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MiRNA-1: Functional roles and dysregulation in heart disease

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ABSTRACT: microRNAs (miRNAs) are a class of small non-coding RNA molecules consisting of 19-22 nucleotides that play important roles in a variety of biological processes, including development, differentiation, apoptosis, cell proliferation and cellular senescence. A growing body of evidence suggests that miRNAs are aberrantly expressed in human cardiac diseases and they play significant roles of the initiation, development. Recently, studies revealed that microRNA-1 (miR-1) was frequently downregulated in various types of cardiac diseases. Here we review recent findings on the aberrant expression and functional significance of miR-1.

KEYWORDS: miR-1, biogenesis, hypertrophy, heart failure, arrhythmia

INTRODUCTION

miRNAs are a class of endogenous small non-coding RNA molecules consisting of 19-22 nucleotides. They play critical roles in a variety of biological processes, including development, differentiation, apoptosis and cell proliferation by regulating gene expression[1-3]. More than 500 miRNAs have been cloned and sequenced in humans, and the estimated number of miRNA genes is as high as1000 in the human genome[4]. Some are shown to play a profound role in human development and disease. An individual miRNA is capable of regulating the expression of multiple genes because a single miRNA may have hundreds of mRNA targets with either an imperfect or a perfect complement [5] . This is an interesting characteristic because a single miRNA becomes functionally important as a transcription factor [6] . MicroRNAs are expressed throughout the body, including the heart, where the most abundant microRNA is recognized as miR-1.

MiR-1 was identified as a muscle-specific microRNA and an important role in cardiac development, function, and disease [7]. RNA sequencing has revealed that miR-1 is the most abundant miRNA in the adult mouse heart, representing up to 40% of all miRNA transcripts[8]. Its expression is detected as early as embryonic day (E) 8.5 in the mouse heart and increases with the progression of differentiation with a dramatic rise in the post-natal period[9].When viewed under the electron microscope, heart muscle from miR-1 double knockout mice lacks the characteristic 'striped', or striated appearance of normal heart muscle. A 50% decrease in total miR-1 results in embryonic death attributable to ventricular septal defects and cardiac dysfunction[10].

1 these authors contributed equally for this work

BIOGENESIS OF MIRNA-1 AND ITS ROLEIN GENE REGULATION

MiRNA genes are transcribed by RNA polymerase II (Pol II). The resulting long transcript is capped with a specially-modified nucleotide at the 5'end, polyadenylated with multiple adenosines and spliced. The product is called primary miRNA (Pri-miRNA). Drosha crops Pri-miRNA into precursor-miRNA (Pre-miRNA). Pre-miRNA hairpins are exported from the nucleus to the cytoplasm by Exportin-5. In the cytoplasm, the pre-miRNA hairpin is cleaved by the RNase III enzyme Dicer. One strand is taken into the RNA-induced silencing complex (RISC), where the miRNA and its target interact. miRNAs that bind to mRNA targets with perfect matching induce mRNA cleavage, whereas translational repression is induced when matching is imperfect[11]. (Fig. 1)

