The role of miRNAs in cardiac hypertrophy

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Abstract

Cardiac hypertrophy and increased cardiomyocytes size are important triggers in the pathophysiology in heart failure. In this review, the role of miRNAs in cardiac hypertrophy is described.

MiRNAs are small non-coding single stranded RNAs of approximately 18-22 nucleotides long. They are able to downregulate target mRNA. Past 10 years, it has become clear that many miRNAs are involved in cardiac diseases. In this review, the influence of miRNAs in cardiac hypertrophy is discussed. Also, the use of miRNA as biomarkers in cardiovascular diseases and as therapeutic potential will be considered.

At the moment, therapeutic potentials of miRNAs in cardiac hypertrophy are not convincing. Only a couple of miRNAs can act as biomarkers. Moreover, only one cardiac specific miRNA is identified, miR-208. Targeting miRNAs *in vivo* is very complicated, concerning to the many possible side effects in other organs. The main challenge for future research will be to determine more cardiac specific miRNAs, as well as to find a way to target miRNAs *in vivo*.

Key words: microRNA, cardiac hypertrophy, fetal gene program

Abbreviations:

miRNA	microRNA
pri-miRA	Primary microRNA
pre-miRNA	Precursor microRNA
RISC	RNA-induced Silencing Complex
ICM	Ischemic Cardiomyopathy
DCM	Dilated Cardiomyopathy
AS	Aortic Stenosis
MHC	Myosin Heavy Chain
NRCM	Neonatal Rat Cardiac Myocytes
TAB	Trans Aortic Binding
TAC	Trans Aortic Constriction
CnA	Calcineurin A
Ca	Calcium
CaM	Calmodulin
CN	Calcineurin
TGF	Transforming Growth Factor
NIDCM	Non Ischemic Dilated Cardiomyopathy

Introduction

MicroRNAs (miRNAs) are non-coding single stranded RNAs of approximately 18-22 nucleotides long. Before miRNAs can interact with the 3'UTR of mRNA and thereby directly inhibiting translation of the mRNA, the miRNA undergoes a couple of processing steps. MiRNA-genes are often found in clusters, so called introns of protein coding genes (miRtrons)[1], but are also found as single genes in the genome, with their own transcriptional control mechanism[2]. During miRNA biogenesis, first primary miRNA (pri-miRNA) is transcripted by RNA polymerase II[3]. This relatively long pri-miRNA is cleaved in the nucleus by the RNase-III protein Drosha[4] into the hairpin folded precursor miRNA (pre-miRNA) of approximately 70 nucleotides. After transportation from the nucleus to the cytoplasm by Exportin-5[5], pre-miRNA is further processed by Dicer. Dicer, another RNase-III protein, cleaves pre-miRNA into a double stranded RNA[6]. This RNA duplex contains the mature miRNA and a complementary RNA sequence (miRNA*), which is in most cases not functional[3]. The mature miRNA is presented to the target mRNA by the RNA-induced Silencing Complex (RISC). After interaction between the miRNA-RISC complex and mRNA, RISC cleaves the mRNA, resulting in break down of the target mRNA.

Since the discovery of miRNAs in 1993 by Lee, Faunbaum and Ambros[7], miRNAs are supposed to be more and more important in gene expression. The first cardiac related miRNA study was published in 2005, describing the role of miRNAs in cardiogenesis[8]. Since 2005, many studies have focused on altered miRNA levels during cardiovascular diseases. With large microarray studies, it became easier to compare huge amounts of RNA levels in diseased and healthy samples. In the landmark paper of Van Rooij *et al.* in 2006, miRNA levels in hypertrophic mice hearts were compared with control mice hearts. This was the first study that linked miRNAs to myocyte hypertrophy and heart failure[9]. Later, it was demonstrated that miRNA levels were altered in left ventricular myocardium of different heart diseases. Samples of patients with ICM (ischemic cardiomyopathy), DCM (dilated cardiomyopathy) or AS (aortic stenosis) were compared to non-failing controls[10]. Levels of 26 miRNAs were significantly changed in the left ventricular myocardium in patients with DCM compared to non-failing hearts[10].

In this review, the role of miRNAs in the hypertrophic heart will be described. Cardiac hypertrophy and enlarged cardiomyocytes are important triggers for the development of heart failure[11]. Many miRNAs are involved in several types of heart failure[10], but also many miRNAs are able to stimulate or repress cardiomyocyte hypertrophy. In this review, the potentials of miRNAs will be discussed. Since it is known that miRNAs fine tune gene expression, it is supposed that they have strong therapeutic potentials[12]. Finally, the role of miRNAs as biomarkers for several forms of heart failure will be discussed[13].

