR-15	Downregulated: VEGF ^b , c-MET ^b , COX-2 ^b , uPAR ^b	Regulates angiogenesis under hypoxia	37
miR-16	Downregulated: VEGF, c-MET, COX-2, uPAR	Regulates angiogenesis under hypoxia	37
miR-20	Downregulated: c-MET ^c , COX-2 ^c	Regulates angiogenesis under hypoxia	37
miR-21	Downregulated: TPM1	Cardiac hypertrophy	50,58
	Downregulated: Thrombospondin-1	Promotes angiogenesis and endothelial migration	66 ^d
	Upregulated: angiotensin receptor Tie-1, interleukin-8.	Capillary sprouting of endothelial cells and tube forming activity	65 ^d ,67
	Downregulated: VEGF, VEGF receptors, endothelial nitric oxide synthase	Endothelial proliferation after balloon angioplasty, antiapoptotic effect	68
	Downregulated: PTEN, Bcl-2		
miR-126	Upregulated: angiotensin receptor Tie-1, interleukin-8, c-Kit. Downregulated: VEGF, VEGF receptors, endothelial nitric oxide synthase	Capillary sprouting of endothelial cells and tube forming activity	65 ^d -67
miR-133	Downregulated: nuclear factor Nelf-A/WHSC2, GDP-GTP exchange protein RhoA, signal transduction kinase Cdc42, SRF, caspase-9 gene	Cardiogenesis, cardiac hypertrophy, prevents differentiation, enhances myoblast proliferation anti-apoptosis	43,51,52
	Downregulated: alpha KvLQT1 subunit of the IKs current	Prolongs action potential	61
	Downregulated: HCN4 subunit of the If	Decreases heart rate	62
	Downregulated: ERG protein responsible of the IKr	Repolarization slowing, QT prolongation	35
	Downregulated: Receptors of angiogenic factors ^g	---	36
miR-195	---	Cardiac hypertrophy with rapid transition to dilated cardiomyopathy and heart failure	54
	Downregulated: VEGF	Regulates angiogenesis under hypoxia	37
miR-206	Downregulated: DNA pol α , B-ind1, Mmd	Promotes differentiation	41
	Downregulated: Connexin 43	Prolongs repolarization, slows conduction velocity, promotes ADs and PVBs	41,59
miR-208	Downregulated: THRAP1	Cardiac remodelling, hypertrophic growth, β -MHC upregulation all of them induced by stress and hypothyroidism	53
miR-221	Downregulated: VEGF, VEGF receptors, angiotensin receptor Tie-1, endothelial NOS, interleukin-8, c-Kit	Capillary sprouting, tube forming activity, and endothelial migration	36,65 ^d -67
miR-222	Downregulated: VEGF, VEGF receptors, angiotensin receptor Tie-1, endothelial NOS, interleukin-8, c-Kit	Controversial results on the capacity of capillary sprouting, tube forming activity, and endothelial migration	36,65 ^d -67

See explanation in the text. Effects on arrhythmogenesis appear highlighted on grey and effect on angio genesis with italics, in contrast to effects on muscle structure (differentiation, proliferation, development...) which are not highlighted. *Specifically described for miR-1-2. ^bSpecifically described for miR-15b. ^cSpecifically described for miR-20a. ^dSpecifically described for miR-20b. ^eSpecifically described for miR-133a. ^fEffect observed in experiments in which Dicer machinery was blocked (either in mice or in endothelial or vascular smooth muscle cell cultures). miRNA, microRNA. mRNA, messenger RNA. HSP, heat shock protein. RasGAP, Ras GTPase-activating protein. Cdk9, cyclin-dependent kinase 9. Rheb, Ras homolog enriched in brain. IK₁, inward rectifier potassium channel. IKs, slow-activating delayed rectifying potassium channel. If, hyperpolarization-activated (pacemaker) channels. AD, afterdepolarization. PVB, premature ventricular beat. VSD, ventricular septal defect. HCN, hyperpolarization-activated cation channel. DNA pol, polymerase. B-ind1, butyrate- induced transcript 1. Mmd, monocyte-to-macrophage differentiation-associated protein. IKr, rapid delayed rectifier potassium current. THRAP1, thyroid hormone receptor associated protein 1. MHC, myosin heavy chain. TPM1, tumour suppressor gene tropomyosin 1. VEGF, vascular endothelial growth factor. NOS, nitric oxide synthase. c-MET, mesenchymal epithelial transition factor. COX-2, cyclooxygenase-2. uPAR, receptor for urokinase-type plasminogen activator. PAI-1, plasminogen activator inhibitor-1. MAPK7, mitogen-activated protein kinase-7. HIF-1 α , hypoxia inducible factor-1 α . PTEN, phosphatase and tensin homolog deleted on chromosome Ten. Bcl-2, B-cell leukemia-2.