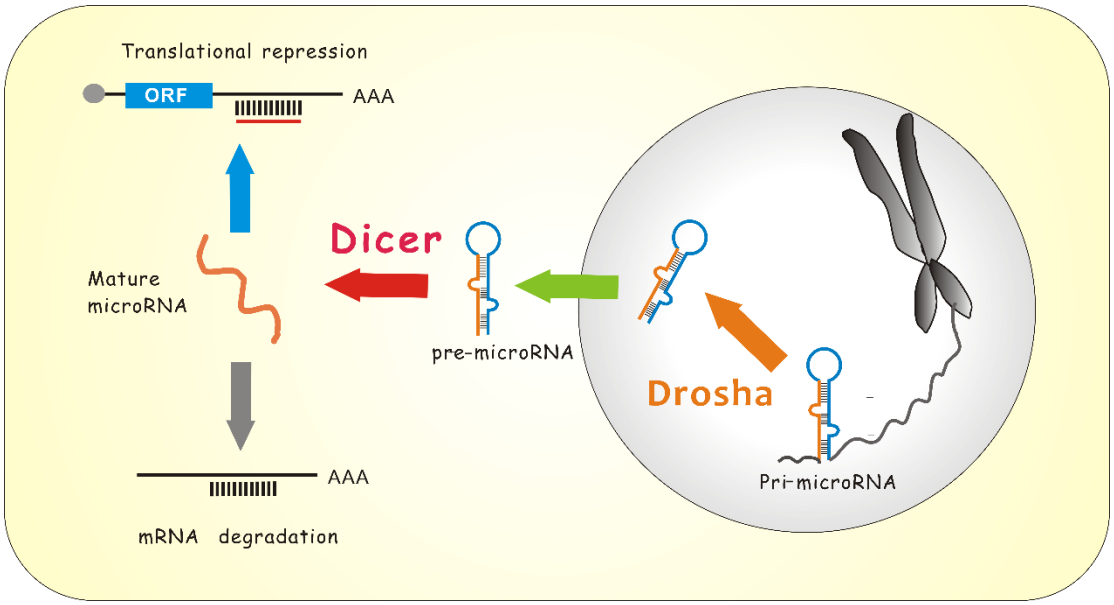

Figure 1 The microRNA biosynthetic pathway

ROLE OF miRNA-1 IN CARDIOVASCULAR CELL BIOLOGY

A fate-switching role of miR-1 is revealed at early human cardiovascular commitment stage in post-transcriptional regulation. It participates in development of embryonic stem (ES) cell and cardiomyocyte progenitor cell (CMPC) alone or with other miRNA [12-13]. (Fig. 2)

The structures of precursor miR-1-1 and miR-1-2 as constructed by the Mfold program (http://mfold.rna.albany.edu/). The respective Pre-miRNA sequences were entered into the program. Each mature miRNA sequence is shown below with red characters indicating variant nucleotides. (Fig. 3)

MiR-1 conducts transition from an immature state characterized by expression of smooth muscle (SM) genes to a more mature fetal phenotype to promote cardiomyocyte differentiation for postnatal cardiac function and reinforces the striated muscle phenotype[12-13]. It was also identified as determinants of electrophysiological maturation of human CMs [14].