MiRNAs and cardiac hypertrophy

To study the role of miRNAs in heart failure, both *in vitro* and *in vivo* models are being used. Expression of specific miRNAs can be simulated by miRNA-mimics[14], but cultured cardiomyocytes may also be infected with adenovirus encoding for a specific miRNA-fragment[9]. Both methods result in higher levels of the specific miRNA, shown by RT-PCR or Northern-Blot respectively[9, 14]. To knock down specific miRNAs, short synthetic RNA strains can be used, complementary to the target miRNA sequence. Next to these so called antagomirs[15] a slightly different oligonucleotide named Antimir could be used to silence a specific miRNA[16]. By targeting the miRNAs, their role in for example cardiac hypertrophy can be studied in cultured cardiomyocytes. By treating cells with a pro- or anti-hypertrophic agents, miRNA levels will change, which can be measured by PCR, Northern Blot or microarray. The same principle applies to *in vivo* studies. Cardiac hypertrophy can be induced by aortic ligation or specific diets. MiRNA levels in the heart, blood, or other organs can be measured with the techniques mentioned above, and the miRNA levels can be compared with sham operated animals or control hearts.

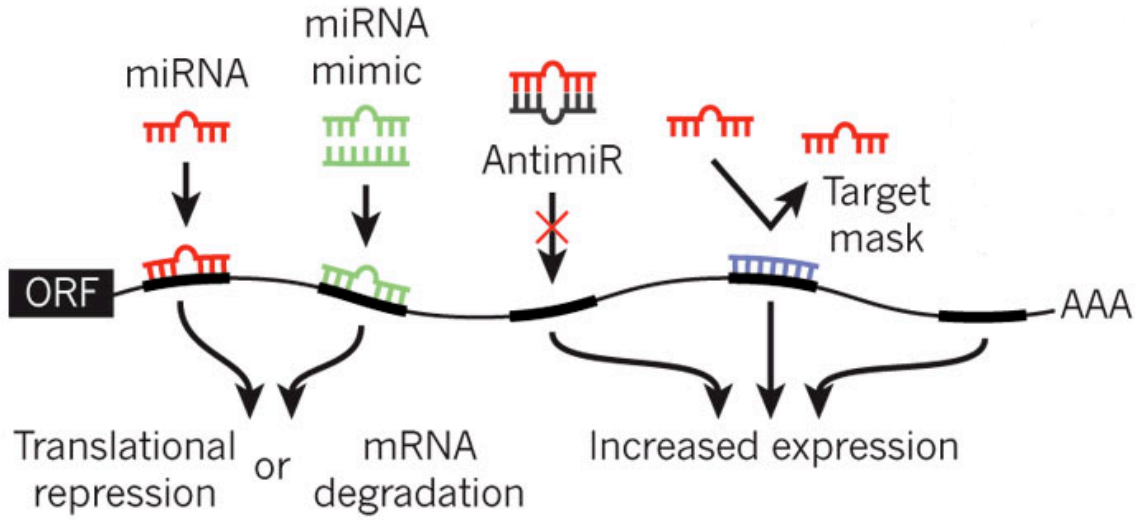

Figure 1: Different methods of how miRNAs regulate mRNA. MiRNAs, shown in red, bind to complementary sequences in the target gene, resulting in translational repression of mRNA degradation. MiRNA mimics are oligonucleotides, corresponding with the same sequence as the miRNA and with comparable effects. Antimirs bind to a specific miRNA and thereby inhibiting their function. A target mask (not discussed in this review) binds to a miRNA target sequence, resulting in less inhibition of miRNAs. The target mask has no effect on mRNA expression itself. [17]
ORF = open reading frame, AAA = poly A tail mRNA

In failing human hearts, the gene expression program has a strong relationship with the fetal gene program. A switch from the adult gene program to the fetal gene program results in hypertrophic cardiomyocytes[18]. The fetal gene program is needed for heart development during embryogenesis and early life[19].
Myosin, a motor protein, is involved in movement in myocytes. The isotype of myosin contributes to the conduction velocity in cardiac muscle fibers. In the adult human heart, mainly α Myosin Heavy Chain (MHC) is expressed. In the failing heart, αMHC is downregulated[20]. Besides, αMHC is upregulated soon after birth and has a high ATPase activity, resulting in fast contractility. βMHC has a low contractility and is mainly present in mononuclear cells[21]. A switch from αMHC to βMHC seems to be correlated with the switch

