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Emerging Roles of Micrornas in Diabetic Cardiomyopathy

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Abstract

Ischaemic heart disease is an increasingly prevalent disease in the developed world, and accounts for a large degree of both morbidity and mortality in many countries. Diabetes Mellitus is also increasing in prevalence and as ischaemic heart disease is associated with Diabetes Mellitus, diabetic cardiomyopathy is an increasing problem globally. MicroRNAs are short, non-coding RNAs which negatively regulate gene expression through either translational repression or mRNA cleavage. These are found to exist in a stable form in both tissue and blood, and are specific to the tissue of origin. Cardiovascular miRNAs have been found to play roles in cardiac arrhythmogenesis, hypertrophy, and cardiac stem cell differentiation, as well as showing considerable links with diabetic cardiomyopathy. This interaction is important when considering the therapeutic potential of cardiovascular miRNAs, with possible therapeutic value in the treatment of vascular dysfunction, as well as the development of biomarkers for the early diagnosis and therefore treatment of the disease.

Keywords: Diabetes; microRNA; Diabetic cardiomyopahy

Introduction

Ischaemic heart disease is a major cause of death in many developed countries and is becoming more often associated with diabetes mellitus, with cardiovascular disease as one of the primary causes of both morbidity and mortality in patients with diabetes, noted as early as 1970 [1]. As the incidence of diabetes rises, with a projected increase from 14% in 2010 to 25-28% in 2050 among United States adults, so does the occurrence of associated heart disease. It is therefore imperative for new and innovative ways to diagnose and treat such complications early on in the disease progression [2].

Diabetic Cardiomyopathy

Diabetic cardiomyopathy was first described in the literature in a study of post-mortem findings carried out on patients with confirmed diabetic glomerulosclerosis. Of the 27 patients, four were found to have cardiomegaly and congestive heart failure without any obvious cause, including hypertension, coronary artery disease, or valvular diseases. Further analysis of cardiac tissue revealed left ventricular hypertrophy and fibrosis, and with the absence of any other causative factor, this heart disease was characterized as diabetic cardiomyopathy [3].

Left ventricular diastolic dysfunction was described to be an early sign of diabetic cardiomyopathy, with even young insulin-dependent diabetic patients with no apparent systolic dysfunction showing diastolic dysfunction, including reduced diastolic filing, increased atrial filling, and longer times for isovolumetric relaxation and deceleration time [4].

The Multicentre Investigation of the Limitation of Infarct Size (MILIS) study included a comparison of left ventricular function in diabetic and non-diabetic patients with acute infarction. Even after adjusting for the baseline differences between the two groups (with diabetic patients presenting with worsened cardiovascular risk factors), results showed an increase in the incidence of adverse outcomes such as mortality, reduction in ejection fraction, and post-operative complications such as post-infarction angina, further infarcts, and intraventricular conduction delay [5]. This has been further investigated, with one group exploring the effect of diabetes on left ventricular function in patients with aortic stenosis. Peri-operative left ventricular biopsies were obtained along with echocardiography results from patients undergoing aortic valve replacement. Hyperphosphorylation of the stiff N2B titin isoform in diabetic patients could explain the increased resting tension of isolated cardiomyocytes. Collagen deposition in the myocardium was also found to be increased, along with glycated end-product deposition in the cardiac vasculature. The combination of all of these factors results in significant left ventricular dysfunction characteristic of diabetic cardiomyopathy [6].

The Framingham study showed that diabetic men of the age bracket 45-74 years had twice the incidence of heart disease than non-diabetic subjects, with diabetic women displaying five times the incidence of heart disease. This risk seemed to be irrespective of other cardiovascular complications and thus supports the characterisation of diabetic cardiomyopathy [7]. Coronary flow is also affected in insulin-dependent diabetics, as shown by a study by Strauer et al., [8] in which echocardiography was employed to measure cardiac function. Both coronary flow and reserve were significantly reduced whilst coronary resistance was increased. It was also shown that diastolic function was impaired with increased relaxation time and reduced diastolic inflow [8]. Increased deposition of collagen fibres leading to fibrosis causes a stiffening of the ventricular wall, resulting in a reduced ability of the heart muscle to effectively contract or pump [9,10]. This translates to a longer relaxation time as previously mentioned coinciding with a reduction in the amount of blood able to flow into the heart. Thus the key feature of diabetic cardiomyopathy seems to involve a loss of function in the left ventricular significantly associated with fibrosis.