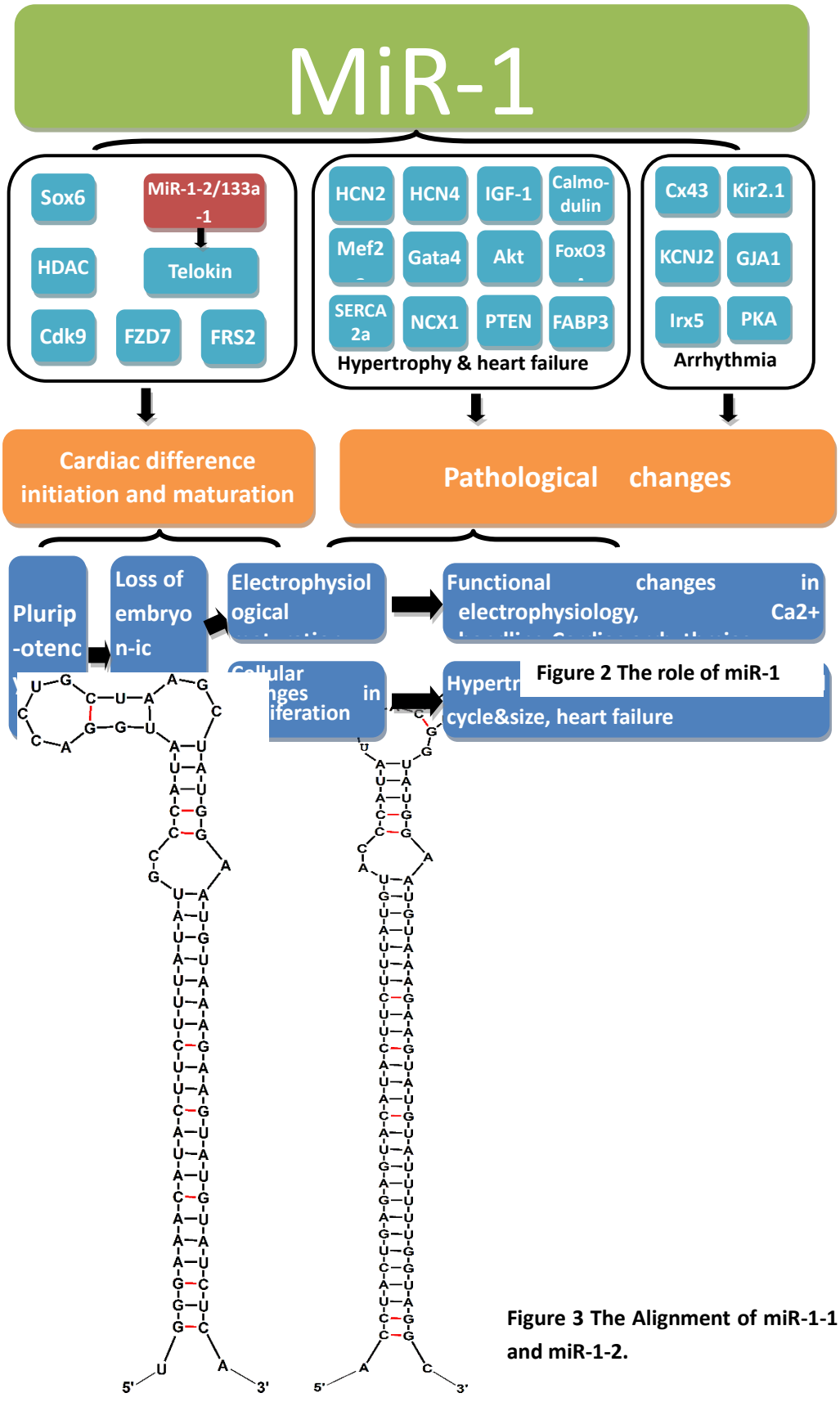

Figure 2 The role of miR-1

Figure 3 The Alignment of miR-1-1 and miR-1-2.

Interestingly, the level of miR-1 in embryonic stem (ES) cells was increased during spontaneous differentiation, but reduced during forced myocardial differentiation. On the other hand miR-1-null cardiomyocytes had abnormal sarcomere organization and decreased phosphorylation of the regulatory myosin light chain-2 (MLC2) [15].

The function of miR-1 is associated with several essential cardiac transcription factors including myocardin, Nkx2.5, SRF, WNT and FGF signaling pathways. Several miR-1-targeted genes play a role in cardiac development or function such as cyclin-dependent kinase-9 (Cdk9), histone deacetylase 4 (HDAC4), Sox6 [16], FZD7, and FRS2.

MiR-1with miR-133 clusters play roles of the specification of embryonic cardiomyocytes. Most mammalian genomes contain two copies of miR-1, miR-1-1 and miR-1-2. Concomitant knockout of miR-1-1/133a-2 and miR-1-2/133a-1 released suppression of the transcriptional co-activator, myocardin. Overexpression of myocardin in the embryonic heart essentially recapitulated the miR-1/133a mutant phenotype at the molecular level, suppressing Telokin expression of embryonic cardiomyocytes in an immature state. And the ability strongly depends on the cellular context [17].

ROLE OF miRNA-1 IN CARDIOVASCULAR DEVELOPMENT

Studies in Drosophila [18-19], *Xenopus laevis* embryo [20]and mice [21] demonstrated the importance of miR-1 (dmiR-1 in Drosophila) during cardiogenesis. Specifically, flies lacking dmiR-1 displayed a loss of differentiation in some muscle, and dmiR-1 deletion resulted in pools of undifferentiated progenitor cells. Conversely, dmiR-1 overexpression disrupted cardioblast patterning in fractions of embryos. The delta/Notch signaling pathway is proposed to mediate the morphologic consequences of dmiR-1 in Drosophila. Several unexpected genes were genetically interacted with dmiR-1, one of which was kayak, encodes a developmentally regulated transcription factor, and the mammalian ortholog of kayak, c-Fos, was dysregulated in hearts of gain- or loss-of-function miR-1 mutant mice in a stress-dependent manner[22].

Similar observations were reported in *Xenopus laevis* and mouse. Injections of miR-1into embryos at the one-cell stage resulted in abnormal cardiac development and decreased cell proliferation. In addition, it was demonstrated that overexpression of miR-1 in mouse hearts causes a decrease of ventricular cardiomyocyte pools through, in part, the targeting of Hand2, ultimately leading to heart failure (Zhao Y, et al., 2005). In the absence of miR-1-2, mouse hearts became hyperplastic. Mutant cardiomyocytes also underwent karyokinesis, indicative of cell cycle dysregulation. These mice also displayed ventricular septal defects (VSDs), possibly due to the subsequent up- regulation of the Notch signaling mediator Hrt2/Hey2, and the bHLH transcription factors Hand 1 and 2[23].