from the adult expression program to the fetal gene program[22]. It has been observed that miRNA levels change in the failing heart and that miRNAs are involved in heart failure[14, 18].
In fetal hearts, both the αMHC isoform as well as the predominant βMHC isoform are expressed, whereas in the adult heart, αMHC is predominantly expressed[22]. During experimental cardiac hypertophy, cells switch to the βMHC isoform[22]. This indicates the possible role of the fetal gene program in hypertrophy, often resulting in heart failure. It must be kept in mind that expression pattern of the different isotypes depends on the species. Most research described here is performed with rats and mice[16, 20].
Targets for the fetal gene program are proteins involved in contraction, calcium handling (SERCA downregulation) and metabolism. Therefore, a switch to the fetal gene program can lead to loss of function of these proteins. Inhibition of the fetal switch can therefore be an interesting target for future therapies[21].
In the nice review of McKinsey and Olson (2005), the central role of fetal gene activation is described. They point out that the switch is normal occurring during postnatal development and in high trained athletes, but physiological hypertrophy in normal adults is mostly not beneficial for the pump function of the heart. Due to acute or chronic damage, changes in the intracellular signalling in cardiomyoctyes will result in fetal gene activation, which plays a central role in altered calcium handling, contractility, and cardiac growth and remodelling (e. g. hypertrophy). Finally, this will result in contractile dysfunction and heart failure (figure 2). Implantation of a left ventricular assist device results in normalized cardiac gene expression**.** The challenge is to interrupt in selective target pathways in physiological mechanisms, but without affecting normal physiological cardiac growth and function[21].

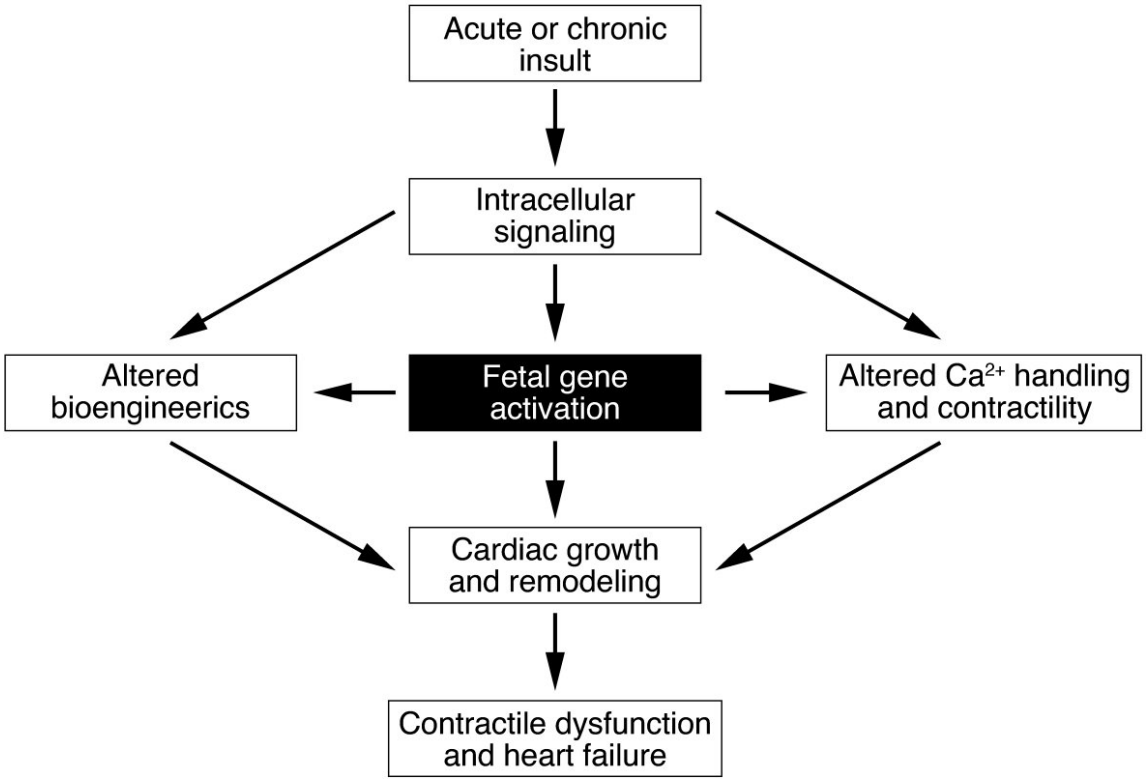

Figure 2: Central role of fetal gene activation [21]

History of miRNAs in cardiac failure

After the discovery of miRNAs as a new mechanism of post transcriptional control[7], it took 12 years before the first study concerning the role of miRNAs in the heart was published. In this study, it was found that miR-1 inhibits ventricular cardiomyocyte proliferation via transcription factor Hand2, but probably also via more factors[8]. Later it became clear that miR-1 was negatively involved in cardiomyocyte hypertrophy[23].

