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MicroRNA-145 regulates disabled-2 and Wnt3a expression in cardiomyocytes under hyperglycaemia

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Abstract

Aims: MicroRNA-145 (miR-145) could protect cardiomyocyte apoptosis against oxidative stress and repair infarcted myocardium. Angiotensin II (Ang II), a pro-inflammatory cytokine could modulate myocardial remodelling. However, the role of hyperglycaemia on miR-145 expression in cardiomyocyte or diabetes is not known. The effect of Ang II on miR-145 expression under hyperglycaemia in cardiomyocytes remains unknown. We sought to investigate the effect of hyperglycaemia and Ang II on miR-145 expression in cardiomyocytes.

Methods: Rat cardiomyocytes were cultured under high glucose concentration (25 mmol/L), and streptozotocin-induced diabetic rats were established. TaqMan® MicroRNA real-time quantitative assay was used to quantitate miR-145.

Results: Sustained high glucose concentration (hyperglycaemia) significantly decreased miR-145 expression in cardiomyocytes. Hyperglycaemia significantly increased Ang II mRNA expression and secretion from rat cardiomyocytes. Ang II suppressed miR-145 expression in cardiomyocytes. Hyperglycaemia increased Dab2 and decreased Wnt3a/ß-catenin expression in cardiomyocytes. Repression of miR-145 expression by Ang II resulted in increased Dab2 and decreased Wnt3a and ß-catenin expression under hyperglycaemia. In contrast, overexpression of miR-145 significantly decreased Dab2 mRNA and protein expression, whereas the mRNA and protein levels for Wnt3a and ß-catenin were significantly reduced in left ventricular myocardium from 5 days to 28 days in diabetic rats. The protein expression patterns of Dab2 and Wnt3a/ß-catenin in left ventricular myocardium of diabetic rats could be reversed upon treatment with valsartan.

Conclusions: Ang II downregulates miR-145 to regulate Dab2 and Wnt3a/ß-catenin expression in cardiomyocytes under high glucose concentration. Ang II plays a critical role in the regulation of miR-145 in cardiomyocytes under hyperglycaemic conditions.

KEYWORDS
angiotensin II, cardiomyocyte, high glucose, microRNA-145

1 | INTRODUCTION

Diabetes is increasingly prevalent and becomes a global emergency.[1] Up to now, diabetes still remains a great challenge for health care providers and givers because cardiovascular complications including micro- and macro-vascular diseases occur frequently, and mortality risk is associated with diabetes.[2,3] To prevent or delay the

cardiovascular complications caused by diabetes mellitus, a better understanding of the mechanisms inducing cardiovascular complications is important to provide effective and novel therapeutic approaches.

MicroRNA (miR) is a newly identified class of small (around 20 to 25 nucleotides) noncoding RNA that transcriptionally or post-transcriptionally modulates the expression of its target genes.[4] MiRs have been shown to play a vital role in cardiovascular diseases.[5] MiRs also play a crucial role in the pathogenesis of hyperglycaemia-induced vascular damage.[6,7] MiR-145 has been demonstrated to protect cardiomyocyte apoptosis against oxidative stress and repair infarcted myocardium by accelerating cardiomyocyte autophagy.[8,9] MiR-145 was decreased in patients with coronary artery disease and in animal model of acute myocardial infarction.[10,11] The downregulation of myocardial miR-145 is followed by increase in disabled-2 (Dab2) expression in cardiomyocytes.[11] The role of hyperglycaemia on miR-145 expression in cardiomyocyte or diabetes is not known.

Dab2 is a tumour suppressor protein and also a transforming growth factor-ß receptor adaptor protein.[12] Dab2 is the target gene of miR-145.[9] Dab2 could directly interact with ß-catenin and inhibit Wnt3a/ß-catenin signalling pathway.[13,14] Hyperglycaemia could inhibit Wnt3a/ß-catenin signalling in mouse myoblasts.[15] How hyperglycaemia affects the expression of Dab2 and Wnt3a/ß-catenin in cardiomyocytes is not known.