Important efforts to screen the clinical consequences of miRNA gene modulation have highlighted their influence on the development of cardiac hypertrophy, an important cardiac adaptive mechanism which, if uncontrolled, can induce itself remodelling and heart failure. In animal models of induced cardiac hypertrophy with calcineurin stimuli or aortic banding, more than 50 miRNA showed progressive changes during the development of hypertrophy [43, 50, 54-56], and forced overexpression of stress-inducible miRNAs was sufficient to induce hypertrophy of cultured cardiomyocytes [43, 54, 56]. Interestingly, many of these miRNAs were similarly regulated in failing human hearts, suggesting that their effects on the regulated target mRNAs contribute to the creation of a fetal-type transcriptosome [5, 26, 45, 57]. Among the miRNA altered in pressure-overload cardiac hypertrophy models, miR-1 was singularly down-regulated, plausibly through a SRF-dependent mechanism, and the relief of the usual repression of miRNA on growth-related target genes allowed an induction of compensatory cardiac hypertrophy [43, 50, 55]. Similar behaviour was described for miR-133 and functional studies confirmed that a gain of function obtained with viral vector transduction prevented cardiomyocyte hypertrophy to take place, whereas a loss of function achieved with the suppression of miR-133 (either by 'decoy' sequences *in vitro* and by infusion of an antagomir *in vivo*) induced hypertrophy, which was more pronounced than that after stimulation with conventional hypertrophy inducers [43, 54, 55]. Unlike miR-1 and miR-133, miR-195 and miR-21 were overexpressed in these models [50, 54-56]. Interestingly, both miRNAs were shown to induce cardiac hypertrophy when overexpressed without pressure-overload [54, 55, 58]. Indeed, the miR-195 forced overexpression was sufficient to induce cardiac hypertrophy with a rapid transition to dilated cardiomyopathy and heart failure [54]. In addition, miR-21 overexpression, stimulated by angiotensin II and epinephrine, induced a hypertrophy in cultured cardiomyocytes which could be blocked with antisense oligonucleotides [55]. The putative mechanism by which miR-21 promotes cardiac hypertrophy could be an antiapoptotic role in early stages [50, 58]. All these assays suggest the possibility that cardiac phenotypes resulting from the regulated expression of endogenous miRNAs most likely reflect the combined actions of multiple miRNAs rather than the effect of a single miRNA. Unfortunately, the study of miRNAs in cultures of cardiomyocytes to address this issue has been troublesome because of their poor transfection capacity. This capacity might improve by using recombinant adenovirus to transfer the stem-loop pre-miRNA to myocytes under the control of the cytomegalovirus promoter [50].

Effects on the Electrical Properties of the Heart

Besides the wide range of actions on the myocardium, several miRNA have been implicated in the maintenance of the electrical properties of the heart. Dysregulations in the fine balance of miRNA homeostasis may lead to electrical instability and to sudden death [59]. The down-regulation of ion channels and proteins localized in gap junctions account for the molecular mechanisms implicated. All of them are of paramount importance in the generation of the action potential and the electromechanical coupling, respectively.

miR-1 is up-regulated in structurally diseased human hearts and in patients with myocardial infarction [34]. Its expression can be localized near the infarct border zone, where it down-regulates the KCNJ2 and GJA1 expressions with the cooperation of miR-206 [60]. The first of these genes encodes the principle pore-forming subunit Kir2.1 of the inwardly rectified ion current (IK₁), while the second encodes connexin43, a constituent gap junction protein, critical for the impulse propagation and electrical synchronization between myocytes [34, 60]. Both reduction in the IK₁ potassium current and loss of connexin43 prolong repolarization and slow the conduction velocity generating re-entry circuits, and enhancement of cellular calcium ion entry, which underlies the phenomena of afterdepolarizations and premature ventricular beats. Other cardiac ion channels are under miRNA control, such as the slow-activating delayed rectifying potassium channel (IKs), hyperpolarization-activated (pacemaker) channels (If), the voltage-gated potassium channel (IKv) and the rapid delayed rectifier potassium current (IKr). In cardiac cells, KCNQ1 assembles with KCNE1 and forms a channel complex constituting the IKs. Similarly, hyperpolarization-activated cation channel 2 (HCN2) and hyperpolarization-activated cation channel 4 (HCN4) constitute the If current. Both the KCNQ1 and HCN2 products have been characterized as targets for the repressive action of miR-1, and KCNE1 and HCN4 of miR-133 [61, 62]. Zhao Y *et al.* demonstrated that miR-1-2 down-regulates Irx5, a transcription factor essential for repressing KCND2, which encodes the Kv4.2 subunit of the IKv current [28]. Hence, in mutant mice lacking miR-1-2, Irx5 levels increased and transcripts from the KCND2 decreased and correlated with both short PR intervals and broad QRS complexes in the electrocardiogram in these animals [28, 63, 64]. Finally, miRNA-133 was shown to down-regulate the expression of HERG, a gene encoding the ion channel responsible for the IKr current, thus, contributing to repolarization slowing, QT prolongation and arrhythmogenesis [35].