With experiments in cultured neonatal rat cardiac myocytes (NRCM), the relation between miRNAs and cardiomyocyte hypertrophy was examined. In the study of Van Rooij *et al.* in 2006, hypertrophy was induced by Trans Aortic Binding (TAB) or chronic Calcineurin A (CnA) activation in mouse hearts. MiRNA levels were defined by microarray after comparing the hypertrophy induced hearts with sham operated or wild type mice respectively. In TAB hearts, 27 miRNAs were upregulated and 15 miRNAs were downregulated compared to the sham operated. In CnA hearts, 33 miRNAs were upregulated and 14 miRNAs were downregulated compared to wild type hearts. In both models together, 21 miRNAs were upregulated and 7 miRNAs were downregulated. This data was compared with altered miRNAs in idiopathic end stage human hearts. It was found that expression of miR-24, miR-125b, miR-195, miR-199a and miR-214 was increased in both mouse hypertrophy models and failing human hearts. Expression of miR-23 was variable in failing human heart group[9].

The upregulated miRNAs found in the *in vivo* study were overexpressed in NRCM, which resulted in morphological changes and hypertrophy. Otherwise, in pro-hypertrophic phenolephrine overexpressed cardiomyocytes, miR-23-a, miR-23b, miR-24, miR-195 and miR-214 were upregulated. Overexpression of miR-150 and miR-181b, which are downregulated in induced hypertrophy mice hearts, resulted in a reduction in cell size in cultured cardiomyocytes[9].

The two studies above opened up a new field of research. It was the base for many studies on miRNAs in heart failure. Now, almost 500 studies are published about miRNAs and there role in the heart. In 2010, more than 120 articles were published, a number which will be beaten in 2011[24]. With the use of microarrays or PCR, it is possible to analyze the expression of many miRNAs in heart failure patients. Ikeda *et al.* described 26 miRNAs that were significantly altered in failing hearts compared with non failing hearts[10]. However, the mechanism of how miRNAs are involved in heart failure and hypertrophy remains unclear. Therefore, specific miRNAs should be up- or downregulated in cardiomyocyte cultures or *in vivo* in the heart.

2007 till now: Get into the mechanism

In cell cultures, it is relatively easy to investigate the influence of single miRNAs on hypertrophy, but also on their capability to repress target mRNA.

For example, Lin *et al*. demonstrated that the nuclear transcription factor NFAT was involved in miR-23a regulation. After stimulating NRCM with isoproterenol, which stimulates hypertrophy and the switch to the fetal gene program, both NFAT and miR-23a levels were increased. Because NFAT regulates the transcription region of miR-23a, this indicates the influence of NFAT on miR-23a expression in isoproterenol induced hypertrophy[25].

The switch to the fetal gene program, resulting in cardiac hypertrophy, is partly regulated by miRNAs. NRCM were treated with pro-hypertrophic isoproterenol, combined with stimulation with miRNA-mimics. MiR-100 stimulation resulted in further downregulation of adult genes and upregulation of fetal genes. MiR-92 was downregulated in failing hearts, but changed expression of miR-92 in the cardiac myocytes had no effects on the adult- or fetal genes. However, stimulation of miR-133b, normally downregulated in failing hearts, indeed inhibited the effect of isoproterenol and the switch to the fetal gene program[14].

After Chen *et al.* investigated the role of miR-1 and miR-133 in skeletal muscle[26], the function of these miRNAs was described in cardiomyocytes[23]. First, hypertrophy was induced by stimulating cardiomyocytes with phenylephrine, resulting in downregulation of miR-133. Stimulation with miR-133 resulted in downregulation of several parameters of hypertrophy and fetal genes. These results showed the negative influence of miR-133 on hypertrophy. By using infection with an adenovirus vector, miR-1 levels were increased in the cell culture, which resulted in downregulation of hypertrophic genes. The effects of miR-1 and miR-133 were similar, suggesting a cooperated role of these miRNAs in cardiac hypertrophy. The fact that miR-1 and miR-133 are in the same transcriptional unit strengthens this theory[23].

In 2009, the role of miR-1 in myocyte hypertrophy was further discussed. The calcium (Ca) binding protein Calmodulin (CaM), together with calcium, activates Calcineurin (CN). The phosphatase CN activates NFAT, which is an important pathway in myocyte hypertrophy[27] and miR-23a expression[25]. Mef2, also a critical protein for myocyte growth, is another target of Ca/CaM. Overexpression of miR-1 resulted in repression of CaM and Mef2a in NRCM as well as reduced cardiomyocyte size. Taken together, this suggests a negative correlation between miR-1 and hypertrophy[27].