Ang II may play a role in the pathogenesis of insulin resistance. Insulin resistance occurs in a wide variety of pathological states and is usually associated with obesity, diabetes mellitus, accelerated atherosclerosis and hypertension.[16] Ang II could modulate cardiac remodelling by causing cardiomyocyte hypertrophy and myocardial fibrosis.[17] The effect of Ang II on miR-145 expression under hyperglycaemic condition in cardiomyocytes remains unknown. We hypothesized that hyperglycaemic condition activates a pro-inflammatory response which mediates the expression of miR-145 in cardiomyocytes. We also sought to explore whether miR-145 serves as a critical regulator to regulate its target Dab2 and Wnt3a activity in cardiomyocytes under hyperglycaemic condition.

2 | MATERIALS AND METHODS

2.1 | Rat cardiomyocytes culture

Rat cardiomyocytes were obtained from ScienCell Research Laboratories (Catalog No. R6200; ScienCell, San Diego, CA, USA). The cardiomyocytes were isolated from postnatal day 2 rat heart. The cardiomyocytes were cryopreserved at P0 and are characterized by immunofluorescence with antibodies specific to smooth muscle actin, sarcomeric alpha-actinin and tropomyosin. The cardiomyocytes were cultured and seeded on a cultured dish with cardiomyocyte medium (Catalog No. 6201; ScienCell) according to the manufacturer's instruction. After 3 days in culture, cells were transferred to serum-free Dulbecco's modified Eagle's medium (DMEM, Catalog No. 31600-034; Invitrogen Corporation, Grand Island, NY, USA) and subjected to experimental condition.

2.2 | Quantification of microRNAs

Total RNA from cultured cardiomyocytes or left ventricular tissue was isolated using Trizol Reagent (Catalog No.15596026; Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instruction. We used TaqMan® MicroRNA Assay (Part No. 4440888; Applied Biosystems Inc., Carlsbad, CA, USA) to quantitate miRs in all of our studies according to the manufacturer's instruction as previously described.[18] The expression levels of target miRs were normalized to U6 (Part No. 4395470; Applied Biosystems Inc.).

2.3 | Construction and delivery of miR-145 expression vector

An 88 bp miR-145 precursor construct was generated as previously described.[19] Antagomir-145 and mutant-miR-145 precursor construct was generated in pmR-ZsGreen1 plasmid vector. The mutant-miR-145 precursor sequence was mutated from GTCCAG of the miR-145 precursor construct to CTGGAC. The constructed plasmid was transfected into cardiomyocytes or left ventricular myocardium using a low pressure-accelerated gene gun (Bioware Technologies, Taipei, Taiwan) essentially following the protocol from the manufacturer. In brief, 2 μg of plasmid DNA was suspended in 20 μL of PBS and was delivered to the cultured cardiomyocytes at a helium pressure of 15 psi. The transfection efficiency of wild-miR-145, mut-miR-145 and antagomir on miR-145 using this method is around 25 to 30%.

2.4 | Western blot analysis

Western blot was performed as previously described.[20] Proteins of interest were revealed with specific antibodies, including Dab2 (Material No. 610465; BD Sciences, San Jose, CA, USA), Wnt3a (sc-26358) and ß-catenin (sc-7199; Santa Cruz Biotechnology Inc., Dallas, TX, USA), as indicated (1:200 dilution) for 1 hour at room temperature followed by incubation with a 1:5000 dilution of horseradish peroxidase-conjugated polyclonal anti-rabbit antibody for 1 hour at room temperature. Equal protein loading of the samples was further verified by staining mouse anti-α-tubulin monoclonal antibody from Santa Cruz Biotechnology Inc.