MicroRNAs

MicroRNAs (miRNAs) are short (~22 nucleotides), noncoding RNAs that modify protein expression by targeting the 3' untranslated region of mRNA, thus regulating specific gene expression at a post-translational level. An early study into Caenorhabditis elegans development reported that the gene lin-14 controlled the timing of developmental sequences in this species by encoding a nuclear protein present only in early stages of development. Deletion of the 3'-untranslated region (present in two types of lin-14 mutation involving gain-of-function) resulted in raised levels of these nuclear proteins in later development stages. This indicated the importance of lin-14 in the temporal switch of development and posed the idea that some factor was responsible for maintaining normal levels of regulatory proteins, potentially through the 3'-untranslated region [11]. The role of the 3'-untranslated region was also reported in 2005, where genes that are involved in basic regulatory cellular processes that were not regulated by miRNA showed short 3'-untranslated regions with a lack of miRNA binding sites [12].

miRNAs were originally identified in the aforementioned *C. elegans* nematode, in which lin-14 translation was regulated via antisense RNA interactions, but have since been shown to exist in many other organisms extending from single-cell algae to humans [13-17]. Whilst originally thought to have developed along with other multicellular organisms, miRNAs have also been found to exist in the single-cell alga Chlamydomonas reinhardtii, functioning in similar ways to those in multi-cell organisms through RNA silencing and are important in processes ranging from Zebrafish embryonic development to cancer and other disease states [18-20].

The existence of miRNAs in such a wide range of species indicates that miRNA function is not only important but also essential for normal cell development and regulation, thus preserved throughout evolution, with species of higher order development displaying more miRNAs. This importance is also evident in the degree of sequence conservation within and across species. A four-genome analysis of 3' untranslated regions showed regulatory relationships in approximately 30% of the human gene set, with more than 5300 human genes (one third) as potential conserved miRNA targets [21].

The pathway which forms mature miRNAs has been well studied and consists of various enzymatic steps, as shown in Figure 1. Most miRNAs are transcribed by RNA polymerase II into primary miRNAs (pri-miRNAs), with some, such as the human chromosome cluster of miRNAs, transcribed by polymerase III [22,23]. The RNase III Drosha processes the pri-miRNAs in the nucleus, resulting in the formation of pre-miRNAs, which are commonly 70-100 nucleotides long, with a characteristic 'hairpin' structure. RNA interference to deplete Drosha levels in HeLa cells causes a loss of function of Drosha, resulting in a build-up of the pri-miRNA, with reduced levels of pre-miRNAs and mature miRNA *in vivo* [24]. These pre-miRNAs move into the cytoplasm where the endonuclease Dicer, a member of the RNase III family of nucleases, develops the pre-miRNA into a double stranded miRNA [25,26]. This is known as a RNA-Induced Silencing Complex (RISC), which is a negative controller of gene expression, through both translational repression and mRNA cleavage [27]. The specificity of this RNA-induced silencing complex is dependent on the type of miRNA incorporated within it, and thus the gene regulation is dependent on the miRNA (Figure 1).