Transforming Growth Factor-β (TGF- β) stimulation in NRCM leads to downregulation of miR-27b. MiR-27b is involved in cardiacmyocyte hypertrophy. Overexpression of miR-27b leads to hypertrophy, almost equal to the phenylephrine induced hypertrophy. The other way around, downregulation of miR-27b resulted in comparable relative cell size with controls. Combination of phenylephrine and downregulation of miR-27b did not lead to a significant increase in relative cell size, suggesting a positive correlation between miR-27b expression and hypertrophy. Also some fetal genes, which were upregulated in hypertrophy, were upregulated in the miR-27b overexpressed cardiomyocytes and phenylephrine treated cardiomyocytes[28]. These data are in contrast with a recent study, where miR-27 was thought to be an anti-hypertrophic miRNA. NRCM were first transfected with synthetic miR-27. Then, myocytes were treated with phenylephrine, resulting in lower levels of miR-27 compared to non transfected myocytes[29]. Unfortunately, Wang *et al.* did not include combined stimulation with phenylephrine and miR-27b myocytes in the study, so the studies are not completely comparable[28, 29].

In the study of Jentzsch *et al.* more miRNAs were evaluated. They showed that miR-22, miR-30c, miR-30d, miR-212 and miR-365 were pro-hypertrophic miRNAs[29]. This was in line with

the study of Xu *et al.* where was shown that overexpression of miR-22 resulted in cardiac myocyte hypertrophy, combined with upregulation of several fetal genes like βMHC. After induced hypertrophy in NRCM, miR-22 was significantly upregulated[11].

Mir-18b and miR-21 were thought to be anti hypertrophic, shown in a study where miRNA levels were downregulated with the use of antisense RNA. After repressing these miRNA levels, NRCM became hypertrophic. By stimulating the cultured cardiomyocytes with miRNA-duplex, the relative cell area was decreased. Relative cell area of cardiomyocytes treated with miR-21 duplex combined with phenylephrine was significant smaller than cells treated with only phenylephrine[30].

MiR-199a, mainly expressed in heart and lungs and easily detectable in cardiomyocytes, was upregulated in hypertrophic mice hearts. Overexpression of miR-199a in NRCM, resulted in significant increased relative cell size. Moreover, αMHC was downregulated and βMHC was upregulated in miR-199a overexpressed cardiomyocytes[31].

Thum *et al.* showed the influence of miRNAs on their targets with a special approach. Other studies only showed up- or downregulation of miRNAs in heart failure, here it was shown that upregulated mRNA had binding sites for miRNAs that were downregulated. This may show that downregulation of miRNA results in less inhibition and thus increased levels of target mRNA. They showed that the fetal miRNA program was reactivated, which resulted in the altered gene expression in failing human hearts. They overexpressed three fetal miRNAs (miR-21, miR-129 and miR-212) in NRCM, which resulted in reactivation of several fetal genes. Overexpression of a single miRNA had almost no effect on the fetal gene expression. The same effect was observed in adult rat cardiomyocytes[18].

MiRNAs and cardiac hypertrophy in *in vivo* models

Shortly after the hallmark paper of Van Rooij *et al.* in 2006[9], mainly large microarray studies were published, evaluating the role of hundreds of miRNAs in cardiovascular diseases. For example in the study of Ikeda *et al,* the altered miRNA expression in left ventricles of patients with three different heart diseases, were compared with left ventricle samples of healthy donor hearts. MiRNA levels were defined by RT-PCR. Of the 428 miRNAs tested, 26 were significantly changed in DCM, 16 in ischemic cardiomyopathy and 35 in aortic stenosis patients[10]. The problem with this approach is that only altered levels of miRNAs are described, and nothing is clear about the target of effects of the single miRNA. The expression of miR-133 and miR-208 was not changed in any of the patients. Only miR-1 level was significantly downregulated in DCM and AS patients[10].

As described earlier, miR-24, miR-195 and miR-214 are upregulated in cardiac hypertrophy. In a mouse model, these miRNAs were overexpressed under the control of the αMHC promoter. MiR-24 overexpression was lethal for the mice. Overexpression of miR-195 resulted in cardiac growth with a dilated phenotype, since organization of cardiomyocytes was disturbed and cardiomyocytes were larger. Ventricular walls were thinner, but the left ventricle diameter was increased. Relative heart weight was increased, indicating the positive effect of miR-195 on cardiac growth. Expression of βMHC was increased in mice overexpressing miR-195. Overexpression of miR-214 had no effect on the morphology of mice hearts. Therefore, it was thought that miR-195 overexpression influences hypertrophic signalling, finally leading to cardiac failure[9]. In addition, miR-195 also plays a role in apoptosis in neonatal mouse cardiomyocytes. After stimulating cultured cardiomyocytes with pro-apoptotic palmitate, miR-195 levels were upregulated. Together with the increased reactive oxygen species production and upregulated caspase-3 activity, it appeared that miR-195 was involved in cardiomyocyte apoptosis. Besides, after stimulating cardiomyocytes with a miR-195 mimic, levels of the anti-apoptotic Sirt1 protein were reduced, again suggesting the positive effect of miR-195 on apoptosis[32]. Taken together, miR-195 drives cardiac hypertrophy, but on the other hand, miR-195 can also induce apoptosis in cardiomyocytes.