2.5 | Real-time PCR

The real-time PCR was performed as described previously.[20] The primers used were as follows: Dab2, 5′-TAA TCCAACAGAAAGCAGAG-3′ (forward) and 5′-GAGGT GACTCCATTTGTTAAG-3′ (reverse); Wnt3a, 5′-AACAC AGCAGCTTAATGAC-3′ (forward) and 5′-ATCTCCACG TAGTTCCTG-3′ (reverse); ß-catenin, 5′-CATCAGGAAG GAGCTAAAATG-3′ (forward) and 5′-AGAATGATGAGC TTGCTTTC-3′ (reverse); GAPDH, 5′-CATCACCATCTTC CAGGAGC-3′ (forward) and 5′-GGATGATGTTCTGGG CTGCC-3′ (reverse).

2.6 | Measurement of Ang II concentration

Conditioned media from cultured cardiomyocytes subjected to hyperglycaemia treatment and were collected with those from control (nontreatment) cardiomyocytes for Ang II measurement. The level of Ang II was measured by a quantitative sandwich enzyme immunoassay kit (Catalog No. EKE-002-12; Phoenix Pharmaceuticals Inc., Burlingame, CA, USA). The lower limit of detection of Ang II was 0.07 ng/mL. Both the intra-observer and inter-observer coefficients of variance were <10%.

2.7 | Streptozotocin (STZ) animal model

Male Wistar rats (220–250 g), age 15 weeks, were kept under conventional conditions with free access to water and standard food. Experiment diabetes was induced in the rats by a single intra-peritoneal injection of streptozotocin (Product No. S0130; Sigma, St Louis, MO, USA), dissolved fresh in sterile citrate buffer 0.01 M, pH4.5, at a dose of 40 mg/kg body weight. Equal volumes of this vehicle were injected into the control rats. At 7 days after induction, blood was collected by tail bleeding and assayed using the glucometer. Diabetes was confirmed by the presence of hyperglycaemia (19.4 mmol/L) for at least 1 week. All animal procedures were performed in accordance with institutional guideline and conformed to Guide for the Care and Use of Laboratory Animals as published by the US National Institutes of Health (NIH publication No. 86-23, revised 2011). Transfection of the different plasmids and valsartan treatment was performed after the treatment with streptozotocin.

2.8 | RNA interference

Left ventricular myocardium was transfected with 800 ng Dab2 small interfering RNA (siRNA) oligonucleotide (Sigma-Aldrich Biotechnology, St. Louis, MD, USA) using a low pressure-accelerated gene gun (Bioware Technologies) essentially following the protocol from the manufacturer. Dab2 siRNA is a target-specific 20-25 nt siRNA designed to knock down Dab2 gene expression. Dab2 siRNA sequences were CAUUUCCCUGUCUGGCAUA [dT][dT] (forward) and UAUGCCAGACAGGGAAAUG [dT][dT] (reverse), respectively.

2.9 | Luciferase assay

To construct reporter vectors bearing miR-145 target site, we synthesized a 500-bp fragment containing the exact target for *miR-145* or the mutated target site, the 3′-UTRs of Dab2. Rat genomic DNA was amplified with forward primer 5′-TGACGCGTTACATAAACTCCTAAGCTCTC-3′ and reverse primer 5′-TGAGATCTAACAATGTGGGG CCCCA-3′. The amplified product was digested with MluI and BglII restriction enzymes and ligated into pGL3-basic luciferase plasmid vector (Catalog No.E1751; Promega Corporation, Madison, WI, USA) digested with the same enzymes. For the mutant, the binding sites were mutated using the mutagenesis kit (Catalog No.200513; Agilent Technologies, La Jolla, CA, USA). Site-specific mutations were confirmed by DNA sequencing. Plasmids were transfected into cardiomyocytes using a low pressure-accelerated gene gun (Bioware Technologies) essentially following the protocol from the manufacturer. In brief, 2 μg of plasmid DNA was suspended in 5 μL of PBS and was delivered to the cardiomyocytes at a helium pressure of 15 psi. The transfection efficiency using this method is 30%. Following 4 hours of hyperglycaemia stimulation, cell extracts were prepared using Dual-Luciferase Reporter Assay System (Promega Corporation) and measured for dual luciferase activity by luminometer (Glomax, Promega Corporation).