miRNA Action

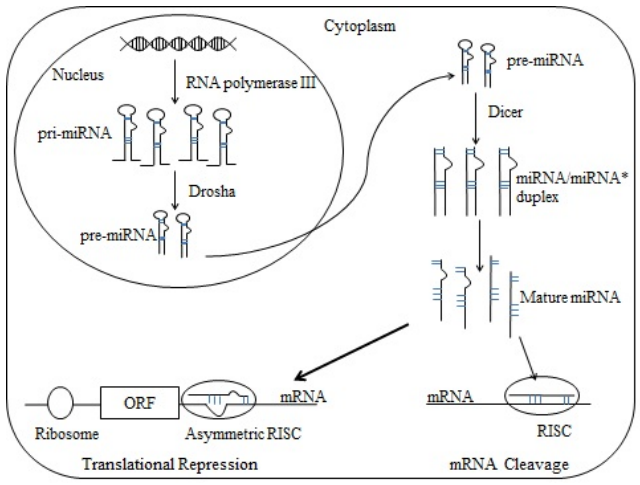

Figure 1: Schematic of the formation of miRNA

miRNA in the Tissue

Many studies have shown that miRNAs are stable in tissue, plasma, and even urine. One such study reported miRNAs (such as miR-141) deriving from human prostate cancer to be detected in human plasma using RT-PCR assays. This was not significantly altered by extended periods up to 24 hours at room temperature, and these endogenous miRNAs remained stable whilst exogenous miRNAs were degraded by plasma RNases. These levels were similar between plasma and serum [28]. Another group showed that even in total RNA samples damaged by heat incubation the miRNA levels were still detectable with no obvious difference to the intact samples, while the mRNAs decreased as the sample integrity decreased [29]. miRNA profiling in diabetic plasma revealed a reduction in miR-126, a potential factor involved in the impaired peripheral angiogenic signalling characteristic of diabetes, with this also detected using qPCR in both human and mice plasma [30]. Circulating miRNAs are also detectable in other disease states, including gastric cancer, and ST Elevation Myocardial Infarction (STEMI) [31,32]. miRNAs have also been detected in saliva with qPCR performed on saliva samples from oral squamous cell carcinoma patients, with a similar result showing that endogenous miRNA degraded to a lesser extent compared to exogenous miRNA [33].

One study used pigs in a coronary occlusion-reperfusion model to study plasma, urine and tissue miRNA expression. Plasma miR-1, miR-133a, miR-208b, and miR-499-5p were all elevated in the porcine model after coronary occlusion was induced, with significant increases also apparent in the plasma of ST-Elevation Myocardial Infarction (STEMI) patients. These correlated with the glomerular filtration rate, indicating a renal elimination route [32]. MiR-133a levels have also been noted to be elevated in patients with STEMI, with increased miR-133a levels correlating with reduced myocardial salvage, worsened and larger infarcts, and greater reperfusion injury, as reported from a study of 216 patients with STEMI who were undergoing primary angioplasty. While the increase meant that miR-133a levels could be used as a prognostic marker, the levels of miR-133a were not detected any earlier than other current biomarkers

such as troponin I and troponin T so could not add independent prognostic information in high-risk populations [34].

As ribonucleases in the plasma would be expected to destroy any miRNA present in the blood, suggestions have been made as to whether miRNAs are protected within vesicles or protein complexes. It was proposed that RNA in the plasma was protected via protein or lipid vesicles, including exosomes, microvesicles, and apoptotic bodies. It was revealed in normal healthy human plasma samples by RT-PCR of the peripheral blood mononuclear cells and plasma microvesicles that miRNAs were co-expressed in both components. Those detected were involved in either blood cell differentiation or immune function, this being the first description of microvesicular compartmentalisation of miRNAs in plasma [35,36]. Whilst exosomes and microvesicles would both involve fusing or blebbing of the plasma membrane, release of miRNAs in apoptotic bodies would indicate cell damage severe enough to activate apoptosis, and is thus likely to be relevant in many disease states. However evidence is showing that miRNAs are more likely to be found bound to RNA-binding proteins, forming protein complexes [37].

The clear stability of miRNAs in the plasma begs the question of why the body has developed to allow such stability. Whilst the potential of using miRNAs as biomarkers arises, another hypothesis involves the ability of miRNAs to act in intercellular messengers which could be to modify the destination environment to somehow reflect the environment from which the miRNAs derive.

miRNA Specificity

Specificity of either the vesicle or the associated protein can indicate where the miRNA originates, and thus most research claims miRNA specificity to certain tissues. Lim et al, 2005, studied the effect of miRNAs on transcription rates in human cells. HeLa cells were transfected with the RNA duplexes of two miRNAs, miR-1 or miR-124. These miRNAs are known for tissue specificity, with miR-1 expressed in heart and skeletal muscle, and miR-124 expressed in the brain [38,39]. The mRNA was purified and profiled on microarrays, resulting in down-regulation of 96 annotated genes by miR-1, and 174 genes by miR-124. Genes down-regulated for miR-1 corresponded with the same genes in cardiac and skeletal tissue with endogenously low levels, with the same effect also noted with miR-124. The authors concluded that the addition of particular miRNAs can shift the expression profile to that of the tissue for which a miRNA is specific, indicating that such tissue specificity is linked to the type of genes regulated by the miRNAs [40].