Modulation of Angiogenesis

Angiogenesis-controlling mechanisms have always attracted the interest of cardiologists because of their crucial role in ischemic heart disease. In recent years, the study of miRNAs has shed new and useful lights on the understanding of the mechanisms underlying angiogenic factors modulation and vessel formation. Thus, silencing the enzymatic processes that intervene in miRNA maturation results in the regulation of the vascular endothelial growth factor (VEGF) and its receptor KDR, the angiopoietin receptor Tie-1, and also the modulation of the endothelial nitric oxide synthase (NOS), interleukin-8, capillary sprouting, tube forming activity, and endothelial migration [65-67]. Based on bioinformatics algorithms, at least 15 miRNAs target up to 5 receptors of angiogenic factors [36], but only 4 of them are overexpressed in endothelial cells, namely miR-21, miR-126, miR-221 and miR-222. Under hypoxia stimulation, different miRNAs may play a predominant role in controlling the key angiogenic factors which are up-regulated in this setting, such as VEGF, mesenchymal epithelial transition factor (c-MET), cyclooxygenase-2 (COX-2), receptor for urokinase-type plasminogen activator (uPAR), plasminogen activator inhibitor-1 (PAI-1), mitogen-activated protein kinase-7

(MAPK7), and angiotensinogen [37]. Indeed, miR-15b, miR-16, miR-20a, and miR-20b, but no others, were observed to specifically regulate these angiogenic factors, probably through either an accumulation of the product of the tumour suppressor gene p53 or a stabilization of the hypoxia inducible factor-1 α (HIF-1 α) during the hypoxic stimuli [37]. Interestingly, several miRNAs with well-known actions in the heart (Table 1) were overexpressed, both under basal conditions (miR-133a, miR-21) [36], and hypoxia (miR-195) [37], and all of them had angiogenic factors as putative targets. Once again, miR-21 was found to be an important regulator of neointima formation after the vascular burden caused by carotid balloon angioplasty in rats [68]. The aberrant increased expression of miR-21 could be suppressed with antisense oligonucleotides, and resulted in a parallel reduction of neointimal proliferation and in an increase in cell apoptosis in a dose-dependent manner. Western blot analysis demonstrated that phosphatase and tensin homolog deleted from chromosome Ten (PTEN) and B-cell leukemia-2 (Bcl-2) were attributable to miR-21 targets which are probably involved in its cellular effects [68].

TANTALIZING HORIZONS

Given the spectrum of miRNAs' actions on the heart (Table 1), it is easily observed that their major areas of influence are cardiac development and performance, arrhythmias and angiogenesis. miRNAs are overexpressed in certain cardiac pathological situations (such as cardiac pressure-overload, idiopathic end-stage failing heart, myocardial infarction and vessel insult due to percutaneous coronary interventions) and being able to attenuate or abolish their actions would reduce their deleterious consequences (such as excessive cardiac hypertrophy and transition to dilated cardiomyopathy, life-threatening electrical perturbations and coronary angioplasty/stent restenosis). The more we know about miRNAs, the more appealing they seem for cardiology researchers. Several clinical issues somehow connected to miRNAs' pathways are remarked below. Their real involvement, though tantalizing, remains to be proved. Several miRNAs, simultaneously involved in the hypertrophic mechanisms and electrical dysbalance of certain diseases (aortic stenosis, idiopathic left ventricle hypertrophy and hypertrophic cardiomyopathy), could turn out to be promising new targets in the future (see Table 1) [69, 70]. The high levels of angiotensin II associated to certain renin-angiotensin-aldosterone system polymorphisms, through the overexpression of miR-21, might help to explain the mechanism by which these genetic traits correlate with higher extent of hypertrophy in patients with hypertrophic cardiomyopathy (see Table 1) [71-73]. Disturbances in the fine network of miRNAs controlling connexin expression (see Table 1) might be responsible for alterations in the embryonic cardiac development [74-78] leading to the development of a large variety of congenital heart diseases. Finally, also the control of cardiac ion channels by certain miRNAs (see Table 1) provides a basis to speculate that a dysregulation in this fine network of miRNAs might mimic channelopathies (which are potentially lethal primary arrhythmic syndromes due to mutations in the coding genes of the cardiac ion channels in the absence of structural heart abnormalities). Whether post-transcriptional effects of these miRNAs can explain the

mechanisms underlying those cases with an electrocardiographic pattern of a given channelopathy and a negative genetic study remains unexplored, a frequent scenario which accounts for up to 80-85% of the cases of electrocardiographical Brugada syndrome [79], for instance.