Endothelin-1 (ET-1) and insulin-like growth factor-1 (IGF-1) are targets of miR-1 which may participate in inhibiting ET-1 gene expression. ET-1 participates in a series of physiologic and pathologic processes including heart development, myocardial hypertrophy and arrhythmia. The inhibitory effect of miR-1 on recombinant luciferase reporter gene was mediated by the target sequence at the 127th nucleotide on ET-1 3’UTR at the post-transcriptional level [24]. IGF-1

inhibits glucose-induced mitochondrial dysfunction, cytochrome-c release and apoptosis and IGF-1's effect is regulated by miR-1[25].The activation state of the IGF-1 signal transduction cascade regulates miR-1 expression through the Foxo3a transcription factor[26]. miR-1 plays a critical role of mediating the effects of the IGF-1 pathway. A feedback loop between miR-1 expression and the IGF-1 signal transduction cascade is demonstrated [27].

ROLE OF miRNA-1 IN CARDIAC HYPERTROPHY AND HEART FAILURE

MiR-1 participates in two segments of hypertrophy development including vascular endothelial dysfunction and regulation of RAAS[28-[29]. Both in vitro and in vivo data suggest that the reduction in miR-1 is required for an increase in cell mass. Some of its validated targets including HCN2, HCN4, IGF-1, calmodulin, and Mef2a play essential roles during cardiac hypertrophy. The targets including calmodulin, Mef2a, Gata4, Akt, FoxO3A, SERCA2a, NCX1, PTEN, FABP3 have great influence in heart failure.

MiR-1 and miR-133 have been implicated in cardiac muscle remodeling and their expression is dysregulated during cardiac hypertrophy and heart failure [30]. In mice, miR-1 is downregulated immediately following surgically induced cardiac hypertrophy, suggesting a role in cardiac remodeling [31]. Whereas, miR-133 suppression was reported to induce hypertrophy in mice by directly targeting RhoA, Cdc42, and Nelf-A/WHSC2[32]. HCN2 was demonstrated as a target for repression by the muscle-specific miRNAs, miR-1 and miR-133 and HCN4 as a target for miR-1 only [33]. Robust increases in HCN2 and HCN4 protein levels are in left ventricular hypertrophy. This paradox of both miR-1 and -133 perhaps sharing a similar function of the specification of embryonic cardiomyocytes in cardiac hypertrophy underscores the complexity of miRNA targeting events that ultimately lead to biological function. It's also established a feedback loop between miR-1/133 expression and HCN2/HCN4. The serum-responsive factor (SRF) protein level was found significantly decreased in hypertrophic hearts, and silencing of this protein by RNA interference resulted in decreased levels of miR-1/miR-133 and concomitant increases in HCN2 and HCN4 protein levels.

MiR-1 regulates cardiomyocyte growth responses by negatively regulating the calcium signaling components including calmodulin, Mef2a, and Gata4[34]. Calmodulin is a critical mediator of calcium signal. In coincidence with the downregulation of these genes, miR-1 attenuated cardiomyocyte hypertrophy in cultured neonatal rat cardiomyocytes and in the intact adult heart. In addition, biochemical assays and an inducible cardiac-specific transgenic mouse model overexpressing miR-1 demonstrated that heart-type fatty acid-binding protein-3(FABP3) is a target of miR-1. An inverse relationship between myocardial expression of miR-1 and circulating levels of FABP3 was found in both mouse models and patient diagnosed with hypertrophy [35]. Targeting cMLCK and CaM likely underlies the detrimental effects of miR-1 on structural components of muscles related to the contractile machinery[36]. MiR-1 and IGF-1 protein are related inversely in cardiac hypertrophy and heart failure models. MiR-1 expression correlates inversely with cardiac mass and thickness in myocardial biopsies of acromegalic patients, in which IGF-1 is overproduced after aberrant synthesis of growth hormone [25].