To investigate the influence of miR-1 and miR-133 on hypertrophy, three different models of cardiac hypertrophy were analyzed; TAC mice, transgenic mice with constitutively active Akt kinase and exercised rats. Both miR-1 and miR-133 were downregulated in the three models. In failing hypertrophic human hearts, expression of these miRNAs was again decreased[23].

Next to silencing miRNAs *in vitro*, it is also possible to target miRNAs *in vivo*. Krützfeldt *et al.* showed that administration of antagomirs in mice resulted in decreased levels of miRNAs in several organs[15]. In 2007, antagomirs were used to target miR-133 in mice. A minipump was implanted to continuous deliver antagomir-133. After one month treatment with antagomir-133, several hypertrophic parameters were increased, like left ventricular wall and septum size. After histological analysis, saline treated mice hearts were less hypertrophic than antagomir-133 treated hearts[23]. These outcomes support the theory that miR-133 is negatively correlated with hypertrophy.

MiR-208 is a cardiac specific miRNA, transcribed within the αMHC gene. Repression of αMHC by propylthiouracil treatment, leads also to decreased miR-208 levels. Simultaneously, βMHC expression was increased during the first nine days treatment in rats. First it was demonstrated that miR-208 was involved in cardiac hypertrophy. Then, a miR-208 knock out mice was generated. After TAB, there was almost no hypertrophy of the cardiac myocytes in the miR-208 knock out mice, although there was hypertrophy in the wild type

cardiomyocytes. Moreover, the knock out mice were not able to upregulate βMHC, but the αMHC levels were increased, suggesting a compensatory mechanism to maintain the MHC expression. Also after stimulating the knock out mice with CN, there was still no hypertrophy in cardiac myocytes observed. Taken together, they showed that miR-208 is required for βMHC upregulation and cardiac hypertrophy[33].

More precisely, miR-208 transcribed within the αMHC gene must be typed as miR-208a. Callis *et al.* investigated that within the βMHC gene, miR-208b is encoded. During embryonic development, miR-208b levels decrease, and during adulthood, miR-208b expression is almost zero. At the same time, levels of miR-208a increase from cardiac development until adulthood. Therefore not surprisingly, levels of βMHC were decreased in miR-208a knock out mice[34].

Within the βMHC gene, two miRNAs are expressed. Next to miR-208b, also miR-499 is found. Both miRNAs are involved in muscle fiber identity. MiR-208b, miR-499 and βMHC are upregulated by miR-208a. MiR-499 expression was extinguished in the heart of a miR-208a knock out mouse. Loss of function experiments with miR-208b and miR-499 knock out mice showed that there was no effect on the expression of both in myosin isoforms. Therefore, miR-208a is seen as the most important regulator in MHC expression[35].

Later, it became clear that miR-208 was upregulated in DCM patients, and βMHC levels were also increased in DCM patients. This strengthened the theory of the regulatory function of miR-208 in βMHC expression[36].

MiR-208 is also a target for therapies, described by Montgomery *et al.* in 2011. They showed that inhibition of miR-208 leads to improved cardiac function. After administration of antimiR-208a, levels of βMHC and miR-499 were donwregulated. Rats were put on high salt diet, which leads to chronic hypertension. In the antimiR-208a treated group, survival was almost 100 percent. Moreover, the antimiR-208a treated mice showed less cardiomyocyte hypertrophy and fibrosis[16].