2.10 | Immunohistochemical analysis

Tissue specimens from heart were fixed in 10% formaldehyde and sliced into 5-μm-thick paraffin sections. For immunohistochemical stain, the slides were post-fixed in 4% paraformaldehyde for 20 minutes, treated in 3% hydrogen peroxide/PBS for 25 minutes, blocked in 5% normal rabbit serum for 20 minutes, blocked with biotin/avidin for 15 minutes each, and incubated with the following: primary antibody for 2 hour at room temperature, biotinylated secondary antibody at 1:400 for 30 minutes and Vector Elite ABC biotin-avidin-peroxidase complex for 30 minutes. Sections were then developed with diaminobenzidine and diaminobenzidine enhancer (Product code. SK-4100; Vector Laboratories, Burlingame, CA, USA), counterstained with haematoxylin. Images were examined with a fluorescence microscope.

2.11 | In situ hybridization assay

In situ hybridization was performed as previously described.[21] FAM-labelled LNA miR-145 probe (Product

No. 88068-04; Exiqon, Vedbæk, Denmark) was used for hybridization.

2.12 | Statistical analysis

All results were expressed as mean ± SEM. Statistical significance was evaluated with analysis of variance (GraphPad Software Inc., San Diego, CA, USA). We used Dunnett's test to compare multiple groups to a single control group and used Tukey-Kramer comparison for pairwise comparisons between multiple groups after the ANOVA. A value of $P < .05$ was considered to denote statistical significance.

3 | RESULTS

3.1 | Sustained high glucose concentration decreases miR-145 expression in neonatal rat cardiomyocytes

To evaluate the effect of high glucose concentration on miR-145 expression in cardiomyocytes, we added different concentrations of glucose to the culture medium for 0.5 hour. MiR-145 was significantly increased from 12.5 mmol/L to 75 mmol/L, and 25 mmol/L high glucose concentration had the maximal effect on miR-145 expression (Figure 1A). Therefore, we used 25 mmol/L high glucose concentration for the following experiments. We have previously proved that miR-145 expression was not caused by the osmotic effect of high glucose concentration.[19] As shown in Figure 1B, 25 mmol/L high glucose concentration increased miR-145 expression maximally at 0.5 hour and decreased gradually and reached significantly less than the control level after 4 to 6 hour.

3.2 | Angiotensin II suppresses miR-145 expression in neonatal rat cardiomyocytes

