MicroRNA Regulation in Cardiovascular Disease

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Abstract: The molecular biology dogma that DNA replicates its genetic information within nucleotide sequences and transcribes it to RNA where it codes for the generation of mRNA, failed to consider a significant part of the genetic code. Although it has been generally assumed that most genetic information is executed by proteins, recent evidence suggests that the majority of the genomes of mammals and other complex organisms is transcribed into non-coding RNA (ncRNA), many of which are alternatively spliced and/or processed into smaller functional RNA molecules. ncRNAs are predominantly involved in processes that require highly specific nucleic acid recognition, revealing a, so far hidden, layer of internal signals that control various levels of gene expression in developmental and (patho)physiological processes. MicroRNAs (miRNAs) are a large class of evolutionary conserved, small ncRNAs, typically 18 to 24 nucleotides in length, that primarily function at the posttranscriptional level by interacting with the 3' untranslated region (UTR) of specific target mRNAs in a sequence-specific manner. Despite the advances in miRNA discovery, the role of miRNAs in physiological and pathological processes is just rising, revealing their cellular functions in proliferation and differentiation, apoptosis, the stress response and tumorgenesis. MiRNA expression profiling and the manipulation of their expression in cardiac tissue has led to the discovery of regulatory roles for these small ncRNAs during cardiac development and disease, implicating them in regulation of cardiac gene expression. Here we review the basic mechanisms by which cardiovascular miRNAs are regulated in the larger context of cardiogenesis and in cardiovascular disease.

Keywords: MicroRNA, transcriptional regulation, cardiac disease.

BIRTH OF microRNAS

MicroRNAs (miRNAs) are currently believed to be mostly transcribed by RNA polymerase II [1], although a few may be transcribed by RNA polymerase III [2]. There are two different classes of miRNAs when discussing their transcriptional mechanism; those found within annotated genes (intronic miRNAs) and those found in intergenic regions of the genome (intergenic miRNAs). It is presently believed that all intronic miRNAs are co-transcribed along with their host gene; this has been shown in both expression correlation studies [3] as well as PCR based biochemical verification [4]. Intergenic miRNAs have been postulated to come from transcripts of up to 50kb in length, allowing for the co-transcription of neighboring miRNAs (polycistronic miRNA clusters) [3].

The identification of sequence features shared by core promoter regions from protein coding genes, has revealed that the transcription start site of miRNA genes share the same sequence features as protein coding genes [5]. These studies exemplify that miRNA genes are transcribed by the same mechanism by which protein-coding genes are transcribed. Given this similarity, the same bioinformatics approaches can be used to identify conserved regulatory elements in the larger miRNA gene regions as described for protein coding genes which may be occupied by transcription factors. The cooperative interaction and combined action between RNA polymerase II and cardiac transcription factors regulate developmental patterning, basal- and stress induced transcription of miRNA genes into primary miRNAs. Below we present some examples of these transcriptional networks regulating cardiac miRNAs.

Serum response factor (SRF) is a cardiac enriched transcription factor responsible for the regulation of organized sarcomeres in the heart [6]. Expression of SRF follows a restrictive pattern during mouse development and finally becomes confined to the heart tube and mesenchymal somites [7]. SRF target genes contain single or multiple copies of the SRF-binding consensus element (known as a *CArG* box), which are usually found in proximity to promoters of genes regulating contractility, cell movement, and growth signaling [8, 9]. The activity of SRF is positively controlled by interactions with other tissue-specific regulatory cofactors, such as GATA4, Nkx2.5 [10], and myocardin [11], while YY1 and HOP are known to restrict SRF activity and SRF-mediated myogenic gene activation [12, 13]. Approximately one-third of all mammalian miRNA genes contain at least one CArG element in or close to their promoter regions, including miR-1-1, miR1-2, miR-21, miR-206, miR-214 [7], but whether this simply reflects the relative high bioinformatics incidence of the CarG sequence throughout genomes, or is indicative of a central role for SRF in controlling basal expression levels of miRNA families, have been scarcely tested experimentally.

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One such example constitutes the functionality of SRF site during control of embryonic expression of miR-1-1/miR-1-2, where the miR-1 gene family titrates the effects of critical cardiac regulatory proteins to control the balance between differentiation and proliferation during cardiogenesis by fine-tuning expression of the basic helix loop helix (bHLH) transcription factor Hand2 [14]. Mutation of the SRF site abolished the cardiac expression of the respective miRNA [14]. SRF is a weak activator of the miR-1 and miR-133 family members, but together with myocardin and potentially other transcription factors and cofactors synergistically induce expression of respective miRNAs [11, 12, 14, 15]. Likewise, myocyte enhancer factor-2 or MEF2, a critical regulator of muscle development and stress-induced cardiac gene expression [16, 17], additionally controls transcription of a bicistronic primary transcript encoding miR-1-2 and miR-133a-1 via an intragenic muscle-specific enhancer located in between coding regions of both miRNAs [18]. As such, the cooperative synergy between MEF2 and the skeletal muscle restricted bHLH factor MyoD, known to regulate various protein coding genes during myogenesis [19, 20], was uncovered to additionally control this miRNA cluster through an intragenic enhancer. When examining the expression pattern and transcriptional regulation of Drosophila miR-1 (DmiR-1), Sokol *et al.* demonstrated that, as in zebrafish, mice, and humans, DmiR-1 is specifically expressed in muscle cells, and its expression regulated by the promesodermal bHLH transcription factor Twist and the promyogenic transcription factor MEF2 [21]. These examples outline a potentially central role for SRF, MEF2 and bHLH factors in both transcriptional and post-transcriptional regulation of genes implicated in proper cardiogenesis and myogenesis.