Cardiovascular miRNAs

As most disease states involve some change in gene expression, the regulation of gene expression by miRNAs is thought to be altered, thus a major focus of miRNA research has been to investigate how specific disease states and miRNAs correlate. Specificity of miRNAs to certain tissues has been well studied, with many cardiovascular miRNAs documented, shown in Table 1.

MicroRNA	Cardiovascular function
miR-1	Involved in cardiac arrhythmogenesis [41,42].
miR-132	Regulation of cardiac hypertrophy, as well as roles in cardiomyocyte autophagy [43].
miR-133	Involved in cardiac hypertrophy and hyperplasia, with regulation of myocardial matrix remodelling, as well as contributions to QT interval prolongation in the diabetic heart [44,45].
miR-206	Involved in cardiac apoptosis during myocardial infarction, as well as a role in cardiac remodelling after a cardiac event [46-48].
miR-208	Upregulated in a myocardial infarction [49,50].
miR-499	Associated with cardiac cell differentiation [51,52].

Table 1: Various cardiac specific microRNAs and their roles in the cardiac system

MiR-1 is considered to be muscle specific and is overexpressed in patients with coronary artery disease [41]. One study used an ischaemia – reperfusion rat model of cardiovascular disease through occlusion of the coronary artery for 30 minutes followed by 24 hours of reperfusion, showing that miR-1 levels in the myocardium were increased while levels of Bcl-2, a key protein involve in apoptosis regulation, were reduced. In in vitro studies based in H9c2 (a rat ventricular cell line) and HEK293 (human embryonic kidney cell line) it was similarly shown that overexpression of miR-1 resulted in a reduction of Bcl-2 mRNA and protein, which could contribute to increased apoptosis in cardiomyocytes [53].

Induction of cardiac hypertrophy in mice resulted in an increased expression of both miR-212 and miR-132, with evidence pointing toward the subsequent down-regulation of FoxO3 transcription factor, which is known to be both anti-hypertrophic and pro-autophagic [43]. It is clear that apoptosis is a major process affected by miRs in cardiovascular disease.

Cardiovascular miRNAs in Diabetic Cardiomyopathy

The role of tissue-specific miRNAs in diabetic cardiomyopathy is becoming an increasingly studied field, with many of these miRNAs identified to be involved in the disease. A study published in 2007 measured the expression of 428 miRNAs found in human left ventricular samples from patients with ischaemic cardiomyopathy, dilated cardiomyopathy, or aortic stenosis. It was reported that 43 miRNAs tested showed differential expression, and concluded that different cardiac diseases are associated with different miRNA profiles [54]. Another study used cardiac tissue from the area bordering the infarcted region from patients who were undergoing heart transplant surgery. This tissue also showed differential expression of miRNAs which was similar to the expression profiles found in mice with induced myocardial infarctions [55].

MiRNAs are therefore differentially expressed in human cardiac disease; however research is limited pertaining to the role of miRNAs

in diabetic heart disease. The interaction of miRNAs and diabetic cardiomyopathy has been investigated further using both *in vitro* and *in vivo* techniques. MiR-1 and miR-206 have been reported to play a role in the expression of Hsp60, subsequently contributing to apoptosis mediated by glucose in cardiomyocytes [56]. An increase in miR-1 in diabetic mice has also been correlated with a decrease in Pim-1, a known pro-survival factor which also interacts with HSP-60, with this reduction in Pim-1 associated with further cardiac apoptosis [57]. Diabetic mice with cardiac hypertrophy have been shown to have reduced levels of miR-133a, which could indicate a role of miR-133a in the development of diabetic cardiac hypertrophy [58].