To investigate the effect of angiotensin II (Ang II) on miR-145 expression in cardiomyocytes, Ang II (Product No. 4006473; Bachem AG, Bubendorf, Switzerland) at 10 nmol/L was added to the culture medium for 0.5 hour to 6 hour. Ang II was found to significantly inhibit miR-
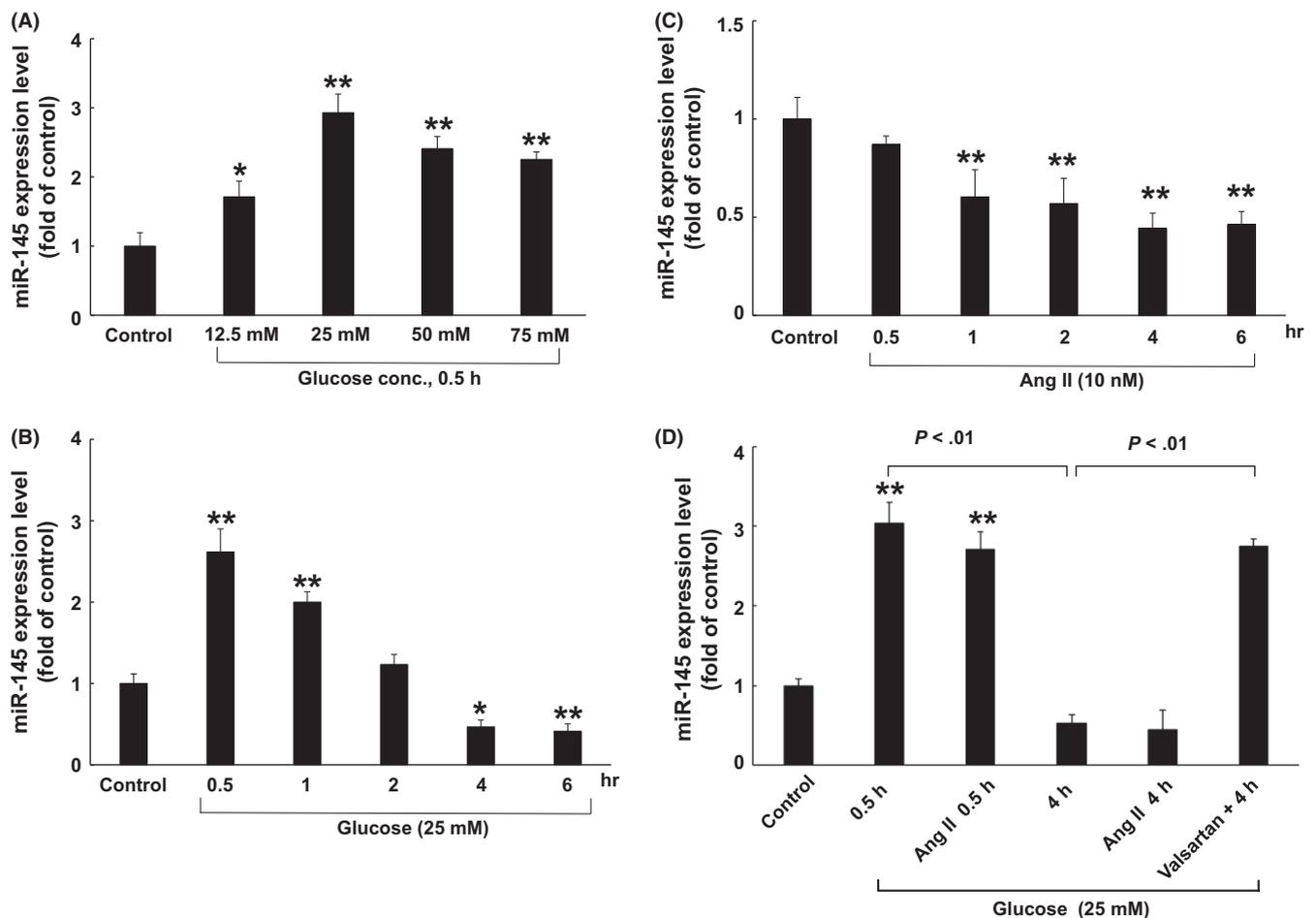
FIGURE 1 Effect of glucose level and angiotensin II (Ang II) on miR-145 expression in cultured rat cardiomyocytes. (A) Treatment of different glucose level for 0.5 hour. (B) Treatment of glucose level at 25 mmol/L for different periods of time. (C) Exogenous administration of Ang II at 10 nmol/L in usual culture medium without extra glucose treatment for different periods of time. (D) Effect of Ang II on miR-145 expression in high glucose stimulation. *$P < .05$ vs. control. **$P < .01$ vs. control. n = 4 per group. n = 3 per group
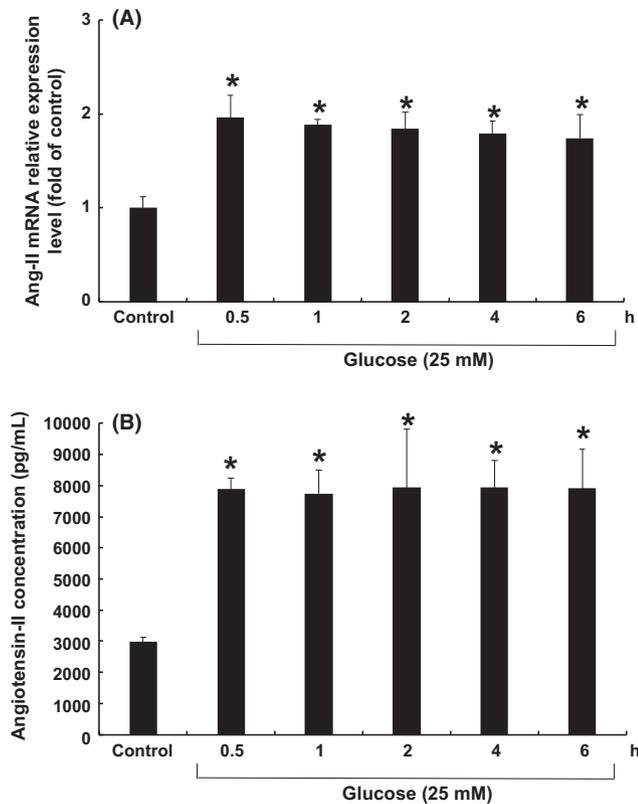
FIGURE 2 Effect of high glucose on angiotensin II expression in cultured rat cardiomyocytes. (A) High glucose concentration increases Ang II mRNA expression. (B) High glucose concentration increases Ang II secretion from cultured rat cardiomyocytes. Ang II was measured by immunosorbent assay. *$P < .01$ vs. control. n = 4 per group