MiR-1 expression was decreased in failing hearts. Mice receiving miR-1-ES cell transplantation post-myocardial infarction had significantly improved fractional shortening and ejection fraction. Transplanted miR-1-ES cells inhibit apoptosis, mediated through the PTEN/Akt pathway, leading to improved cardiac function in the infarct myocardium [37]. Increased Akt activation in cultured cardiomyocytes led to phosphorylation of FoxO3A and subsequent exclusion from the nucleus, resulting in miR-1 gene silencing[38]. In vitro a feedback loop between miR-1 expression and SERCA2a expression is demonstrated in failing cardiomyocytes. In vivo, Akt and FoxO3A were highly phosphorylated in failing hearts. SERCA2a gene therapy of failing hearts restores miR-1 expression by an Akt/FoxO3A- dependent pathway, which is associated with normalized NCX1 expression and improved cardiac function[39].

ROLE OF miRNA-1 IN CARDIAC ARRHYTHMIAS

MiR-1 exerts multiple but specific effects on a range of ion channel, Ca^{2+}-handling and contractile proteins and uniquely facilitates maturation by altering the expression levels of several immature related components (I_{to}, I_{Kr}, I_{Kr} and I_f) to levels closer to those of adults. It enhances excitation-contraction coupling by increasing phosphorylation of L- type and RyR2 channels and augmented the immature Ca^{2+} transient amplitude and kinetics[40].

MiR-1 has great influence in the development of cardiac electrical conduction, and on the other hand it has the responsibility in arrhythmogenesis. It's recognized that Cx43 and Kir2.1 are the crucial protein in cardiac arrhythmias. MiR-1 overexpression slowed conduction and depolarized the cytoplasmic membrane by post-transcriptionally repressing KCNJ2 (which encodes the K+ channel subunit Kir2.1) and GJA1 (which encodes connexin 43), and this likely accounts at least in part for its arrhythmogenic potential, and elimination of miR-1 in infarct rat hearts relieved arrhythmogenesis[41].

MiR-1 participates in regulating Cx43 expression and activity. Treatment of pressure overload-induced myocyte hypertrophy reduces the risk of life-threatening tachyarrhythmia by normalizing miR-1 expression levels with the consequent stabilization of Cx43 expression and activity within the gap junction[42]. In addition, miR-1 overexpression may contribute to the increased susceptibility of the heart to viral myocarditis and atrioventricular block in the setting of myocardial ischemia via post-transcriptional repression of Cx43[43]. Cx43 protein was downregulated in the cardiac-specific miR-1transgenic (Tg) mouse which exhibited a significant decrease of the systolic [Ca^{2+}] ventricular myocytes, but a prominent increase of the resting [Ca^{2+}] was lower in ventricular myocytes from miR-1 Tg mice[44]. miR-1-2 has been shown to directly target Irx5, a repressor of the potassium channel Kcnd2, leading to increased arrhythmias [10].

In addition, miR-133 also has close cooperation in arrhythmia with miR-1. Down-regulation of miR-1/miR-133 levels promotes automaticity via up-regulation of HCN2/HCN4, but this defect can be reversed by forced expression of miR-1/miR-133[45-46]. The high glucose exposure leads to miR-1/133-dependent changes in the electrophysiological properties of CPCs, including the targeted suppression of KCNE1 and KCNQ1. Moreover, the high glucose-triggered dysfunction of potassium channels in human CPCs suggests that a CPC myopathy may be induced as part of

diabetic cardiomyopathy[47].

The β-adrenoceptor–cAMP–Protein Kinase A (PKA) signaling pathway can stimulate expression of miR-1 and SRF, contributing to ischemic arrhythmogenesis, and β-blockers produce their beneficial effects partially by inhibition of the β-adrenoceptor–cAMP–PKA signaling pathway, suppression of SRF expression and down-regulating miR-1, which might be a novel strategy for ischemic cardioprotection[48].

FUTURE DIRECTIONS

Researches in animal experiments on miR-1 have made the preliminary results. And human trials are in progress. The levels of circulating miR-1 and miR-133a are elevated early after the onset of chest pain when there was no elevation in CPK or cTnT. But compared with miR-1, elevated levels of circulating miR-133a in patients with cardiovascular diseases originate mainly from the injured myocardium[49]. Serum and urine miR-1could be a novel sensitive biomarker for acute myocardial infarction. Serum and urine miR-1 levels in 20 patients with elective mitral valve surgery were measured at pre-surgery, pre-CPB, 60min post-CBP, and 24h post-CBP. Compared with these in pre-operative and pre-CPB groups, the levels of miR-1 in serum and urine from patients after open-heart surgeries and CPB were significant increased at all observed time points. A similar pattern of serum cTnI levels and their strong positive correlation with miR-1 levels were identified in these patients [50].