Table 1: Overview of miRNAs involved in cardiac hypertrophy

miRNA	Experiment	Result	Reference
In vitro			
1	miR-1 levels ↑	Ventricular myocyte proliferation ↓	8
1	PE stimulation	miR-1 levels ↓	22
1	miR-1 ↓	hypertrophy ↓	22
1	miR-1 ↑ in NRCM	Calcium signalling↓, cardiomyocyte cell size↓	26
18b, 21	miRNAs ↓	Relative cell size ↑	29
22	miR-22 ↑ NRCM	Cardiomyocyte hypertrophy, fetal genes↑	11
22, 30d, 212, 365	PE stimulation in NRCM	miRNAs expression↑	28
23a	ISO stimulation in NRCM	NFAT↑ results in miR-23a↑ and cell size ↑	24
23a, 23b, 24, 195, 214	miRNA levels ↑ in NRCM	Cardiomyocyte hypertrophy ↑	9
27a, 27b, 133a	PE stimulation in NRCM	miRNAs ↓	28
27b	TGF-β stimulation	miR-27b↑	27
27b	miR-27b ↑ in NRCM	Cardiomyocyte hypertrophy, fetal genes↑	27
92	miRNA ↑ compared to ISO stimulation NRCM	No effect on fetal or adult gene expression	14
100	miRNA ↑ compared to ISO stimulation NRCM	Adult genes↓ and fetal genes↑	14
133	PE stimulation	miR-133 levels ↓	22
133	miR-133 ↓	hypertrophy ↓	22
133b	miRNA ↑ compared to ISO stimulation NRCM	Effect ISO↓ and fetal genes↓	14
150, 181b	miRNA levels ↑ in NRCM	Cardiomyocyte cell size ↓	9
199a	miR-199a ↑ NRCM	Relative cell size↑, adult genes↓ , fetal genes↑	30
In vivo			
1, 133	Hypertrophy induced in mice by TAC, Akt and exercised rats	miR-1 and 133 ↓	22
1	aortic constriction in mouse	miR-1 level decreased significantly already after 1 day and even more after 7 days	36
24, 125b, 195, 199a, 214	Hypertrophy induced in mice hearts by TAB or CnA	miR-24, 125b, 195, 199a, 214↑ miR-21, 27, 29c, 93, 150, 181b — miR-23 ↔	9
133	antagomir-133	Left ventricular wall and septum thickness↑ Hypertrophy ↑	22
208	Induced hypertrophy by TAB and CN in miR-208 KO mice	Almost no cardiac hypertrophy No upregulation of βMHC, αMHC↑	32
208	Expression in human DCM patients hearts	miR-208↑ βMHC↑	35
208	Antimir-208 in rats with HS diet	Survival↑ Cardiomyocyte hypertrophy↓	16

PE = phenylephrine, NRCM = neonatal rat cardiomyocyte, ISO = isoproterenol, TAB = thoraric aortic binding, CnA = chronic Calcineurin A activation, TAC = trans aortic constriction, Akt = constitutively active Akt kinase, CN = calcineurin stimulation, HS = high salt diet

Therapeutic potential

In the first part of this thesis, the role of miRNAs in cardiac hypertrophy was discussed. Now, the importance of miRNAs in the clinic will be mentioned. Can miRNAs have a predictive potentials as biomarkers and can the miRNA biology be manipulated?

Biomarkers

In the past years, more and more possible biomarkers were introduced, resulting in new methods to trace heart failure[37]. Also circulating miRNAs have potentials as biomarkers, free dissolved in the blood or in blood cells. It is remarkable that the relatively short miRNAs were found to be present in human blood samples in a stable form. Most likely, miRNAs are packed in exosomes, to be protected from endogenous RNAse activity. Also RNA-protein complexes and modifications of miRNAs could contribute to stability of miRNAs in the blood. Although the study was based on tumor specific miRNAs, it was clear that miRNAs could be used as possible biomarkers. A nice advantage is that it is relatively easy to quantify circulating miRNA levels in the blood by RT-PCR for example[38]. It is supposed that miRNAs can be used as biomarkers for heart failure as well[39]. At this moment, only a few studies have described a correlation between miRNAs in the blood and heart failure.

Next to circulating biomarkers, miRNAs can also be characterized in blood cells. Voellenkle *et al.* showed that miRNA expression was altered in peripheral blood mononuclear cells of heart failure patients (ICM and NIDCM) compared to controls without cardiac diseases[40].

For this review, the focus will be on the miRNAs that can be used as biomarkers in cardiac hypertrophy patients. MiR-208 is specific for the heart, and could therefore be a potential biomarker. It has been shown that plasma levels of miR-208 were increased in rats in isoproterenol induced cardiac injury. Levels of miRNA-208 in the blood were correlated with the classic marker for cardiac injury cTnI. Together with the fact that miR-208 is easier to detect than cTnI, miR-208 might be a marker for cardiac hypertrophy[41].

Plasma levels of more than thousand miRNAs in heart failure patients were analyzed with a microarray and compared to healthy controls and dyspneal (air hunger) patients without heart failure. MiR-423-5p was significantly increased in heart failure patients compared with healthy controls and dyspneal patients[42]. This is in disconcordance with other findings, where was shown that plasma levels of miR-423-5P were decreased in a rat model with congestive heart failure[16].