Diverse forms of injury and stress evoke a hypertrophic growth response in adult cardiac myocytes, which is characterized by an increase in cell size, enhanced protein synthesis, assembly of sarcomeres, and reactivation of fetal genes, often culminating in heart failure and sudden death [6]. Given the emerging roles of miRNAs in modulation of gene expression that accompany cellular phenotypes, it can be anticipated that miRNAs are also differentially expressed during cardiac hypertrophy and heart failure. Indeed, multiple groups have now described sets of miRNAs that are differentially expressed in cardiac tissue from human heart failure patients and in mouse models of cardiac disease compared to the healthy myocardium [22-30]. In contrast, limited information is available how stress signals can mechanistically influence miRNA expression (Fig. **1**).

A recent report demonstrated that miR-23a is a pro-hypertrophic miRNA, and its expression regulated by the stress-induced, calcineurin responsive transcription factor, Nuclear Factor of Activated T cells (NFATc3), a transcriptional cascade that is intimately linked to myogenesis and

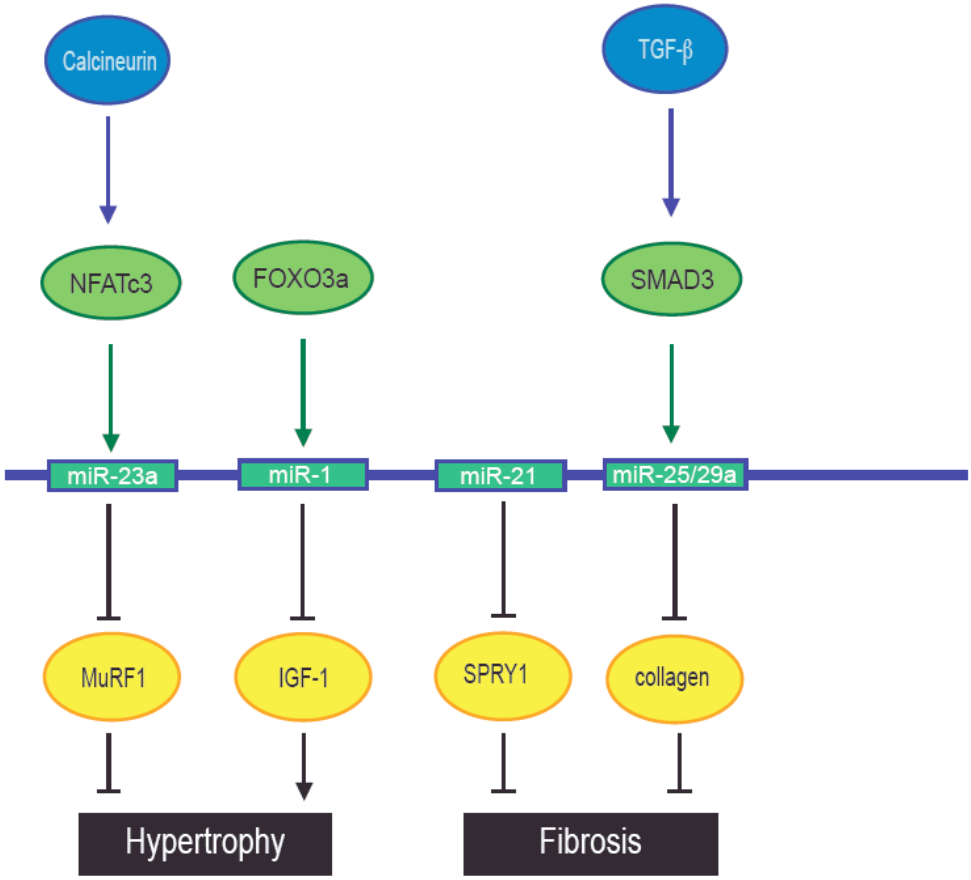

Fig. (1). Transcriptional regulation of stress induced microRNAs.