The researches have formed the relatively fixed pattern. The role of miR-1 in cardiovascular development is always certified by loss of function strategy. Gain of function strategy is applied for verification of therapeutical effect of miR-1. The relative articles adopt these research methods. In addition, miR-1 doesn't influence downstream genes totally independently. It always plays a part in cardiac development and diseases associated with other miRNAs, such as miR-133, miR-208.

Herb medicine has some good effects in cardiac disease. Cardiac diseases are related with miR-1. So it's inferred that miR-1 may be a target of herb medicine for cardiac disease. For this hypothesis, our team have a study of a clinical experience prescription Bu shen jiang ya decoction (BSJYD). It's obtained that BSJYD reverses LVH, which may be related to up-regulating miR-1 to inhibit the expression of ERK signaling pathway and its key targets. BSJYD has shown some effect on reducing blood pressure and heart rate, reversing LVH, up-regulating miR-1, and inhibiting the expression of ERK signaling pathway.

Taken with the above studies about miR-1 in models of cardiac hypertrophy, heart failure and cardiac arrhythmia, it is clear that miR-1 has profound influence by specifically regulating multiple targets that coordinate within a common biological function, maintaining the homeostasis required for normal processes .it's predicted that miR-1 will have therapeutic potential either by being used to target specific genes, or by becoming therapeutic targets itself.

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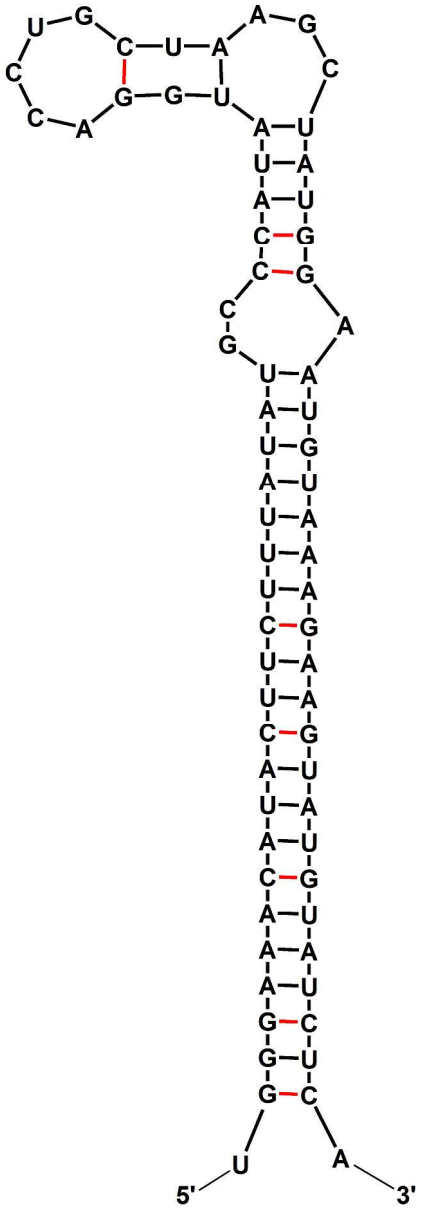

The Alignment of miR-1-1 and miR-1-2.
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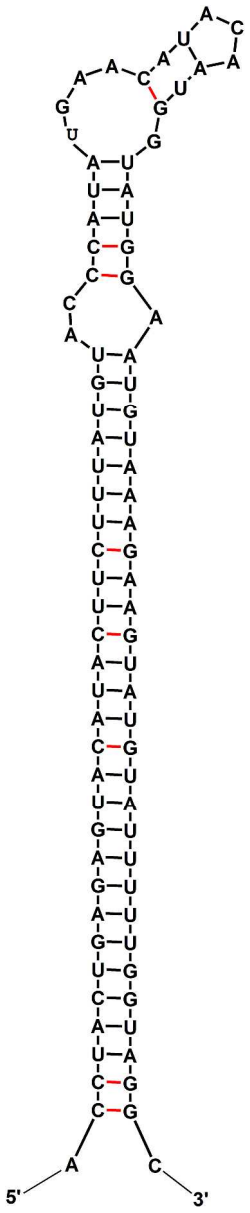

The Alignment of miR-1-1 and miR-1-2.
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