The current rise in papers on miRNA reflects the strong interest of researchers on this field, based on the hope that miRNAs hold promise as novel diagnostic tool and possibly as therapeutic targets or drug agents [80]. As diagnostic tools, miRNAs should aim to anticipate the development of severe cardiac hypertrophy and end-stage dilated cardiomyopathy on one hand, and also shed some light on the current risk stratification for sudden death, on the other. Current approaches towards a therapeutic use of miRNAs include the use of antisense oligonucleotides to inhibit miRNA function and siRNA-like technologies to replace miRNAs [34, 62, 74, 80]. Few companies have jumped into the miRNA field as suppliers of tools and information. However, debate has been sparked about future patents and biotech industry seems to be awaiting more data on mechanism and some clarity on the intellectual property situation (a list of interested firms is updated elsewhere [80]). It is well-known that diseased tissues reveal aberrant expression patterns that implicate many genes, including miRNAs, and the feasibility to utilise single molecules to modulate most of them looks quite reasonable and attractive. At least theoretically, targeting specific miRNAs implicated in ischemic heart disease, cardiac hypertrophy, heart failure, angiogenesis and ion channel perturbances could turn out to be an attractive therapeutic tool in the future, aiming to prevent the development of heart failure, angioplasty/stent restenosis and ventricular arrhythmias or to promote neoangiogenesis for ischemic myocardium. Moreover, attending to the properties of miR-1 and miR-133 in modifying the If current (Table 1), they could both be used to modulate heart rate in sinus rhythm, in order to increase heart rate in patients with sick sinus syndrome or to decrease it in patients with inappropriate sinus tachycardia or ischemic heart disease, by blocking or replacing these miRNAs, respectively. However, the *in vivo* final effect of the modulation of miRNAs remains unclear, since each of them can target hundreds of genes with different intensity. Concerns about delivery of antisense oligonucleotides into cells, distribution in blood, clearance pathways and toxicity still remain unsolved [80]. Nonetheless, *microRNAs* really do seem to have a *macro* effect on cardiac structure (differentiation, development and hypertrophy) and arrhythmogenesis (sudden death). Further research will hopefully address these and other outstanding issues with success and provide the basis to implement all this knowledge in our future daily practice. Although cancer will surely be the first field where miRNAs will be assayed in clinical trials (hopefully within five years' time [80]), lethal cardiopathies will patiently wait their turn throughout the next decade.

ACKNOWLEDGMENTS

This work has been supported by research grants from Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III (FIS PI08/0185, PI07831, CP07/00326 and Red RECAVA), and Fundación para la Investigación del Hospital Universitario La Fe. Authors acknowledge the interest of coroners and pathologists from the Institute of Legal Medi-

cine of Castellón, Valencia and Alicante in promoting clinical and translational research on the field of sudden cardiac death. Authors state that there are no potential conflicts of interest.

ABBREVIATIONS

Ago	=	Argonaute
Bcl-2	=	B-cell leukemia-2
Cdk9	=	Cyclin-dependent kinase 9
c-MET	=	Mesenchymal epithelial transition factor
COX-2	=	Cyclooxygenase-2
HCN	=	Hyperpolarization-activated cation channel
HIF-1 α	=	Hypoxia inducible factor-1 α
Id1-3	=	Inhibitor of differentiation-3 protein
If	=	Hyperpolarization-activated (pacemaker) current
IK ₁	=	Inwardly rectified ion current
IKr	=	Rapid delayed rectifier potassium current
IKs	=	Slow-activating delayed rectifying potassium current
IKv	=	Voltage-gated potassium current
MAPK7	=	Mitogen-activated protein kinase-7
miRNA	=	microRNA
mRNA	=	Messenger RNA
Mef2	=	Myocyte enhancer factor 2
MHC	=	Myosin heavy chain
MyoD	=	Myogenic differentiation 1
MyoR	=	Myogenic repressor
PAI-1	=	Plasminogen activator inhibitor-1
PTEN	=	Phosphatase and tensin homolog deleted on chromosome Ten
RAAS	=	Renin-angiotensin-aldosterone system
RasGAP	=	Ras GTPase-activating protein
rasRNA	=	Repeat-associated siRNA
Rheb	=	Ras homolog enriched in brain
scnRNA	=	Small scanRNA
siRNA	=	Short interfering RNA
SRF	=	Serum response factor
THRAP1	=	Thyroid hormone receptor-associated protein
uPAR	=	Receptor for urokinase-type plasminogen activator
UTR	=	Untranslated region