A subset of stress activated transcription factors have been revealed to regulate miRNA transcription, mechanistically linking miRNA downstream genes with molecular anti-hypertrophic and anti-fibrotic targets in the setting of adult onset heart failure.

pathological cardiac growth [31-34]. The authors demonstrated that miR-23a expression was increased upon treatment with the hypertrophic agonists isoproterenol and aldosterone, while knockdown of miR-23a attenuated pathological hypertrophy, suggesting that miR-23a is able to convey a hypertrophic signal [35]. A conserved NFAT site was identified upstream of the miR-23 cluster, demonstrated to bind NFATc3 in this genomic region *in vivo* and able to regulate the expression of the primary miRNA [35]. As a molecular mechanism by which the NFATc3 responsive miR-23a regulates hypertrophy, muscle specific ring finger protein 1 (MuRF1), an anti-hypertrophic protein [36, 37], was identified to be a target of miR-23a, a finding that is in line with the exaggerated hypertrophy response of MuRF1 knockout mice [37]. Likewise, Elia and colleagues recently demonstrated the existence of functional binding sites in the murine miR-1 promoter for the transcription factor Foxo3a, and convincingly showed that the mature form of miR-1 is able to target insulin-like growth factor-1 (IGF-1) [30]. Since it is known that IGF-1 signaling provokes hypertrophic growth of the myocardium through activation of protein kinase B or AKT, and the activity of Foxo3a is controlled by AKT, this study establishes the existence of a reciprocal, regulatory master circuitry between miR-1 and IGF-1 in controlling growth of striated muscles [30].

Diminution of contractility, a hallmark feature of heart failure, partially results from down-regulation of α–myosin heavy chain (αMHC, encoded by the myh6 gene) and up-regulation of βMHC (encoded by the myh7 gene), the primary contractile proteins of the heart. A cardiac-specific miRNA (miR-208a) embedded within and encoded by an intronic region of the αMHC gene, which in turn is controlled by the thyroid hormone receptor (TR) [38], was demonstrated to be necessary for βMHC upregulation in response to stress and hypothyroidism [39]. More recently, it became clear that not only αMHC, but three myosin encoding genes, the cardiac Myh6 (αMHC), Myh7 (βMHC), and the skeletal muscle myosin heavy chain 7b (Myh7b), all share similar intronic miRNAs, together termed MyoMiRs [40]. The Myh6 gene, expressed in the adult heart, encoding a fast myosin, coexpresses miR-208a, which, remarkably, regulates the expression of two slow myosins and their intronic miRNAs, Myh7/miR- 208b and Myh7b/miR-499, respectively. In turn, miR-208b and miR-499 play overlapping roles in the specification of muscle fiber identity by activating slow and repressing fast myofiber types [40]. These findings exemplify how single miRNA genes, or clustered miRNA/protein encoding genes, are regulated by the cooperative interaction of conserved transcription factor families that regulate developmental patterning, basal expression and stress induced transcription of miRNA genes with broad control on muscle gene expression and performance. The future will likely further provide many more examples of shared similarities between the transcription of protein coding genes and miRNAs.

STEPWISE PROCESSING OF miRNAS

MiRNAs are genomically encoded and their biogenesis starts with transcription by RNA Polymerase II [1] or III [2] creating long primary miRNA transcripts (pri-miRNAs). Most miRNAs derive from the exons or introns of noncoding RNAs, but about one-third are located in the introns of protein coding host genes [4].

Still in the nucleus, pri-miRNAs are recognized and cleaved into a 70 nucleotide hairpin-shaped precursor miRNA (pre-miRNA) in a two-step mechanism by a microprocessor complex which consists of the double-stranded RNA-specific nuclease Drosha and its cofactor, DiGeorge syndrome critical region 8 (DGCR8, Pasha) [3, 41, 42]. First, DGCR8 recognizes and binds tightly at the junction between to the stem loop structure, consisting of double stranded RNA, and the neighboring single stranded fragments following cleavage by recruited Drosha [43]. In addition, recent studies have shown that miRNA specific processing steps can precede and facilitate Drosha cleavage [44, 45]. For example, hnRNP A1 contributes to the pri-miR-18a processing by binding to it and supporting its processing by Drosha [44]. However, an alternative non-canonical pathway for miRNA biogenesis has been described, in which certain debranched introns, called mitrons, mimic the structural features of pre-miRNAs to enter the miRNA-processing pathway without Drosha-mediated cleavage [46].

The resulting pre-miRNA, whether formed by Drosha cleavage or splicing, is then transported to the cytoplasm by a nuclear transport receptor, exportin-5, which recognizes a 2- to 3-bp overhang of the pre-miRNA stem-loop structure [47, 48]. Further processing by a complex of the RNase III ribonuclease Dicer and the transactivator RNA-binding protein (TRBP) will cleave the pre-miRNA to release the mature miRNA duplex [49, 50]. Thermodynamic stability of the 5' ends of the miRNA duplex will decipher the faith of each strand with the less stable one being preferentially loaded into the RNA-induced silencing complex (RISC) and the other strand being degraded [51, 52].

In RISC, the short mature miRNA will be incorporated in a protein complex containing, among others, the protein *Argonaut* (Ago). Members of the Ago family are involved in silencing the mRNA translation with Ago2 being the only human Ago protein that contains intrinsic endonuclease activity [53]. Once the mature miRNAs have become associated with the RISC and Argonaut proteins they bind to their target sites through base complementarity on the 3' untranslated region of mRNA transcripts, typically 7 – 8nt in length [54]. This binding can result in either the full degradation of the target mRNA transcript or the blocking of its translation. However, several additional roles for Ago protein have been described in miRNA processing and posttranscriptional regulation, upstream in the pathway of miRNA maturation and function [55]. Since all Ago proteins are capable of binding miRNAs, but not on the RNase active site of Ago2, they are able to increase miRNA stability and abundance at posttranscriptional level. Loss of Ago2 results in reduced expression of mature endogenous miRNAs. On the other hand, Ago2 can generate an additional miRNA precursor called ac-pre-miRNA, a pathway intermediate in miRNA biogenesis that is generated from the pre-miR by Ago2 and serves as a substrate for Dicer to mature into the active miRNA. These multiple roles of Ago proteins suggest regulation of miRNA expression and function at multiple levels.

