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The two faces of miR-29

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Abstract

Diabetes mellitus is a metabolic homeostasis disease that contributes to additional comorbidities such as cardiovascular disease (CVD) and cancer. It has a long undiagnosed latent period during which there can be irreparable damage to the pancreas and cardiovascular tissues. Recent studies have highlighted the roles of several microRNAs in CVD. Determining the microRNAs that link diabetes mellitus and CVD is an important topic to be explored. In the present review, we discuss the microRNAs that contribute to the progression of diabetes mellitus and CVD and focus on the miR-29 family microRNAs whose expression is upregulated by hyperglycemia and proinflammatory cytokines, the hallmarks of diabetes mellitus. Upregulation of miR-29 expression is a key factor in the loss of pancreatic β cells and development of the first stage of type 1 diabetes mellitus (T1DM). Additionally, miR-29-mediated suppression of myeloid cell leukemia 1 (MCL-1), an important prosurvival protein, underlies Marfan's syndrome, abdominal aortic aneurysm, and diabetes mellitus-associated cardiomyocyte disorganization. Suppression of miR-29 expression and subsequent increase in the prosurvival MCL-1, however, promotes tumor development. Therefore, miR-29 mimics that suppress MCL-1 are hailed as tumor suppressors. The critical question is whether an increase in miR-29 levels is well tolerated in conditions of comorbidities in which insulin resistance is an underlying disease. In light of increasing awareness of the interconnection of diabetes mellitus, CVD, and cancer, it is of utmost importance to understand the mechanism of action of current treatment options on all of the comorbidities and careful evaluation of cardiovascular toxicity must accompany any treatment paradigm that increases miR-29 levels.

The diabetic heart

Diabetes mellitus has reached epidemic proportions in the United States, with 25.8 million (8.3% of the population) diagnosed as having diabetes mellitus and a staggering 79 million

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with prediabetes who are on the 'fast track' to type 2 diabetes mellitus (T2DM).[1,2] Diabetes has been described as an independent risk factor for cardiovascular disease (CVD); however, the underlying mechanisms are unclear.[3–7] Diabetes mellitus exacerbates coronary heart disease, cardiovascular remodeling, and hypertrophy, as well as CVD-associated mortality.[6,8–10] One cannot look at diabetes mellitus as an isolated condition, and it is important to realize that diabetes mellitus is in many patients part of the metabolic syndrome 'package,' and, assuch, will lead to or is accompanied by other disease conditions, among them cancer. This situation makes it imperative that every novel treatment approach has to be cross-checked for potential interference with comorbidities.

Traditionally, diabetes has been classified mainly as a disorder of metabolic homeostasis characterized by inappropriate hyperglycemia.[6,11–13] Recent research, however, strongly implicates chronic inflammation as the underlying disease of diabetes mellitus.[14–18] In the early stages of diabetes mellitus, hyperinsulinemia is a compensatory mechanism to regulate hyperglycemia. At this point, the disease is developing, but goes mostly unnoticed, because glucose levels remain within the normal range. Even in this asymptomatic early stage of impaired glucose tolerance, which is usually brought about by overnutrition or age-related changes, however, damage in the tissue takes place, predisposing it to failure on further insults. Most patients of T2DM are diagnosed after the age of 40, which is usually preceded by years of asymptomatic hyperglycemia and compensatory hyperinsulinemia.[11,12,15,19] During this stage, insulin itself as well as increased nutrients and angiotensin II signals to activate, among others, the metabolic sensor mammalian target of rapamycin complex 1 (mTORC1), which leads to compensatory cardiac hypertrophy.[20–24] Excessive activation of mTORC1 is implicated in the development of insulin resistance and cardiovascular dysfunction.[25,26] Shende *et al.*,[27] however, have shown that targeted ablation of cardiac *Raptor*, an essential component of mTORC1, results in rapid deterioration of cardiac functions and lethal dilated cardiomyopathy. In these animals, adaptive cardiomyocyte growth in response to aortic banding-induced pathological overload was lost.[27] Similarly, Zhang *et al*.[28] showed that inducible and cardiac-specific *Mtor-cKO* knock-out mice also experienced lethal dilated cardiomyopathy. These animals also were unable to develop adaptive hypertrophy when subjected to pressure overload. It was concluded that heart failure associated with the loss of mTOR activity is due to an increase in the activity of eukaryotic translation initiation factor 4E-binding protein 1.[27,28] These observations highlight the critical role of mTORC1 signaling in normal cardiac function.

In both type 1 diabetes mellitus (T1DM) and T2DM, loss of pancreatic islet function and autoimmune destruction of the insulin-producing pancreatic β cells underlie the development of insulinopenia, which exacerbates hyperglycemia and damages vital organs such as the heart.[29] We have previously shown that cardiac mTOR is phosphorylated at Ser^{2448} and mTORC1 signaling is activated in heart tissues of young hyperinsulinemic Zucker obese rats.[25,30] In a 22-week-old Zucker diabetic fatty (ZDF) rat, however, a rodent model for severe hyperglycemia and reduced insulin levels, we observed that Ser^{2448} phosphorylation was significantly lower.[25] Given the significance of mTORC1 in cardiomyocyte protection,[28] it is conceivable that a reduction in cardiac mTOR activation during diabetes mellitus progression can have a critical role in diabetic heart disease.