The link between miRs and increased apoptosis demonstrated in cardiac disease is also seen in diabetic cardiomyopathy, with one study reporting in cardiomyocytes exposed to high glucose conditions increased apoptosis, which was linked to the activity miR-1 [59]. Another study investigated the levels of miR-320 in myocardial microvascular endothelial cells from type-2 diabetic rats, with an increase in miR-320 correlating with a decrease in the expression of insulin-like growth factor 1 which acts to inhibit apoptosis [60]. This evidence, along with the previously mentioned effect of miR-1 on Hsp60 expression in glucose induced cardiac apoptosis, indicates that the increased apoptotic drive seems to be a key component of miRNA involvement in diabetic cardiomyopathy [56].

Mice with streptozotocin-induced diabetes have been shown to develop cardiomyopathy after only 2 months of diabetes duration, with miRNA analysis in cardiac tissue from these mice showing that miR-133a contributes significantly to the characteristic cardiac changes, with down-regulation of miR-133a occurring in diabetic cardiomyopathy. Further in vitro studies in neonatal rat cardiomyocytes using a miR-133a mimic demonstrated the causal relationship of miR-133a in the glucose-induced cardiomyocytes hypertrophy. Results from both *in vivo* and *in vitro* models showed that the miR-133a end-targets IGF1R and SGK1 are both up-regulated in diabetic cardiomyopathy which could be a potential way in which miR-133a mediates cardiac hypertrophy in diabetes [58].

Hsp60 is a known component of protection against myocardial injury but is seen to be reduced in the diabetic myocardium. Cardiomyocytes cultured in hyperglycaemic conditions show a significant up-regulation of miR-1 and miR-206, with post-transcriptional modification of Hsp60. Both serum response factor and the MEK ½ pathway were also linked to the miR-1 and miR-206 involvement in diabetic cardiomyopathy, although the major role of these particular miRNAs in the associated apoptosis of diabetic cardiomyopathy seems to be through the regulation of Hsp60 [56].

Therapeutic Potential of miRNAs

The potential of using miRNAs as therapeutic targets in diabetic cardiomyopathy is a relatively new concept in this field. A recent study induced cardiac hypertrophy in mice by TAC and injected antagomirs, oligonucleotides that are complementary to miRNA targets with a mispairing at cleavage sites or base modification to inhibit base cleavage, into these mice intravenously. This successfully knocked-down endogenous miR-132 levels in cardiac tissue, resulting in improved preservation of cardiac function and less dilatation. This could be a potential method to be investigated as a therapeutic intervention to prevent development of hypertrophy and heart failure in diabetic cardiomyopathy [43]. In another study miR-221 was found to be increased in hyperglycaemic conditions in Human Umbilical Vein Endothelial Cells (HUVECs), mimicking the endothelial cell dysfunction observed in diabetics, a condition which can contribute to diabetic cardiomyopathy. This study went on to postulate that by manipulating the miR-221 and associated c-kit pathway could lead to potential treatment of such vascular dysfunction [61].

A clear advantage of miRNA involvement in diabetic cardiomyopathy is the use of miRNAs as biomarkers for the disease, especially in the earlier stages where diabetic cardiomyopathy often goes unnoticed. Diabetic cardiomyopathy is difficult to diagnose early, as many pathological changes do not manifest as symptoms until later in the disease progression. Diagnostic methods are helpful in general cardiac dysfunction, but are not specific to diabetic cardiomyopathy, with no single tool for this diagnosis readily available [62,63]. As the major symptom of diabetic cardiomyopathy is a myocardial infarction, most damage has already occurred, and this damage is irreversible.

Several miRNAs, including miR-454, miR-500, miR-1246, and miR-142-3p/5p, have been identified in patients with diastolic dysfunction in plasma samples, using standard miRNA profiling techniques, with different profiles of up or down regulation in the disease state seen among the different miRNAs [64].

miRNAs are a new and exciting field in the context of disease states, with much research investigating the roles they play in diseases including cancer and cardiomyopathies. By modifying protein expression through negative control of gene expression, miRNAs have the ability to alter significant cellular processes. This is part of normal cellular activity but has been shown to become deregulated in various disease states, becoming both markers for disease, as well as causing further damage. The potential for these relatively tissue-specific regulatory molecules to be used as biomarkers and even therapeutic targets for diseases such as diabetic cardiomyopathy is a widely promising research focus, and remains an open question to be answered. There is a great need for further research in this area, with the role of miRNAs in human diabetic cardiomyopathy an emerging and exciting topic of research.

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