MiRNA target prediction methods have shown that each miRNA gene may be able to target many mRNAs, while

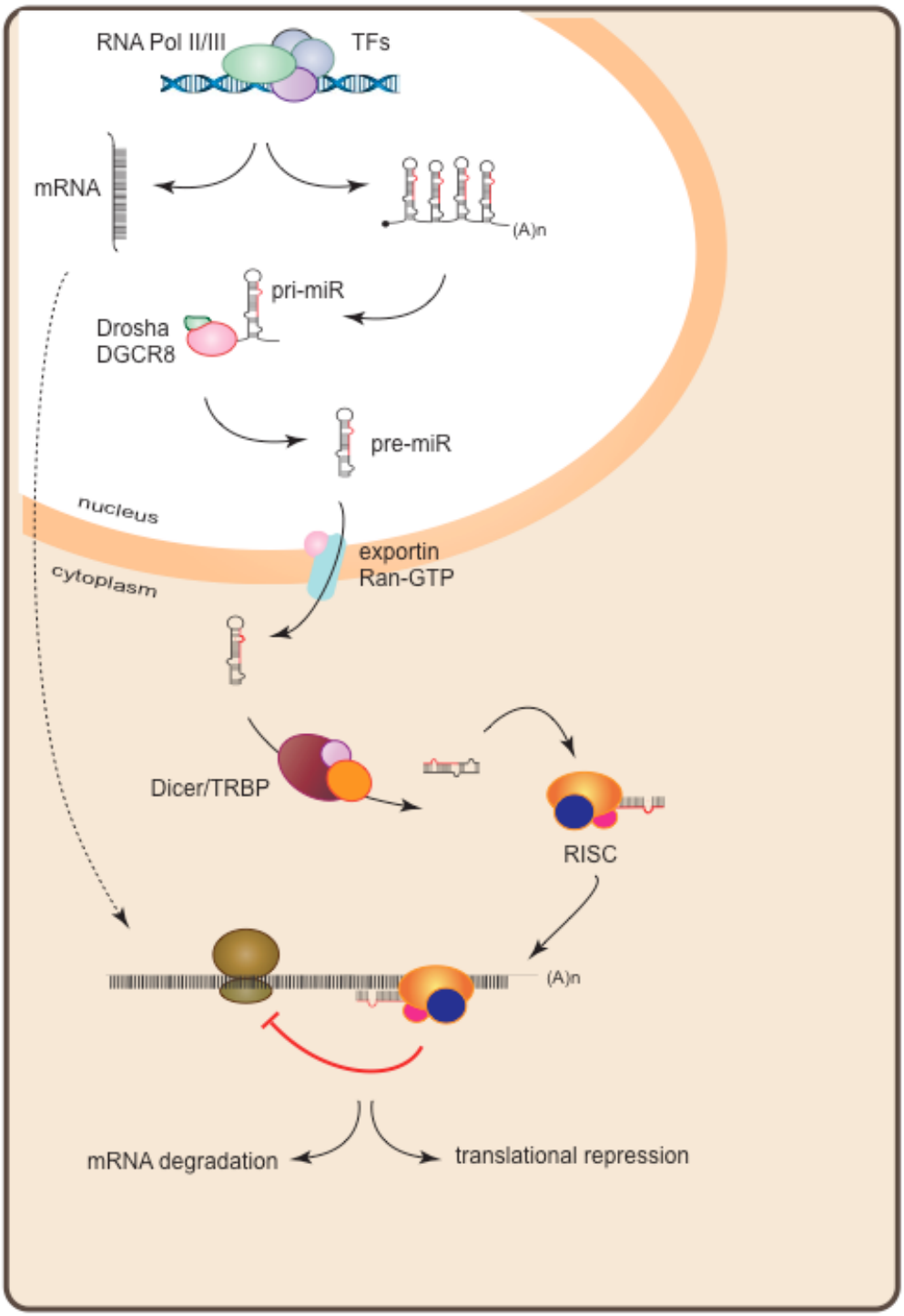

Fig. (2). MicroRNA biogenesis.

The primary transcripts of miRNAs, called pri-miRNAs, are transcribed as individual miRNA genes, from introns of protein-coding genes, or from polycistronic transcripts. The RNase Drosha further processes the pri-miRNA into 70–100 nucleotide, hairpin-shaped precursors, called pre-miRNA, which are exported from the nucleus by exportin 5. In the cytoplasm, the pre-miRNA is cleaved by Dicer into an miRNA:miRNA* duplex. Assembled into the RISC, the mature miRNA negatively regulates gene expression by either translational repression or mRNA degradation, which is dependent on sequence complementarity between the miRNA and the target mRNA. ORF, open reading frame.

each mRNA may be the target of multiple miRNAs (Fig. **2**) [56, 57]. Although miRNAs have been implicated in developmental processes as well as a variety of human diseases, there is still surprisingly little known about the precise biochemical regulation of either one of the above steps.