VEGF = Vascular endothelial growth factor

WHSC = Wolf-Hirschhorn syndrome complex

REFERENCES

- [1] Bartel, D.P. MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell*, **2004**, *116*, 281.
- [2] Fitzgerald, K. RNAi versus small molecules: Different mechanisms and specificities can lead to different outcomes. *Curr. Opin. Drug Discov. Devel.*, **2005**, *8*, 557.
- [3] Stanislawski, J.; Olszewski, W.L. RNA interference--significance and applications. *Arch. Immunol. Ther. Exp. (Warsz)*, **2005**, *53*, 39.
- [4] Kim, V.N. Small RNAs: Classification, biogenesis, and function. *Mol. Cells*, **2005**, *19*, 1.
- [5] Latronico, M.V.; Catalucci, D.; Condorelli, G. Emerging role of microRNAs in cardiovascular biology. *Circ. Res.*, **2007**, *101*, 1225.
- [6] Katiyar-Agarwal, S.; Morgan, R.; Dahlbeck, D.; Borsani, O.; Villegas, A., Jr.; Zhu, J.K.; Staskawicz, B.J.; Jin, H. A pathogen-inducible endogenous siRNA in plant immunity. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 18002.
- [7] Ruby, J.G.; Jan, C.; Player, C.; Axtell, M.J.; Lee, W.; Nusbaum, C.; Ge, H.; Bartel, D.P. Large-scale sequencing reveals 21U-RNAs and additional microRNAs and endogenous siRNAs in *C. Elegans*. *Cell*, **2006**, *127*, 1193.
- [8] Sontheimer, E.J.; Carthew, R.W. Silence from within: endogenous siRNAs and miRNAs. *Cell*, **2005**, *122*, 9.
- [9] Pillai, R.S. MicroRNA function: multiple mechanisms for a tiny RNA? *RNA*, **2005**, *11*, 1753.
- [10] Zhang, B.; Wang, Q.; Pan, X. MicroRNAs and their regulatory roles in animals and plants. *J. Cell Physiol.*, **2007**, *210*, 279.
- [11] Hannon, G.J.; Rossi, J.J. Unlocking the potential of the human genome with RNA interference. *Nature*, **2004**, *431*, 371.
- [12] Griffiths-Jones, S. The microRNA Registry. *Nucleic Acids Res.*, **2004**, *32*, D109.
- [13] Griffiths-Jones, S.; Grocock, R.J.; van Dongen, S.; Bateman, A.; Enright, A.J. miRBase: microRNA sequences, targets and gene nomenclature. *Nucleic Acids Res.*, **2006**, *34*, D140.
- [14] Lee, R.C.; Feinbaum, R.L.; Ambros, V. The *C. elegans* heterochronic gene *lin-4* encodes small RNAs with antisense complementarity to *lin-14*. *Cell*, **1993**, *75*, 843.
- [15] Ambros, V. The functions of animal microRNAs. *Nature*, **2004**, *431*, 350.
- [16] Rodriguez, A.; Griffiths-Jones, S.; Ashurst, J.L.; Bradley, A. Identification of mammalian microRNA host genes and transcription units. *Genome Res.*, **2004**, *14*, 1902.
- [17] Li, S.C.; Tang, P.; Lin, W.C. Intronic microRNA: discovery and biological implications. *DNA Cell Biol.*, **2007**, *26*, 195.
- [18] Lund, E.; Güttinger, S.; Calado, A.; Dahlberg, J.E.; Kutay, U. Nuclear export of microRNA precursors. *Science*, **2004**, *303*, 95.
- [19] Obernosterer, G.; Leuschner, P.J.; Alenius, M.; Martinez, J. Post-transcriptional regulation of microRNA expression. *RNA*, **2006**, *12*, 1161.
- [20] Kawahara, Y.; Zinshteyn, B.; Sethupathy, P.; Iizasa, H.; Hatzigeorgiou, A.G.; Nishikura, K. Redirection of silencing targets by adenosine-to-inosine editing of miRNAs. *Science*, **2007**, *315*, 1137.
- [21] Tomari, Y.; Zamore, P.D. Perspective: machines for RNAi. *Genes Dev.*, **2005**, *19*, 517.
- [22] Carmell, M.A.; Xuan, Z.; Zhang, M.Q.; Hannon, G.J. The Argonaute family: tentacles that reach into RNAi, developmental control, stem cell maintenance, and tumorigenesis. *Genes Dev.*, **2002**, *16*, 2733.
- [23] Liu, J.; Carmell, M.A.; Rivas, F.V.; Marsden, C.G.; Thomson, J.M.; Song, J.J.; Hammond, S.M.; Joshua-Tor, L.; Hannon, G.J. Argonaute2 is the catalytic engine of mammalian RNAi. *Science*, **2004**, *305*, 1437.
- [24] Lewis, B.P.; Burge, C.B.; Bartel, D.P. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell*, **2005**, *120*, 15.
- [25] Berezikov, E.; Guryev, V.; van de Belt, J.; Wienholds, E.; Plasterk, R.H.; Cuppen, E. Phylogenetic shadowing and computational identification of human microRNA genes. *Cell*, **2005**, *120*, 21.
- [26] Lagos-Quintana, M.; Rauhut, R.; Yalcin, A.; Meyer, J.; Lendeckel, W.; Tuschl, T. Identification of tissue-specific microRNAs from mouse. *Curr. Biol.*, **2002**, *12*, 735.