It is increasingly apparent that we need to find early markers for damages occurring during the early asymptomatic stages of diabetes mellitus, as well as other incrementally developing diseases associated with diabetes mellitus. We also need to understand the critical switching point between the benign adaptive mechanisms and those that are able to compensate, but at the same time set the stage for irreversible tissue damage. What are the signals that control and balance these stages and how do drugs commonly prescribed for accompanying conditions affect the outcome of long-term compensatory hyperinsulinemia? MicroRNAs, each of which regulates hundreds to thousands of genes and responds to tight transcriptional and posttranscriptional regulation through many central pathways itself, are promising candidates as such wide-reaching master switches.

MicroRNA precursors are short transcripts of approximately 70 nucleotides, which get exported from the nucleus as a hairpin or stem-loop and processed to mature miRNAs of 22–24 nucleotides. These are then able to regulate the expression of numerous genes by complete or partial complementary binding to mRNA to inhibit translation or cause degradation of the targeted transcript.[31] These small molecules can be actively or passively released into the circulation and can serve as markers for various diseases, including diabetes mellitus and CVD.[32,33] Because diabetes mellitus is an independent risk factor for CVD, an intriguing question is which of the miRNAs that serve as a marker for diabetes mellitus also contribute to CVD progression.

MicroRNAs that link diabetes mellitus and cardiovascular disease

Several recent papers and comprehensive reviews have discussed the roles of different microRNAs (miRs) in cardiomyocyte death,[34] cardiac fibroblast signaling,[35] heart failure,[36] cardiac development and regeneration,[37] and atherosclerosis and myocardial infarction (MI).[38] In diabetic cardiomyopathy, the expression of microRNAs miR-1, miR-133, miR-141, miR-206, and miR-223 is increased, whereas the expression of miR-133a, miR-373, and miR-499 is suppressed.[39] Zampetaki *et al.*[40] reported deregulation of 12 miRNAs (miR-24, miR-21, miR-20b, miR-15a, miR-126, miR-191, miR-197, miR-223, miR-320, miR-486, miR-150, and miR-28-3p), and Kong *et al.*[41] reported deregulation of another seven miRNAs (miR-9, miR-29a, miR-30d, miR-34, miR-124, miR-146a, and miR-375) in the plasma of patients with diabetes mellitus. It is unknown, however, which of these miRs serve as a marker for both diabetes mellitus and CVD. The miR-29 family miRNAs is of special interest in this context because an increase in miR-29 family miRNAs is associated with diabetes mellitus[41–44] and miR-29 suppresses expression of myeloid cell leukemia 1 (MCL-1), a prosurvival protein, which is essential for the survival and function of cardiomyocytes,[45–47] vascular smooth muscle cells,[48–50] and pancreatic β cells.[51]

The miR-29 family

The human miR-29 family consists of three closely related precursors, with miR-29a and miR-29b1 being transcribed from chromosome 7 (7q32.3), and miR-29b2, which has an identical sequence to miR-29b1, as well as miR-29c being transcribed from chromosome 1 (1q32.2).[52] Even though the two miR-29 gene-coding fragments are in each case located more than 1kb away from each other, both clusters of miR-29 are transcribed together as

polycistronic primary transcripts.[53–55] In rats, the same two clusters, 29a with 29b1 and 29b2 with 29c, map to chromosomes 4q22 and 13q27, respectively, whereas, in mice, the miR-29 miRNAs cluster on chromosomes 6qA3.3 and 1qH6, respectively. The mature sequences of miR-29 family members are conserved among humans, rats, and mice and include the identical seed region AGCACC.[52]

miR-29 and cancer

Members of the miR-29 family have gained interest as tumor suppressors because they are silenced or downregulated in several types of cancer.[55,56] Even though all of the miR-29 family members are downregulated together in a concerted fashion, the stability of the individual miR-29 family members might vary between tissues and specific cellular environments.[57] Interestingly, in many patients, the expression of these miRNAs follows the pattern of dysregulated developmental pathways paralleled during carcinogenesis. Members of the miR-29 family are downregulated during development in response to Hedgehog signaling, and both transcripts, miR-29a/b1 and miR-29b2/c, carry Hedgehog-responsive glioblastoma transcription factor binding sites.[55] In aggressive B-cell lymphomas, miR-29 has been described to be repressed by MYC via a corepressor complex with histone deacetylase 3 and enhancer of zeste homolog 2.[58] In osteoblasts, Wnt signaling promoted miR-29 expression, whereas the recombinant mouse dickkopf-1 treatment repressed miR-29a and miR-29c expression.[59] Additionally, miR-29 itself is able to repress dickkopf-1, Krement2, and secreted frizzled-related protein 2 and thus provides a positive feedback loop for Wnt signaling as observed during osteoblast development.[60]