ARREST OF miRNA BIOGENESIS: CARDIOVASCULAR CONSEQUENCES

The first observations confirming the key significance of miRNA in the regulation of mammalian cardiovascular biology derived from miRNA biogenesis arresting experiments and, consequently, depletion of miRNA pools in vascular cells and tissues [58-61]. Dicer, a key enzyme involved in miRNA biogenesis was reported to be abundantly expressed at embryonic day 11 (E11) remaining constant to E17 and being uniformly expressed through the different tissues [60]. A dicer mutant mouse, lacking two essential exons for protein function, exhibited growth and developmental defects vascular malformation, the first evidence for the involvement of miRNAs in angiogenesis during embryogenesis. Subsequent studies followed this hallmark finding, further strengthening a strong involvement of miRNAs in blood vessel development. Apart from Dicer, the endonuclease activity of Drosha also constitutes a rate-limiting processing step during the maturation of miRNAs. In endothelial cells (EC) silencing of Dicer and/or Drosha dramatically disturbed capillary sprouting and tube-forming capacities while upon Dicer silencing only EC migration was affected [58, 59].

Although downregulation of the miRNA-processing enzymes Dicer and Drosha is known to impair angiogenesis and 15 of the most abundant miRNAs in HECs are predicted to target receptors of angiogenic factors, only a few specific miRNAs have been identified to influence endothelial cell function and angiogenesis. For further details about the role of miRNAs in angiogenesis see this issue [62, 63]. Among the highly expressed miRNAs in human umbilical vein endothelial cells (HUVEC), miR-221 and miR-222 are known to exert anti-angiogenic effects [64] since transfection of endothelial cells with miR-221 and miR-222 inhibits tube formation, migration, and wound healing of endothelial cells *in vitro*. In line, another study demonstrated the anti-angiogenic function of miR-221/222 in endothelial cells [59]. The core mechanism involves downregulation of the protein expression levels, without affecting the mRNA level, of the predicted target c-kit, the receptor for stem cell factor, suggesting posttranscriptional regulation [64]. In haemato-poietic progenitor cells, the miR-221/222 family also reduces c-kit expression and as a functional consequence cell proliferation [65,66]. Although the precise role of miR-221 and miR-222 has not been described so far and since c-kit is an important marker of cardiac stem cells [65, 66] it is appealing to speculate a possible role of these miRNAs in cardiac stem cell differentiation or function.

ARREST OF miRNA BIOGENESIS: CARDIAC DEMISE

In mice, targeted deletion of *Dicer* causes lethality at E7.5, just before body axis formation [67]. Cardiac-specific deletion of Dicer by expressing Cre recombinase under control of the endogeneous Nkx2.5 regulatory elements also results in embryonic lethality at E12.5, with hearts displaying pericardial edema and a poorly developed ventricular myocardium. Interestingly, just prior to death these hearts showed increased expression of adult cardiac stress-related genes. Nkx2.5-Cre is active from E8.5 during heart patterning and differentiation, throughout initial commitment of cardiac progenitor cells. Although Dicer activity is known to be required for embryonic stem cell differentiation, it still remains unclear whether Dicer is required before E8.5 during the primordial stages of cardiogenesis such as cardiac lineage specification.

To address the role of Dicer activity in the mature heart, researchers had to circumvent the embryonical lethality associated with germline null mutations for Dicer. Two similar studies have cardiac specifically deleted Dicer in the postnatal heart with distinct results. Da Costa Martins *et al.* [68] used a tamoxifen-inducible Cre recombinase in the postnatal murine myocardium to target Dicer deletion in 3-week- and 8-week-old mice. Dicer deletion provoked premature death within 1 week accompanied by mild ventricular remodeling and dramatic atrial enlargement in the juvenile mice. In the older animals (adult myocardium), loss of Dicer induced rapid and dramatic biventricular enlargement, accompanied by myocyte hypertrophy, myofiber disarray, ventricular fibrosis, and strong induction of fetal gene transcripts. Furthermore, a comparative miRNA profiling revealed a set of miRNAs entailing causality between miRNA depletion and spontaneous cardiac remodeling underlining that modifications in miRNA biogenesis affect postnatal myocardial morphology and function.

In a similar study, Wang and coworkers [69] used a similar Cre-loxP system to disrupt Dicer tissue-specifically in the heart by the use of constitutive expression of a MHC-Cre deleter model, and a significant reduction of most miRNAs analyzed was observed. Moreover, all animals died within 4 days after birth displaying heart failure phenotypes. Interestingly, while Dicer expression is decreased in end-stage human dilated cardiomyopathy (DCM), in failing human hearts a concomitant increase in Dicer expression was observed after insertion of left ventricle assist devices to improve patient cardiac function, suggesting a reciprocal relationship between Dicer expression and the severity of heart disease. Altogether, alterations in Dicer function and/or expression may explain the miRNA signature of failing hearts, have clinical implications in the onset and development of heart failure and provide a therapeutic entry point to approaches aiming at restore functional Dicer activity in the failing heart.