- [27] Rao, P.K.; Kumar, R.M.; Farkhondeh, M.; Baskerville, S.; Lodish, H.F. Myogenic factors that regulate expression of muscle-specific microRNAs. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 8721.
- [28] Zhao, Y.; Ransom, J.F.; Li, A.; Vedantham, V.; von Drehle, M.; Muth, A.N.; Tsuchihashi, T.; McManus, M.T.; Schwartz, R.J.; Srivastava, D. Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. *Cell*, **2007**, *129*, 303.
- [29] Valadi, H.; Ekström, K.; Bossios, A.; Sjöstrand, M.; Lee, J.J.; Lötvall, J.O. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.*, **2007**, *9*, 654.
- [30] Yekta, S.; Shih, I.H.; Bartel, D.P. MicroRNA-directed cleavage of HOXB8 mRNA. *Science*, **2004**, *304*, 594.
- [31] Meister, G.; Landthaler, M.; Patkaniowska, A.; Dorsett, Y.; Teng, G.; Tuschl, T. Human Argonaute2 mediates RNA cleavage targeted by miRNAs and siRNAs. *Mol. Cell*, **2004**, *15*, 185.
- [32] Calin, G.A.; Dumitru, C.D.; Shimizu, M.; Bichi, R.; Zupo, S.; Noch, E.; Aldler, H.; Rattan, S.; Keating, M.; Rai, K.; Rassenti, L.; Kipps, T.; Negrini, M.; Bullrich, F.; Croce, C.M. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 15524.
- [33] Jing, Q.; Huang, S.; Guth, S.; Zarubin, T.; Motoyama, A.; Chen, J.; Di Padova, F.; Lin, S.C.; Gram, H.; Han, J. Involvement of microRNA in AU-rich element-mediated mRNA instability. *Cell*, **2005**, *120*, 623.
- [34] Yang, B.; Lin, H.; Xiao, J.; Lu, Y.; Luo, X.; Li, B.; Zhang, Y.; Xu, C.; Bai, Y.; Wang, H.; Chen, G.; Wang, Z. The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat. Med.*, **2007**, *13*, 486.
- [35] Xiao, J.; Luo, X.; Lin, H.; Zhang, Y.; Lu, Y.; Wang, N.; Zhang, Y.; Yang, B.; Wang, Z. MicroRNA miR-133 represses HERG K⁺ channel expression contributing to QT prolongation in diabetic hearts. *J. Biol. Chem.*, **2007**, *282*, 12363.
- [36] Poliseno, L.; Tuccoli, A.; Mariani, L.; Evangelista, M.; Citti, L.; Woods, K.; Mercatanti, A.; Hammond, S.; Rainaldi, G. MicroRNAs modulate the angiogenic properties of HUVECs. *Blood*, **2006**, *108*, 3068.
- [37] Hua, Z.; Lv, Q.; Ye, W.; Wong, C.K.; Cai, G.; Gu, D.; Ji, Y.; Zhao, C.; Wang, J.; Yang, B.B.; Zhang, Y. MiRNA-directed regulation of VEGF and other angiogenic factors under hypoxia. *PLoS ONE*, **2006**, *1*, e116.
- [38] Humphreys, D.T.; Westman, B.J.; Martin, D.I.; Preiss, T. MicroRNAs control translation initiation by inhibiting eukaryotic initiation factor 4E/cap and poly(A) tail function. *Proc. Natl. Acad. Sci. USA*, **2005**, *102*, 16961.
- [39] Hwang, H.W.; Wentzel, E.A.; Mendell, J.T. A hexanucleotide element directs microRNA nuclear import. *Science*, **2007**, *315*, 97.
- [40] Bentwich, I. Prediction and validation of microRNAs and their targets. *FEBS Lett.*, **2005**, *579*, 5904.
- [41] Kim, H.K.; Lee, Y.S.; Sivaprasad, U.; Malhotra, A.; Dutta, A. Muscle-specific microRNA miR-206 promotes muscle differentiation. *J. Cell Biol.*, **2006**, *174*, 677.
- [42] Krutzfeldt, J.; Rajewsky, N.; Braich, R.; Rajeev, K.G.; Tuschl, T.; Manoharan, M.; Stoffel, M. Silencing of microRNAs *in vivo* with 'antagomirs'. *Nature*, **2005**, *438*, 685.
- [43] Care, A.; Catalucci, D.; Felicetti, F.; Bonci, D.; Addario, A.; Gallo, P.; Bang, M.L.; Segnalini, P.; Gu, Y.; Dalton, N.D.; Elia, L.; Latronico, M.V.; Hoydal, M.; Autore, C.; Russo, M.A.; Dorn, G.W., 2nd; Ellingsen, O.; Ruiz-Lozano, P.; Peterson, K.L.; Croce, C.M.; Peschle, C.; Condorelli, G. MicroRNA-133 controls cardiac hypertrophy. *Nat. Med.*, **2007**, *13*, 613.
- [44] Ebert, M.S.; Neilson, J.R.; Sharp, P.A. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat. Methods*, **2007**, *4*, 721.
- [45] Mann, D.L.; Bristow, M.R. Mechanisms and models in heart failure: the biomechanical model and beyond. *Circulation*, **2005**, *111*, 2837.
- [46] Zhao, Y.; Samal, E.; Srivastava, D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. *Nature*, **2005**, *436*, 214.
- [47] Nakajima, N.; Takahashi, T.; Kitamura, R.; Isodono, K.; Asada, S.; Ueyama, T.; Matsubara, H.; Oh, H. MicroRNA-1 facilitates skeletal myogenic differentiation without affecting osteoblastic and adipogenic differentiation. *Biochem. Biophys. Res. Commun.*, **2006**, *350*, 1006.