Following these observations, several patent applications and clinical studies involving miR-29 as diagnostic targets, as well as miR-29 mimics, as treatment options are under way.[61–63] Patients with acute myeloid leukemia with downregulated miR-29b and miR-29c were found to be resistant to chemotherapy treatment, presumably because of the upregulation of the antiapoptotic MCL-1.[64] Even as anticancer therapies with miR-29 analogues are being considered, there is some evidence suggesting that in some instances miR-29 might have tumor-promoting activities and antiapoptotic behavior.[65,66] In non-small cell lung carcinoma, suppression of miR-29b expression by c-Myc promotes tumor progression.[66] Consequently, use of miR-29 for cell protection has been described in a 2013 patent (US 20130178514 A1) by Deshmukh *et al.*[67]

These variations in miR-29 regulation of cell survival are because of the fact that the miR-29 family has been reported to potentially regulate more than 4000 gene products, which are likely to differ between tissues and immediate cellular milieu. Pathological significance of direct and indirect targets of miR-29 is highlighted by the selection of miR-29 targets shown in Table 1.

miR-29 and fibrosis

Among the many targets of miR-29 are multiple collagens and integrins and several metalloproteases, including those belonging to a disintegrin and metalloprotease domain family. All of these proteins are involved in profibrotic events.[71,72] Therefore, it is not surprising that endogenous miR-29 has been found to be reduced in several fibrotic events,

notably in the lung,[73] kidney, liver, and heart,[74,75] and that the addition of exogenous miR-29 decreases fibrosis, as was demonstrated in the liver[76] and heart.[74] Moreover, upregulation of miR-29b expression in kidney by the Dipeptidyl peptidase-4 (DPP-4) inhibitor Linagliptin is involved in linagliptinmediated suppression of fibrosis in diabetic rats.[75]

Conversely, anti-miR-29b anti-miR treatment induces excess fibrosis.[71]

miR-29 and diabetes

In diabetic rodent models and humans, an increase in miR-29 family miRNAs is reported in different tissues, including liver (miR-29a–c[44,77]), β cells (miR-29a–c[51,78]), kidney (miR-29c[79]), skeletal muscle (miR-29c[43]), and adipose tissue (miR-29c[43]). Importantly, an increase in miR-29 levels is seen in the serum of children diagnosed as having T1DM[80] and adult patients with T2DM.[41] Both hyperglycemia and proinflammatory cytokines, the hallmarks of diabetes mellitus, upregulate the expression of miR-29 family miRNAs.[51,79] Roggli *et al.*[51] have shown that treatment with a proinflammatory cytokine cocktail increased the expression of miR-29 family members in human and mouse pancreatic islets and caused cell death. Moreover, an increase in the expression of miR-29 family miRNAs is associated with the first stage of T1DM in nonobese diabetic mice.[51] Suppression of miR-29 by anti-miR-29 oligomers protects against diabetic nephropathy.[79] Collectively, these observations strongly support the idea that miR-29 is a diabetic marker. In islets of pancreas, coordinated upregulation of miR-29a–c and subsequent suppression of *Mcl-1*, a prosurvival gene, by miR-29 underlies β-cell death and marks the first stage of T1DM.[51] Because miR-29 is associated with inflammatory microvesicles,[81] it is conceivable that uptake of microvesicles containing miR-29 from blood can occur in various tissues of diabetic individuals. Given the pathological effect of miR-29 upregulation in the pancreas, it is of interest to investigate whether miR-29 upregulation is a common mechanism that underlies development of insulinopenia and diabetes mellitus-associated progression of CVDs.

miR-29 and the diabetic heart

It is interesting to note that quantitative trait loci associated with rat miR-29 highlight potential involvement of miR-29 in CVDs.[47] miR-29b was found to be downregulated in the infarcted region of mouse hearts subjected to induced MI by occlusion of the left coronary artery, as well as in the cardiac tissues from the border zone of the infarcted region from patients with MI.[72] Because cardiac fibroblasts express high levels of miR-29 and downregulation of miR-29 expression correlates with derepression of collagens and matrix metalloproteinases, suppression of miR-29 is implicated in cardiac fibrosis.[72] Transfection of fibroblasts with miR-29 mimic, however, exerted only a modest suppression of collagen, implying that reduction in miR-29 may not be the primary mechanism for cardiac fibrosis after MI.[72] Interestingly, Ye *et al.*[82] showed that in rat hearts subjected to ischemiareperfusion injury, mir-29 antagomiRs significantly reduced myocardial infarct size and apoptosis. These observations favor the notion that suppression of miR-29 is protective for cardiac tissue in stress conditions such as ischemia-reperfusion injury.