An alternative to Dicer and/or Drosha silencing is to disrupt the biogenesis pathway of miRNAs by targeting other intervening proteins. Deficiency in Ago2 impairs miRNA biogenesis from precursor miRNAs followed by a reduction in miRNA expression levels and in mice disruption of Ago2 leads to embryonic lethality early in development [70]. On the other hand, Ago-2 overexpression has been shown to enhance cell proliferation and increase cell migration in both *in vitro* and *in vivo* wound healing settings [71].

Drosha is known to form a protein complex with DGCR8, interaction required for proper processing of pri-miRNAs. Similarly, a DGCR8 knockout model has been created to study the role of miRNAs in ES cell differentiation. DGCR8 was originally identified as a gene whose monoallelic deletion accounts for >90% of patients with DiGeorge Syndrome, the most common human genetic deletion syndrome that affects around 1 in 3.000 live births [72]. The clinical manifestations of the disease are highly

variable, with 75% of the patients displaying congenital heart defects. The fact that the disease associates with haploinsufficiency of a typical deleted region (1.5 Mb) that covers around 30 genes has made the identification of these genes a crucial step in better understanding the pathology. Given the emerging role of miRNAs in regulating different aspects of disease, a provocative hypothesis could constitute that defects in miRNA processing secondary to primary defects in DGCR8 function may contribute to the developmental and cardiac abnormalities affecting DiGeorge syndrome patients.

Although many studies have been designed to modulate miRNA expression by interfering with specific steps in their biogenesis, we are far from being presented with all the main players. Just recently a mechanism has been described to promote specific changes in target mRNA expression affecting specific biological programs where KH-type splicing regulatory protein (KSRP) serves as a component of both Drosha and Dicer complexes regulating a specific cohort of miRNAs [73]. We can expect to witness the discovery of additional levels of complexity for miRNA-dependent regulation of gene expression that contributes to the modulation of different biological programs. Our collective understanding of these post-transcriptional gene regulators function in cellular networks may provide new molecular horizons for cures or therapies to a variety of human diseases (see this issue [74].

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REFERENCES

[1] Lee Y, Kim M, Han J, *et al.* MicroRNA genes are transcribed by RNA polymerase II. EMBO J 2004; 23: 4051-60.

[2] Borchert GM, Lanier W, Davidson BL. RNA polymerase III transcribes human microRNAs. Nat Struct Mol Biol 2006; 13: 1097-101.

[3] Baskerville S, Bartel DP. Microarray profiling of microRNAs reveals frequent coexpression with neighboring miRNAs and host genes. RNA 2005; 11: 241-7.

[4] Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification of mammalian microRNA host genes and transcription units. Genome Res 2004; 14: 1902-10.

[5] Naguibneva I, Ameyar-Zazoua M, Polesskaya A, *et al.* The microRNA miR-181 targets the homeobox protein Hox-A11 during mammalian myoblast differentiation. Nat Cell Biol 2006; 8: 278-84.

[6] Olson EN, Schneider MD. Sizing up the heart: development redux in disease. Genes Dev 2003; 17: 1937-56.

[7] Niu Z, Li A, Zhang SX, Schwartz RJ. Serum response factor micromanaging cardiogenesis. Curr Opin Cell Biol 2007; 19: 618-27.

[8] Sun Q, Chen G, Streb JW, *et al.* Defining the mammalian CArGome. Genome Res 2006; 16: 197-207.

[9] Parlakian A, Charvet C, Escoubet B, *et al.* Temporally controlled onset of dilated cardiomyopathy through disruption of the SRF gene in adult heart. Circulation 2005; 112: 2930-9.

[10] Sepulveda JL, Vlahopoulos S, Iyer D, Belaguli N, Schwartz RJ. Combinatorial expression of GATA4, Nkx2-5, and serum response factor directs early cardiac gene activity. J Biol Chem 2002; 277: 25775-82.

[11] Pipes GC, Creemers EE, Olson EN. The myocardin family of transcriptional coactivators: versatile regulators of cell growth, migration, and myogenesis. Genes Dev 2006; 20: 1545-56.

[12] Lee TC, Shi Y, Schwartz RJ. Displacement of BrdUrd-induced YY1 by serum response factor activates skeletal alpha-actin transcription in embryonic myoblasts. Proc Natl Acad Sci USA 1992; 89: 9814-8.

[13] Chen F, Kook H, Milewski R, *et al.* Hop is an unusual homeobox gene that modulates cardiac development. Cell 2002; 110: 713-23.

[14] Zhao Y, Samal E, Srivastava D. Serum response factor regulates a muscle-specific microRNA that targets Hand2 during cardiogenesis. Nature 2005; 436: 214-20.

[15] Xiao J, Luo X, Lin H, *et al.* MicroRNA miR-133 represses HERG K+ channel expression contributing to QT prolongation in diabetic hearts. J Biol Chem 2007; 282: 12363-7.

[16] Kim Y, Phan D, van Rooij E, *et al.* The MEF2D transcription factor mediates stress-dependent cardiac remodeling in mice. J Clin Invest 2008; 118: 124-32.