- [48] Mishima, Y.; Stahlhut, C.; Giraldez, A.J. miR-1-2 gets to the heart of the matter. *Cell*, **2007**, *129*, 247.
- [49] Sokol, N.S.; Ambros, V. Mesodermally expressed Drosophila microRNA-1 is regulated by Twist and is required in muscles during larval growth. *Genes Dev.*, **2005**, *19*, 2343.
- [50] Sayed, D.; Hong, C.; Chen, I.Y.; Lypowy, J.; Abdellatif, M. MicroRNAs play an essential role in the development of cardiac hypertrophy. *Circ. Res.*, **2007**, *100*, 416.
- [51] Xu, C.; Lu, Y.; Pan, Z.; Chu, W.; Luo, X.; Lin, H.; Xiao, J.; Shan, H.; Wang, Z.; Yang, B. The muscle-specific microRNAs miR-1 and miR-133 produce opposing effects on apoptosis by targeting HSP60, HSP70 and caspase-9 in cardiomyocytes. *J. Cell Sci.*, **2007**, *120*, 3045.
- [52] Chen, J.F.; Mandel, E.M.; Thomson, J.M.; Wu, Q.; Callis, T.E.; Hammond, S.M.; Conlon, F.L.; Wang, D.Z. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. *Nat. Genet.*, **2006**, *38*, 228.
- [53] van Rooij, E.; Sutherland, L.B.; Qi, X.; Richardson, J.A.; Hill, J.; Olson, E.N. Control of stress-dependent cardiac growth and gene expression by a microRNA. *Science*, **2007**, *316*, 575.
- [54] van Rooij, E.; Sutherland, L.B.; Liu, N.; Williams, A.H.; McAnally, J.; Gerard, R.D.; Richardson, J.A.; Olson, E.N. A signature pattern of stress-responsive microRNAs that can evoke cardiac hypertrophy and heart failure. *Proc. Natl. Acad. Sci. USA*, **2006**, *103*, 18255.
- [55] Cheng, Y.; Ji, R.; Yue, J.; Yang, J.; Liu, X.; Chen, H.; Dean, D.B.; Zhang, C. MicroRNAs are aberrantly expressed in hypertrophic heart: do they play a role in cardiac hypertrophy? *Am. J. Pathol.*, **2007**, *170*, 1831.
- [56] Tatsuguchi, M.; Seok, H.Y.; Callis, T.E.; Thomson, J.M.; Chen, J.F.; Newman, M.; Rojas, M.; Hammond, S.M.; Wang, D.Z. Expression of microRNAs is dynamically regulated during cardiomyocyte hypertrophy. *J. Mol. Cell Cardiol.*, **2007**, *42*, 1137.
- [57] Thum, T.; Galuppo, P.; Wolf, C.; Fiedler, J.; Kneitz, S.; van Laake, L.W.; Doevendans, P.A.; Mummery, C.L.; Borlak, J.; Haverich, A.; Gross, C.; Engelhardt, S.; Ertl, G.; Bauersachs, J. MicroRNAs in the human heart: a clue to fetal gene reprogramming in heart failure. *Circulation*, **2007**, *116*, 258.
- [58] Zhu, S.; Si, M.L.; Wu, H.; Mo, Y.Y. MicroRNA-21 targets the tumor suppressor gene tropomyosin 1 (TPM1). *J. Biol. Chem.*, **2007**, *282*, 14328.
- [59] Anderson, M.E.; Mohler, P. MicroRNA may have macro effect on sudden death. *J. Nat. Med.*, **2007**, *13*, 410.
- [60] Anderson, C.; Catoe, H.; Werner, R. MIR-206 regulates connexin43 expression during skeletal muscle development. *Nucleic Acids Res.*, **2006**, *34*, 5863.
- [61] Luo, X.; Xiao, J.; Lin, H.; Li, B.; Lu, Y.; Yang, B.; Wang, Z. J. Transcriptional activation by stimulating protein 1 and post-transcriptional repression by muscle-specific microRNAs of IKs-encoding genes and potential implications in regional heterogeneity of their expressions. *Cell Physiol.*, **2007**, *212*, 358.
- [62] Xiao, J.; Yang, B.; Lin, H.; Lu, Y.; Luo, X.; Wang, Z. Novel approaches for gene-specific interference *via* manipulating actions of microRNAs: examination on the pacemaker channel genes HCN2 and HCN4. *J. Cell Physiol.*, **2007**, *212*, 285.
- [63] Costantini, D.L.; Arruda, E.P.; Agarwal, P.; Kim, K.H.; Zhu, Y.; Zhu, W.; Lebel, M.; Cheng, C.W.; Park, C.Y.; Pierce, S.A.; Guerschicoff, A.; Pollevick, G.D.; Chan, T.Y.; Kabir, M.G.; Cheng, S.H.; Husain, M.; Antzelevitch, C.; Srivastava, D.; Gross, G.J.; Hui, C.C.; Backx, P.H.; Bruneau, B.G. The homeodomain transcription factor *Irx5* establishes the mouse cardiac ventricular repolarization gradient. *Cell*, **2005**, *123*, 347.
- [64] Gottlieb, P.D.; Pierce, S.A.; Sims, R.J.; Yamagishi, H.; Weihe, E.K.; Harriss, J.V.; Maika, S.D.; Kuziel, W.A.; King, H.L.; Olson, E.N.; Nakagawa, O.; Srivastava, D. Bop encodes a muscle-restricted protein containing MYND and SET domains and is essential for cardiac differentiation and morphogenesis. *Nat. Genet.*, **2002**, *31*, 25.
- [65] Yang, W.J.; Yang, D.D.; Na, S.; Sandusky, G.E.; Zhang, Q.; Zhao, G. Dicer is required for embryonic angiogenesis during mouse development. *J. Biol. Chem.*, **2005**, *280*, 9330.