We have explored the fate of miR-29 expression in heart tissues of ZDF rats to gain a better understanding of the role of this diabetic marker in heart disease.[47] We observed a coordinated upregulation of all of the three miR-29 family members in the hearts of insulinopenic, hyperglycemic male ZDF rats. Conversely, we observed an insulin-mediated concerted suppression of miR-29 family members in female mouse atrial cardiomyocytes HL-1 cells.[47] Additionally, we were able to demonstrate how treatment with rapamycin resulted in a significant upregulation of miR-29a–c, in both male ZDF rat heart and female mouse cardiomyocytes. Although rapamycin is expected to have cardioprotective effects, we observed that rapamycin-induced increase in miR-29 levels correlated with a significant cardiomyocyte disorganization, indicative of myocardial damage. These observations lead us to posit that suppression of miR-29 in cardiomyocytes by hyperinsulinemia could be a part of an adaptive mechanism to protect the heart from structural damage in the prediabetic stage.[47]

Increase in miR-29b expression augments expansion of abdominal aortic aneurysm (AAA) in mouse models.[48,49] Conversely, in-vivo administration of locked nucleic acid anti-miR-29b greatly increased collagen expression and promoted an early fibrotic response in abdominal aortic wall that reduced expansion of AAA. Thus, in the context of AAA, suppression of miR-29b and subsequent profibrotic response is a protective mechanism. Increase in miR-29b is also associated with aortic aneurysm in Marfan's syndrome, and blockade of miR-29b expression is known to have protective effects in Marfan's syndrome.[50] Elastin is also an interesting miR-29 target. It was found that inhibition of miR-29 could increase elastin levels in the cells from patients haploinsufficient for *ELN* and in bioengineered human vessels.[83,84]

Also of note, ischemic conditions create a short-term oxygen and glucose deprivation state. In rat brains, miR-29c was downregulated after focal ischemia.[85] Interestingly, in high-glucose conditions, HK-2 cells displayed downregulation of miR-29a, leading to derepression of collagen IV deposition in proximal tubule cells as seen during diabetic nephropathy.[86] A report also described the observed upregulation of miR-29b in response to short-term starvation in rat liver.[87] The same authors pointed to miR-29b as a 'female-predominant' miRNA. Another group of miR-29 targets includes genes involved in metabolism and metabolic disorders, specifically those involved in glucose transport,[88] such as the transmembrane protein insulin-induced gene 1,[43] *CAV2*,[89] monocarboxylate transporter 1 (*SLC16A1*, also known as *MCT1*),[90] and *PIK3R1*,[91] which further confirm its critical role in diabetes mellitus.

MCL-1 as a crucial target of miR-29

Among the many targets of the miR-29 family miRNAs, the antiapoptotic MCL-1 caught our attention as a critical protein for cardiac health in the diabetic context. MCL-1 is a BCL-2 family member that plays a critical role in cardioprotection.[45,46] It was reported that cardiac-specific ablation of Mcl-1 causes fatal dilated cardiomyopathy, lethal cardiac failure, and mitochondrial dysfunction. The observations that blocking cell death is not sufficient to overcome the cardiac disease caused by the loss of MCL-1, and that MCL-1 has a specific

role in mitochondrial function, have raised concerns regarding the proposed use of MCL-1-inhibiting therapeutics to treat cancer because they can cause cardiotoxicity.[45,46]

Evidence suggests that MCL-1 is a direct target of miR-29 in pancreatic β cells[51] and in cholangiocarcinoma cells.[92] The human *Mcl-1* gene encodes three transcripts. Isoform 1, also known as the long isoform (NM_021960), is the prosurvival protein.[93] Recently, however, two alternatively spliced isoforms have been identified: isoform 2 (extra short; NM_182763)[85] and isoform 3 (short; NM_001197320),[94–96] both of which are promoting apoptosis, which explains conflicting reports on MCL-1 and cell survival. MCL-1 is regulated on multiple levels, and an orchestrated fine-tuning of transcription, alternative splicing, translation, stabilizing, or prodegradation phosphorylation determines its proapoptotic versus antiapoptotic function.[96,97]

We have scanned all of the three human MCL-1 mRNA isoforms, as well as the rat transcript NM_021846.2, and the mouse transcript NM_008562.3 using the Regulatory RNA Motifs and Elements Finder RegRNA,[98] which uses the miRanda algorithm for target prediction,[99] to detect potential miR-29 binding sites. This in-silico analysis revealed two potential binding sites for miR-29 on the human MCL-1 mRNA, one within the coding region and another within the 3′untranslated region (UTR). Interestingly, only the site within the 3′ UTR is recognized by all of the miR-29s. The other site seems to be exclusively regulated via miR-29b and was found only on transcript isoforms 1 and 3 (Fig. 1a). In mouse, only the common site for all of the three miR-29 miRNAs located within the 3′UTR is present (Fig. 1b). In rat, however, our analysis yielded one recognition site for miR-29b within the MCL-1 protein-coding region (Fig. 1c).