In the study of Tijsen *et al,* five more miRNAs were significantly changed in the heart failure versus healthy control group. It is likely that this group of miRNAs is not specific for heart failure, because no significant difference was found when levels were compared with dyspneal patients. Interestingly, levels of miR-208a seemed to be decreased in heart failure patients whereas levels of miR-208b did not change in this study[42]. These data differ from the study described earlier, were miR-208 seemed to be a potential biomarker. Possibly, this discrepancy is because of the study investigated more general heart failure patients, and not only cardiac hypertrophy[41, 42].

More miRNAs were identified as possible biomarker. Plasma levels of miR-1 were investigated in patients with acute myocardial infarction. It was found that in acute myocardial infarction patients, levels of plasma miR-1 were significantly increased. After treatment, levels of plasma miR-1 were restored to baseline levels. It was most likely that miR-1 in the plasma was cardiac specific. Probably, miR-1 was released by necrotic myocytes in the infracted area[43]. MiR-1 is negatively correlated with cardiac hypertrophy[8, 23, 27], which hypothetically would result in decreased plasma levels of miR-1. This makes it harder to use

as a biomarker, because it is more difficult to determine a predictive value since very low plasma levels of miRNA will be even lower.
Plasma levels of miR-499 were significantly decreased in a rat model for congestive heart failure, when treated with antimiR-208a. Since there was more cardiac remodeling in the control group, a negative correlation between miR-499 and remodeling has been suggested.
In the control group was more cardiac remodeling, suggesting a negative correlation of miR-499 and cardiac remodeling[16]. This suggests miR-499 can be used as a biomarker for cardiac remodelling and hypertrophy.
The use of miRNAs as biomarkers in cardiac diseases is a very new field of research. Consequently, only a couple of articles are published about useful miRNAs in cardiac diseases. The only known biomarker for cardiac hypertrophy is miR-208[41]. Because miRNAs are stable and easily detectable, they seem to be potential biomarkers for the diagnosis and prognosis of heart failure[38]. Looking at the expansively growing number of publications about miRNAs in cardiac diseases, studies describing miRNAs as potential biomarkers will probably be published in the coming years.

Potentials in the clinic

According to the online miRNA database miRBase, the total number of miRNAs expressed in humans is around 1500 and this number is still growing. Whether miRNAs can be used as biomarkers needs to be further investigated. The question remains: what are the potentials of miRNAs in the clinic?

In a recent animal study, Montgomery *et al.* showed that administration of antimiR-208 leads to increased survival in rats when hypertrophy was induced[16]. Targeting miRNAs *in vivo* can result in less hypertrophy and therefore be beneficial for the heart. It is important that the miRNAs used as possible therapies, are specific for the target organ. For example, miR-1 and miR-133 are involved in hypertrophy, but expression is not cardiomyocyte but myocyte specific[26]. Targeting these miRNAs will theoretically have effect in cardiomyocytes, but also in other myocytes in the body.

Another possibility can be local administration to a specific organ, via viral or nonviral vectors. Administration of the drug only in the heart will overcome the possible toxic side effects for other organs[44]. Delivering of a miRNA to the specific target is nicely described in the review of Montgomery and Van Rooij in 2011. Also more examples of miRNA targeting in non cardiac diseases are described[45].

Concluding remarks

In this review, the role of miRNAs in cardiac hypertrophy was discussed. Many *in vitro* studies demonstrated that targeting specific miRNAs could reduce cardiomyocyte hypertrophy.

At this moment, it is hard to conclude if miRNAs have potentials in a clinical setting. Most studies about miRNAs and biomarkers are cancer related[38, 39]. Only levels of miR-208[41], miR-423-5p[42] and miR-499[16] were shown to be altered in different models of heart failure. Only miR-208 seemed to be specific for cardiac hypertrophy, and therefore be the only suitable biomarker in cardiac hypertrophy[41, 46]. Furthermore, some studies were contradictory to each other[41, 42]. Besides, comparing between various types of heart failure in different species is not very reliable[16, 42].

Taken together, it is tremendously important to do more research with the aim to discover cardiac specific miRNAs. This applies not only to biomarkers, but also for the potentials of miRNAs as possible drugs. It is clear that many miRNAs are involved in cardiac hypertrophy, but only a few studies demonstrated positive results after targeting miRNAs *in vivo*. It appears that it is much harder to target miRNAs *in vivo* than *in vitro*. Only one recent study showed improved survival rates in rats treated with antimir-208, after inducing hypertrophy. Although it is only one promising report, it is a strong indication of the therapeutic potentials of targeting miRNAs[16]. This new approach with current therapies against cardiac hypertrophy and heart failure needs further testing. To overcome the problem of unspecific miRNAs, more research must be performed to find a way of local drug delivery, for example with virus particles. The quest to more cardiac specific miRNAs needs to be continued.

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