- [66] Kuehbachner, A.; Urbich, C.; Zeiher, A.M.; Dimmeler, S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. *Circ. Res.*, **2007**, *101*, 59.
- [67] Suárez, Y.; Fernández-Hernando, C.; Pober, J.S.; Sessa, W.C. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. *Circ. Res.*, **2007**, *100*, 1164.
- [68] Ji, R.; Cheng, Y.; Yue, J.; Yang, J.; Liu, X.; Chen, H.; Dean, D.B.; Zhang, C. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. *Circ. Res.*, **2007**, *100*, 1579.
- [69] Virmani, R.; Burke, A.P.; Farb, A.; Kark, J.A. Causes of sudden death in young and middle-aged competitive athletes. *Cardiol. Clin.*, **1997**, *15*, 439.
- [70] Suárez-Mier, M.P.; Aguilera, B. Causes of sudden death during sports activities in Spain. *Rev. Esp. Cardiol.*, **2002**, *55*, 347.
- [71] Tesson, F.; Dufour, C.; Moolman, J.C.; Carrier, L.; al-Mahdawi, S.; Chojnowska, L.; Dubourg, O.; Soubrier, E.; Brink, P.; Komajda, M.; Guicheney, P.; Schwartz, K.; Feingold, J. The influence of the angiotensin I converting enzyme genotype in familial hypertrophic cardiomyopathy varies with the disease gene mutation. *J. Mol. Cell Cardiol.*, **1997**, *29*, 831.
- [72] Ortlepp, J.R.; Vosberg, H.P.; Reith, S.; Ohme, F.; Mahon, N.G.; Schröder, D.; Klues, H.G.; Hanrath, P.; McKenna, W.J. Genetic polymorphisms in the renin-angiotensin-aldosterone system associated with expression of left ventricular hypertrophy in hypertrophic cardiomyopathy: a study of five polymorphic genes in a family with a disease causing mutation in the myosin binding protein C gene. *Heart*, **2002**, *87*, 270.
- [73] Perkins, M.J.; Van Driest, S.L.; Ellsworth, E.G.; Will, M.L.; Gersh, B.J.; Ommen, S.R.; Ackerman, M.J. Gene-specific modifying effects of pro-LVH polymorphisms involving the renin-angiotensin-aldosterone system among 389 unrelated patients with hypertrophic cardiomyopathy. *Eur. Heart J.*, **2005**, *26*, 2457.
- [74] Esau, C.C.; Monia, B.P. *Adv. Drug Deliv. Rev.*, **2007**, *59*, 101.
- [75] Britz-Cunningham, S.H.; Shah, M.M.; Zuppan, C.W.; Fletcher, W.H. Mutations of the Connexin43 gap-junction gene in patients with heart malformations and defects of laterality. *N. Engl. J. Med.*, **1995**, *332*, 1323.
- [76] Krüger, O.; Maxeiner, S.; Kim, J.S.; van Rijen, H.V.; de Bakker, J.M.; Eckardt, D.; Tiemann, K.; Lewalter, T.; Ghanem, A.; Lüderitz, B.; Willecke, K. Cardiac morphogenetic defects and conduction abnormalities in mice homozygously deficient for connexin40 and heterozygously deficient for connexin45. *J. Mol. Cell Cardiol.*, **2006**, *41*, 787.
- [77] Kołcz, J.; Drukała, J.; Bzowska, M.; Rajwa, B.; Korohoda, W.; Malec, E. The expression of connexin 43 in children with Tetralogy of Fallot. *Cell Mol. Biol. Lett.*, **2005**, *10*, 287.
- [78] Reaume, A.G.; De Sousa, P.A.; Kulkarni, S.; Langille, B.L.; Zhu, D.; Davies, T.C.; Juneja, S.C.; Kidder, G.M.; Rossant, J. Cardiac malformation in neonatal mice lacking connexin43. *Science*, **1995**, *267*, 1831.
- [79] Priori, S.G.; Napolitano, C.; Gasparini, M.; Pappone, C.; Della, B.P.; Brignole, M.; Giordano, U.M.; Giovannini, T.; Menozzi, C.; Bloise, R.; Crotti, L.; Terreni, L.; Schwartz, P.J. Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: A prospective evaluation of 52 families. *Circulation*, **2000**, *102*, 2509.
- [80] Mack, G.S. MicroRNA gets down to business. *Nat. Biotechnol.*, **2007**, *25*, 631.