Thus, a site for miR-29b in the coding region of MCL-1 is conserved in all of the three species. What is intriguing about the fact that miR-29b may have a stronger impact on the expression of MCL-1 is the previously reported observations on its involvement in aneurysms[48–50] and a very recent paper that described miR-29b as a potential antifibrotic therapeutic agent in the heart.[74] The observation that human MCL-1 has two sites, one specifically for miR-29b and other for all of the three miR-29 family miRNAs, may imply that human MCL-1 is tightly controlled by events that not only change the expression levels of miR-29 family miRNAs but also specifically influence the stability of miR-29b. Because high glucose and proinflammatory cytokines upregulate miR-29 family miRNAs, this diabetic marker may be an important factor that modulates MCL-1 expression in heart and vasculature in diabetic patients and contributes to the progression of CVD.

The rapamycin dilemma

Rapamycin is a macrolide that has both immunosuppressant and antiproliferative properties.[100–105] Interestingly, it has several modes of action because it has been independently studied for its antifungal[106] and antiproliferative properties.[107,108] Rapamycin was used as the immunosuppressant for the Edmonton immunosuppression protocol that was designed to avoid the diabetogenic effects of corticosteroids as well as to minimize the adverse effects of tacrolimus. This was based on early studies that showed rapamycin had a few adverse effects and under the assumption that rapamycin would not

have detrimental effects on pancreatic islet survival and function. Rapamycin is produced commercially as sirolimus and its derivative everolimus, and these drugs are widely used as prophylaxis in organ transplantation and to prevent vascular restenosis. Several studies are also exploring the efficacy of rapamycin in antitumor treatments.

Many patients who develop ischemic vascular disease as a consequence of diabetes as well as those who eventually will require heart, kidney, or liver transplants will receive rapamycin treatment. Rapamycin is an original inhibitor for the nutrient sensor kinase mTORC1 and has been widely used in transplant prophylaxis and the standard of care to prevent vascular restenosis.[103–106] An earlier observed adverse effect of sirolimus (rapamycin) treatment was hyperlipidemia.[109] Sirolimus is also implicated in new-onset diabetes in patients receiving organ transplants.[110,111] Studies on obese sand rats (*P. obesus*), a model of nutrition-dependent T2DM, showed that rapamycin treatment worsened hyperglycemia and impaired insulin biosynthesis as well as glucose-stimulated insulin secretion from pancreatic islets.[112] The metabolic effects of long-term rapamycin treatment (2 mg/kg/day) in an obese animal model, KK/HIJ mice, included reduction in body weight and adiposity, coupled with impaired glucose tolerance and increase in plasma reactive oxygen species.[113] Similarly, in Sprague–Dawley rats, Houde *et al*.[114] reported that rapamycin treatment (2 mg/kg/day) induced increased gluconeogenesis in addition to severe glucose intolerance and insulin resistance. These researchers found that increases in the expression of hepatic gluconeogenic master genes, *PEPCK* and *G6Pase*, and transcriptional regulator peroxisome proliferator-activated receptor-γ coactivator-1α combined with an enhanced nuclear recruitment of FoxO1, CRTC2, and CREB contribute to the rapamycin-induced increase in gluconeogenesis. In both these studies, rapamycin treatment also induced hyperinsulinemia.

We have shown recently that in male ZDF rats, 6 weeks of a lower-dose rapamycin treatment (1.2 mg/kg/day) resulted in a significant suppression of fasting plasma insulin levels.[47] The obese male ZDF rat has become a widely used animal model of T2DM that exhibits a full course of diabetes mellitus development starting with hyperinsulinemia, progression of diabetes mellitus to diminished plasma insulin and uncontrollable hyperglycemia, and other diabetes mellitus–associated conditions such as retinopathy and neuropathy.[25,30,43,44,52,53,115,116] Hyperglycemia develops between 7 and 9 weeks of age in ZDF rats along with compensatory hyperinsulinemia. Plasma insulin levels are initially normal, then elevated, and finally decreased by 20 weeks. This is similar to the progressive loss of glucose-induced INS secretion seen in diabetic humans. We observed that rapamycin treatment expedited diabetes mellitus progression that resulted in a quicker loss of compensatory hyperinsulinemia.[47] Loss of compensatory hyperinsulinemia was observed within 1 week of rapamycin treatment in 9-week-old ZDF rats (Fig. 2). This is not surprising because disruption of FKBP12.6 impairs glucoseinduced insulin secretion.[117,118] These findings are in accordance with growing evidence indicating that rapamycin promotes diabetes mellitus by inhibiting pancreatic β-cell proliferation and β-cell adaptation to hyperglycemia.[110,112–114,118–128]

Rapamycin treatment increased in the expression of cardiac miR-29 family miRNAs in ZDF rats.[47] Such an increase in miR-29 levels may regulate fibrosis and atrial fibrillation.[129,130]

It also suppresses, however, the expression of MCL-1. We observed that an increase in miR-29 family miRNA expression in ZDF rats correlated with progression of diabetes mellitus, loss of cardiac Mcl-1 expression, and increase in the magnitude of cardiomyocyte disarray.[47] Thus, upregulation of cardiac miR-29 expression in response to the progression of diabetes mellitus or mTORC1 inhibition by rapamycin treatment can promote myocardial damage and diabetes mellitusassociated CVD (Fig. 3).