FIGURE 3 Effect of high glucose concentration at 25 mmol/L on Dab2 and wnt3a/ß-catenin mRNA expression in cultured rat cardiomyocytes. Quantitative analysis of Dab2 (A) wnt3a (B) and ß-catenin (C) mRNA levels. The values from cardiomyocytes under high glucose treatment have been normalized to be matched GAPDH measurement and then expressed as a ratio of normalized values to mRNA in control group. *$P < .05$ vs. control. **$P < .01$ vs. control. n = 4 per group

145 expression from 1 hour to 6 hour (Figure 1C). Addition of valsartan at 10 µmol/L (Product No. SML0142; Sigma-Aldrich), an angiotensin II receptor antagonist, significantly increased miR-145 expression in high glucose concentration as compared to high glucose concentration only for 4 hour (Figure 1D). The expression of miR-145 under high glucose concentration for 4 hour was similar to that of high glucose concentration + Ang II for 4 hour. Addition of valsartan in cardiomyocytes not treated with high glucose concentration did not affect miR-145 expression (data not shown). High glucose concentration at 25 mmol/L did not significantly affect miR-145 expression after 0.5 hour in cardiomyocytes pretreated with Ang II. High glucose concentration also increased mRNA expression and secretion of Ang II from cultured cardiomyocytes from 0.5 to 6 hour (Figure 2). These results indicate that Ang II plays an important role in miR-145 expression in cardiomyocytes under high glucose concentration. The expression level of atrial natriuretic factor, brain natriuretic peptide and ß-myosin heavy chain on neonatal rat cardiomyocytes under Ang II treatment is dose-dependent (Figure S1).

3.3 | High glucose concentration increases Dab2 and decreases Wnt3a and ß-catenin expression in cardiomyocytes