[17] van Oort RJ, van Rooij E, Bourajjaj M, *et al.* MEF2 activates a genetic program promoting chamber dilation and contractile dysfunction in calcineurin-induced heart failure. Circulation 2006; 114: 298-308.

[18] Liu N, Williams AH, Kim Y, Bezprozvannaya S, Sutherland LB, Richardson JA, *et al.* An intragenic MEF2-dependent enhancer directs muscle-specific expression of microRNAs 1 and 133. Proc Natl Acad Sci USA 2007; 104: 20844-9.

[19] Molkentin JD, Olson EN. Combinatorial control of muscle development by basic helix-loop-helix and MADS-box transcription factors. Proc Natl Acad Sci USA 1996; 93: 9366-73.

[20] Molkentin JD, Black BL, Martin JF, Olson EN. Cooperative activation of muscle gene expression by MEF2 and myogenic bHLH proteins. Cell 1995; 83: 1125-36.

[21] Sokol NS, Ambros V. Mesodermally expressed Drosophila microRNA-1 is regulated by Twist and is required in muscles during larval growth. Genes Dev 2005; 19: 2343-54.

[22] 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: 1263-71.

[23] Sayed D, Hong C, Chen IY, Lypowy J, Abdellatif M. MicroRNAs play an essential role in the development of cardiac hypertrophy. Circ Res 2007; 100: 416-24.

[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: 258-67.

[25] 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 USA 2006; 103: 18255-60.

[26] van Rooij E, Sutherland LB, Thatcher JE, *et al.* Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc Natl Acad Sci USA 2008; 105: 13027-32.

[27] Elia L, Contu R, Quintavalle M, *et al.* Reciprocal regulation of microRNA-1 and insulin-like growth factor-1 signal transduction cascade in cardiac and skeletal muscle in physiological and pathological conditions. Circulation 2009; 120: 2377-85.

[28] Ikeda S, Pu WT. Expression and function of MicroRNAs in heart disease. Curr Drug Targets 2010;XXX.

[29] Latronico MVG, Condorelli G. MicroRNAs and cardiac conduction - Therapeutic options. Curr Drug Targets 2010;XXX.

[30] Jazbutyte V, Thum T. MicroRNA-21: from cancer to cardiovascular disease. Curr Drug Targets 2010;XXX.

[31] Bueno OF, van Rooij E, Molkentin JD, Doevendans PA, De Windt LJ. Calcineurin and hypertrophic heart disease: novel insights and remaining questions. Cardiovasc Res 2002; 53: 806-21.

[32] De Windt LJ, Lim HW, Bueno OF, *et al.* Targeted inhibition of calcineurin attenuates cardiac hypertrophy *in vivo*. Proc Natl Acad Sci USA 2001; 98: 3322-7.

[33] Armand AS, Bourajjaj M, Martinez-Martinez S, *et al.* Cooperative synergy between NFAT and MyoD regulates myogenin expression and myogenesis. J Biol Chem 2008; 283: 29004-10.

[34] Bourajjaj M, Armand AS, da Costa Martins PA, *et al.* NFATc2 is a necessary mediator of calcineurin-dependent cardiac hypertrophy and heart failure. J Biol Chem 2008; 283: 22295-303.

[35] Lin Z, Murtaza I, Wang K, Jiao J, Gao J, Li PF. miR-23a functions downstream of NFATc3 to regulate cardiac hypertrophy. Proc Natl Acad Sci USA 2009; 106: 12103-8.

[36] Fielitz J, van Rooij E, Spencer JA, *et al.* Loss of muscle-specific RING-finger 3 predisposes the heart to cardiac rupture after myocardial infarction. Proc Natl Acad Sci USA 2007; 104: 4377-82.

[37] Willis MS, Ike C, Li L, Wang DZ, Glass DJ, Patterson C. Muscle ring finger 1, but not muscle ring finger 2, regulates cardiac hypertrophy *in vivo*. Circ Res 2007; 100: 456-9.

[38] Morkin E. Control of cardiac myosin heavy chain gene expression. Microsc Res Tech 2000; 50: 522-31.

[39] van Rooij E, Sutherland LB, Qi X, Richardson JA, Hill J, Olson EN. Control of stress-dependent cardiac growth and gene expression by a microRNA. Science 2007; 316: 575-9.

[40] van Rooij E, Quiat D, Johnson BA, *et al.* A family of microRNAs encoded by myosin genes governs myosin expression and muscle performance. Dev Cell 2009; 17: 662-73.

[41] Gregory RI, Yan KP, Amuthan G, *et al.* The Microprocessor complex mediates the genesis of microRNAs. Nature 2004; 432: 235-40.

[42] Han J, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The Drosha-DGCR8 complex in primary microRNA processing. Genes Dev 2004; 18: 3016-27.

[43] Han J, Lee Y, Yeom KH, *et al.* Molecular basis for the recognition of primary microRNAs by the Drosha-DGCR8 complex. Cell 2006; 125: 887-901.

[44] Guil S, Caceres JF. The multifunctional RNA-binding protein hnRNP A1 is required for processing of miR-18a. Nat Struct Mol Biol 2007; 14: 591-6.