Inhibition of mTORC1 by rapamycin has several beneficial effects in organ transplant prophylaxis, cancer treatment, and other conditions such as restenosis. Rapamycin can, however, weaken myocardium because it increases the expression of miR-29 family miRNAs in cardiomyocytes and suppresses the prosurvival MCL-1 (Fig. 3b). Therefore, if rapamycin is used in cancer therapy, or in transplant rejection prophylaxis, it needs to be used in conjunction with antidiabetic prophylactic treatment.

Chronic inflammation is a hallmark of diabetes mellitus. Oxidized low-density lipoprotein (oxLDL) that promotes atherosclerosis also enhances proinflammatory response. Both proinflammatory cytokines and oxLDL are known activators of miR-29 expression.[51,131–133] In human aortic smooth muscle cells, oxLDL-induced increase in miR-29b expression plays an important role in epigenetic modifications of *MMP-2/MMP-9* genes seen in atherosclerosis. This is another example of the role of miR-29 in promoting CVD.

An increase in miR-29 in the liver was originally considered to be a beneficial effect because loss of miR-29 expression in liver contributes to fibrosis. Kurtz *et al.*,[134] however, showed that in male ZDF rats and female C57BL/6J mice that were fed a high-fat diet, hepatic miR-29 levels were significantly increased. This observation further confirms the idea that upregulation of miR-29 in insulin sensitive tissues is a common phenomenon in diabetes mellitus. Importantly, the insulin-sensitizing drug pioglitazone reversed this effect. These authors showed that the insulin-regulated transcription factor FOXA2 is a modulator of hepatic miR-29 expression and that miR-29 serves as a feedforward negative modulator.[134]

Suppression of miR-29 by either anti-miR-29 oligomers or drugs to mitigate diabetes mellitus progression and diabetes mellitus-associated CVD, however, must be handled with caution. This is because lowering miR-29 expression is likely to result in cancer. Thus, the treatment paradigms must be focused on normalizing/balancing miR-29 levels in different tissues to achieve overall health status and prevent development of negative adverse effects.

Conclusion

In summary, miR-29 family members are regulated in a concerted fashion in multiple tissues, including heart and pancreas, and are early markers of diabetes mellitus. They are downregulated under hyperinsulinemic conditions, but increase dramatically in response to loss of hyperinsulinemia and elevated plasma glucose levels. Rapamycin treatment results in miR-29 upregulation and can lead to new-onset diabetes. Increased miR-29 will, on one hand, suppress profibrotic gene expression, but in the heart it is associated with myofibril

disorganization. Moreover, suppression of miR-29 that causes a profibrotic effect is beneficial in conditions of aortic aneurysm and prevents expansion of AAA. Even though it appears in a central position to regulate whole myriads of genes to regulate proapoptotic/antiapoptotic and fibrotic programs, miR-29 appears too capricious a master switch to target lightheartedly.

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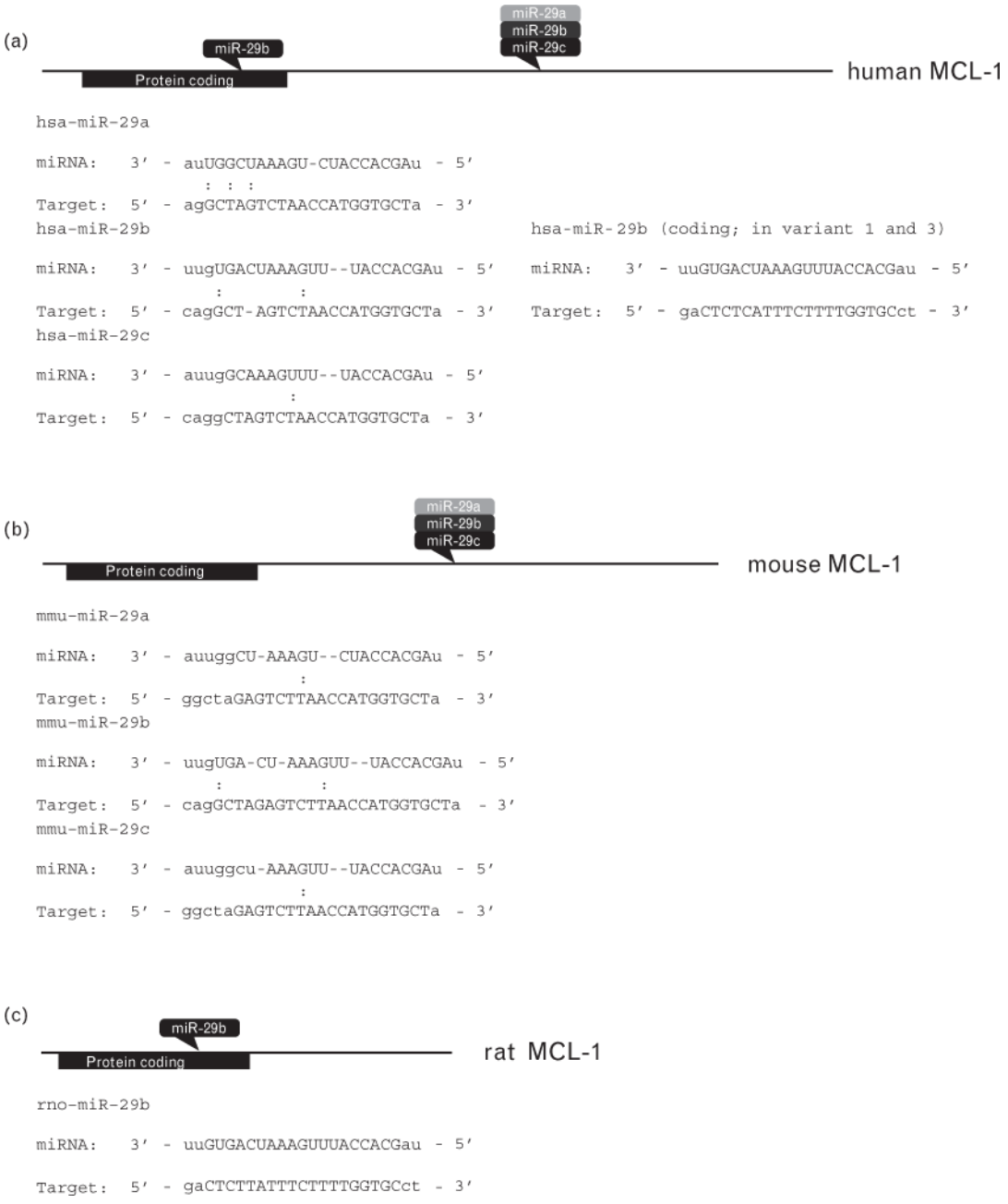

Fig. 1.

Human (a), mouse (b), and rat (c) transcripts of MCL-1 with miR-29 miRNA binding sited, shown to scale. MCL-1, myeloid cell leukemia 1.

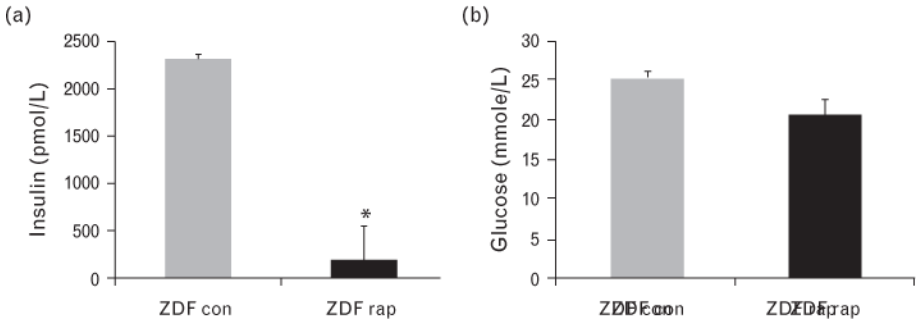

Fig. 2.

Suppression of fasting plasma insulin in male ZDF rats at 9 weeks of age treated by subcutaneous implanting of pellets designed to deliver rapamycin at a concentration of 1.2 mg/kg/day. Procedures are described previously[64]. Control rats (ZDF Con) received placebo pellets. n = 6 for fasting plasma analysis (6 hours of fasting) for ZDF Rap and ZDF Con rats. Glucose and insulin were measured by an automated hexokinase G-6-PDH assay and an ELISA kit specific for rat insulin, respectively as described previously[64]. After only one week, plasma insulin levels were dramatically reduced ($P<0.001$). Plasma glucose levels showed a trend in reduction, with $P = 0.059884$.