[45] Okamura K, Hagen JW, Duan H, Tyler DM, Lai EC. The mirtron pathway generates microRNA-class regulatory RNAs in Drosophila. Cell 2007; 130: 89-100.

[46] Ruby JG, Jan CH, Bartel DP. Intronic microRNA precursors that bypass Drosha processing. Nature 2007; 448: 83-6.

[47] Bohnsack MT, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. RNA 2004; 10: 185-91.

[48] Zeng Y, Cullen BR. Structural requirements for pre-microRNA binding and nuclear export by Exportin 5. Nucleic Acids Res 2004; 32: 4776-85.

[49] Ambros V. The functions of animal microRNAs. Nature 2004; 431: 350-5.

[50] Du T, Zamore PD. microPrimer: the biogenesis and function of microRNA. Development 2005; 132: 4645-52.

[51] Gregory RI, Chendrimada TP, Cooch N, Shiekhattar R. Human RISC couples microRNA biogenesis and posttranscriptional gene silencing. Cell 2005; 123: 631-40.

[52] Wang HW, Noland C, Siridechadilok B, *et al.* Structural insights into RNA processing by the human RISC-loading complex. Nat Struct Mol Biol 2009; 16: 1148-53.

[53] Song JJ, Smith SK, Hannon GJ, Joshua-Tor L. Crystal structure of Argonaute and its implications for RISC slicer activity. Science 2004; 305: 1434-7.

[54] Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-97.

[55] Diederichs S, Haber DA. Dual role for argonautes in microRNA processing and posttranscriptional regulation of microRNA expression. Cell 2007; 131: 1097-108.

[56] Griffiths-Jones S, Grocock RJ, van Dongen S, Bateman A, Enright AJ. miRBase: microRNA sequences, targets and gene nomenclature. Nucleic Acids Res 2006; 34: D140-4.

[57] Kiriakidou M, Nelson PT, Kouranov A, *et al.* A combined computational-experimental approach predicts human microRNA targets. Genes Dev 2004; 18: 1165-78.

[58] Kuehbacher A, Urbich C, Zeiher AM, Dimmeler S. Role of Dicer and Drosha for endothelial microRNA expression and angiogenesis. Circ Res 2007; 101: 59-68.

[59] Suarez Y, Fernandez-Hernando C, Pober JS, Sessa WC. Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circ Res 2007; 100: 1164-73.

[60] Yang WJ, Yang DD, Na S, Sandusky GE, Zhang Q, Zhao G. Dicer is required for embryonic angiogenesis during mouse development. J Biol Chem 2005; 280: 9330-5.

[61] Shilo S, Roy S, Khanna S, Sen CK. Evidence for the involvement of miRNA in redox regulated angiogenic response of human microvascular endothelial cells. Arterioscler Thromb Vasc Biol 2008; 28: 471-7.

[62] Bonauer A, Boon RA, Dimmeler S. Vascular microRNAs. Curr Drug Targets 2010;XXX.

[63] Weber C, Schober A, Zernecke A. MicroRNAs in arterial remodelling, inflammation and atherosclerosis. Curr Drug Targets 2010;XXX.

[64] Poliseno L, Tuccoli A, Mariani L, *et al.* MicroRNAs modulate the angiogenic properties of HUVECs. Blood 2006; 108: 3068-71.

[65] Beltrami AP, Barlucchi L, Torella D, *et al.* Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 2003; 114: 763-76.

[66] Orlic D, Kajstura J, Chimenti S, *et al.* Bone marrow cells regenerate infarcted myocardium. Nature 2001; 410: 701-5.

[67] Zhao Y, Ransom JF, Li A, *et al.* Dysregulation of cardiogenesis, cardiac conduction, and cell cycle in mice lacking miRNA-1-2. Cell 2007; 129: 303-17.

[68] da Costa Martins PA, Bourajjaj M, Gladka M, *et al.* Conditional dicer gene deletion in the postnatal myocardium provokes spontaneous cardiac remodeling. Circulation 2008; 118: 1567-76.

[69] 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 USA 2008; 105: 2111-6.

[70] Morita S, Horii T, Kimura M, Goto Y, Ochiya T, Hatada I. One Argonaute family member, Eif2c2 (Ago2), is essential for development and appears not to be involved in DNA methylation. Genomics 2007; 89: 687-96.

[71] Adams BD, Claffey KP, White BA. Argonaute-2 expression is regulated by epidermal growth factor receptor and mitogen-activated protein kinase signaling and correlates with a transformed phenotype in breast cancer cells. Endocrinology 2009; 150: 14-23.

[72] Shiohama A, Sasaki T, Noda S, Minoshima S, Shimizu N. Molecular cloning and expression analysis of a novel gene DGCR8 located in the DiGeorge syndrome chromosomal region. Biochem Biophys Res Commun 2003; 304: 184-90.

[73] Trabucchi M, Briata P, Garcia-Mayoral M, *et al.* The RNA-binding protein KSRP promotes the biogenesis of a subset of microRNAs. Nature 2009; 459: 1010-4.

[74] Montgomery RL, van Rooij E. MicroRNA Regulation as a Therapeutic Strategy for Cardiovascular Disease. Curr Drug Targets 2010;XXX.

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