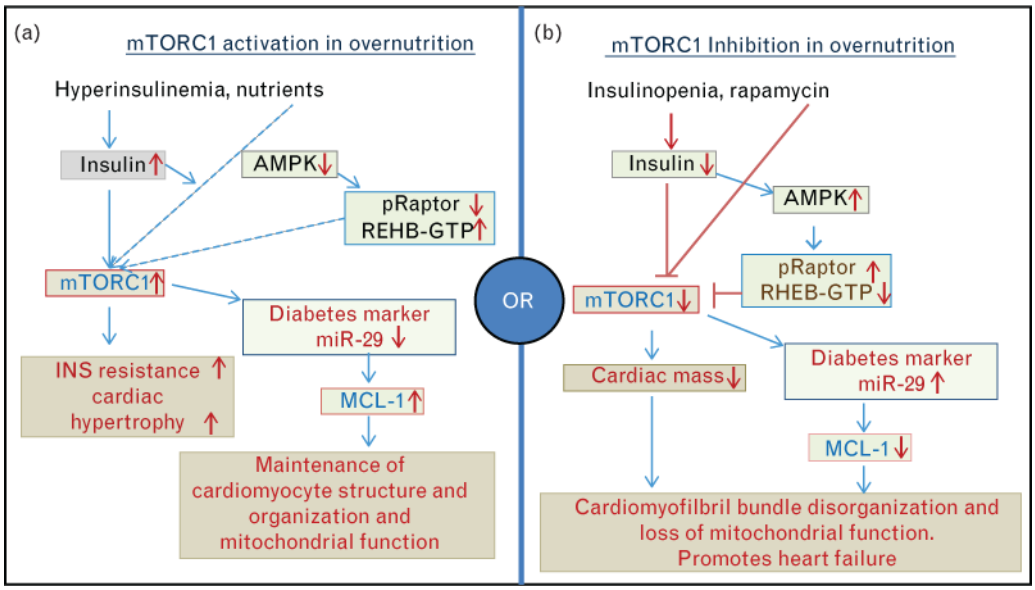

Fig. 3.

Cartoon diagram showing the effect of insulin and rapamycin on the expression of miR-29 family miRNAs and MCL-1 in cardiomyocytes and their implications in cardiac pathology associated with overnutrition and insulin resistance. (A): Steps in insulin-induced mTORC1 activation in hyperinsulinemia. mTORC1 inhibits AMP Kinase (AMPK). AMPK phosphorylates Tuberin (TSC2) that inactivates RHEB (Ras Homolog Enriched in Brain), an activator of mTORC1. Inhibition of AMPK by insulin further stabilizes mTORC1 signaling. In cardiomyocytes, mTORC1 signaling suppresses miR-29 family miRNAs and improves expression of MCL-1 that maintains cardiomyocyte structure and function. (B):Effects of Insulinopenia and rapamycin treatment on mTORC1. mTORC1 inhibition increases expression of miR-29 family miRNAs, and suppresses MCL-1, and results in myocardial damage and heart failure.

Table 1

Selected direct and indirect targets of miR-29

Target genes	Organism	Tissue/disease	miR-29	References
ATS5, B4GT1, C1RA, CALU, CERU, CFAB, CO1A1, CO1A2, CO3, CO3A1, CO4A1, CO4A2, CO5A1, CO5A2, CO6A1, CO6A2, CO8A1, CPXM1, CSF1, CYTC, DKK3, EPDR1, FBN1, FBLN3, FBLN4, FSTL1, GELS, IBP7, INHBA, LAMA4, LAMB2, LAMC1, LEG1, LG3BP, LOXL1, LOXL2, LOXL3, LTBP2, LYOX, NEUS, NID2, MMP2, PCSK5, PEDF, PGS1, PPIA, PRELP, PXDN, SAP, SPA3N, SPRC, SVEP1, TIMP2, VEGFD, QSOX1	Mouse	Cardiac fibroblasts	29b	[71]
COL1A1, COL1A2, COL3A1, FBN1, ELN	Human, mouse	Myocardial ischemia–reperfusion injury	29a, b	[72,82]
INSIG1, CAV2, SLC16A1 (MCT1), PIK3R1	Human, mouse, rat	Glucose transport	29a–c	[43,88–90]
Mcl-1	Human, mouse, rat	Islets, β cells, vascular smooth muscles, cardiomyocytes	29a–c	[47,51,92]
COL4	Human	Proximal tubule cells; diabetic nephropathy	29a	[78]
DKK1, Kremen2, *sFRP2*	Human, mouse	Osteoblasts	29a, c	[60]
TCL1A	Human	B-cell chronic lymphocytic leukaemia	29b	[68]
DNMT3A, DNMT3B	Human	Lung cancer	29a–c	[69]
ATPSF1, ATPSG1, ATPSG2, ATPSG3, BSG, CCDC56, CHCHD#, DDX17, DKC1, EIF4A3, HNRNPM, HIST1H1E, HIST1H28D, HIST1H28K, HIST2H2AB, HK1, MT-ATP6, PHB2, PRDX3, RPL7, RPL14, SLC25A12, SNRPD1, SRSF3, UQCR10, VDAC1, VDAC2	Human	HEK293T cells	29a	[70]

Table shows the involvement of miR-29 family miRNAs in cardiovascular diseases, diabetes and cancer and its wide spread expression in different tissues of human, mouse and rat based on the literature. Both direct and indirect targets of miR-29 are listed.