Because Dab2 is the target gene of miR-145, and Dab2 inhibits Wnt3a/ß-catenin signalling,[9,13] we sought to investigate the effect of high glucose concentration on Dab2 and Wnt3a expression in cardiomyocytes. High glucose concentration initially did not have effect on Dab2 mRNA expression as shown in Figure 3A. However, Dab2 mRNA began to increase after 2 hour of high glucose concentration and high glucose concentration significantly increased Dab2 mRNA expression after 2 to 6 hour (Figure 3A). The Dab2 protein expression after high glucose concentration is similar to the mRNA expression (Figure 4). In contrast to Dab2 expression, high glucose concentration significantly
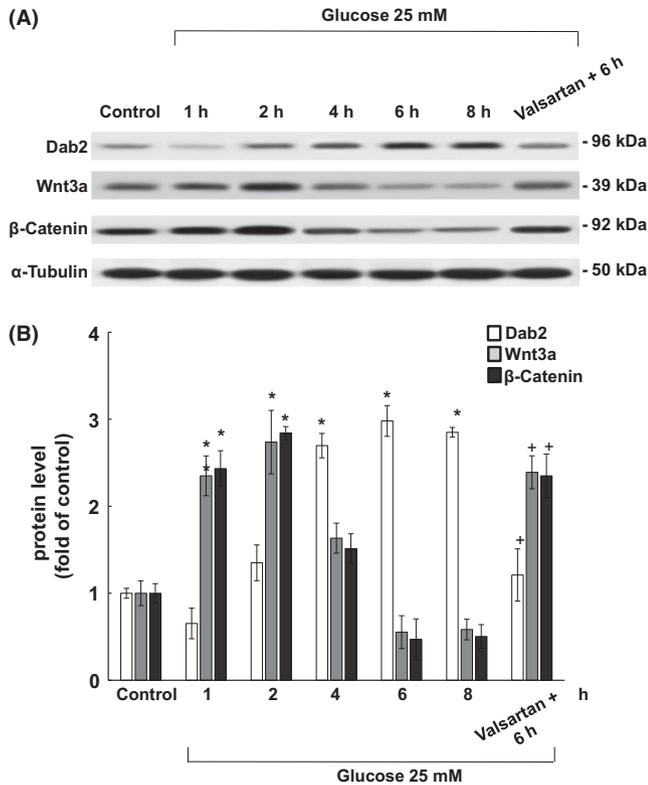
FIGURE 4 Effect of high glucose concentration at 25 mM on Dab2 and wnt3a/ß-catenin protein expression in cultured rat cardiomyocytes. (A) Representative western blots for Dab2 and wnt3a/ß-catenin in rat cardiomyocytes after high glucose stimulation at 25 mmol/L for various periods of time. (B) Quantitative analysis of Dab2 and wnt3a/ß-catenin protein levels. The values from cardiomyocytes after high glucose treatment have been normalized to matched α-tubulin measurement and then expressed as a ratio of normalized values to protein in control group. $*P < .01$ vs. control. $+P < .01$ vs. 6 hour. n = 4 per group

increased Wnt3a and ß-catenin mRNA and protein expression for 0.5 to 1 hour, and then gradually decreased after 2 to 6 hour (Figure 3B, C and Figure 4). Addition of valsartan before high glucose concentration significantly impaired the increased Dab2 protein by high glucose concentration for 6 hour and significantly increased Wnt3a and ß-catenin protein expression (Figure 4). When Wnt protein expression increased, total form of ß-catenin expression increased while active form of ß-catenin expression decreased, and when Wnt protein expression decreased, total form of ß-catenin expression decreased while active form of ß-catenin expression increased (data not shown). Luciferase assay showed that overexpression of miR-145 significantly decreased luciferase activity after high glucose concentration for 4 hour, and overexpression of mutant-miR-145 did not alter the luciferase activity induced by hyperglycaemia stimulation for 4 hour (Figure S2). This result confirms that Dab2 is a target gene of miR-145.

3.4 | MiR-145 decreases while Dab2 increases in left ventricular myocardium in diabetic rats

As shown in Figure 5A, miR-145 expression in left ventricular myocardium was gradually decreased from 3 days to 28 days after diabetic induction by streptozotocin and significantly decreased after 5 days after diabetic induction by streptozotocin as compared to sham rats without hyperglycaemia. The miR-145 expression was significantly enhanced by treatment with valsartan in diabetic rats as compared with diabetic rats without valsartan treatment. The finding indicates that miR-145 responds in vivo similar to that in vitro. Dab2 mRNA and protein expression was significantly increased in left ventricular myocardium from 5 days to 28 days in diabetic rats (Figure 5B–D). Wnt-3a and ß-catenin mRNA expression was significantly reduced from 5 to 28 days in diabetic rats (Figure 5B), and protein expression was significantly decreased from 14 to 28 days in diabetic rats (Figure 5C and D). As shown in Figure 6, overexpression of miR-145 with wild-type miR-145 plasmid significantly decreased Dab2 protein expression and increased Wnt3a and ß-catenin protein expression induced by diabetic rats at 28 days. Treatment with valsartan also significantly decreased Dab2 protein expression and increased Wnt3a and ß-catenin protein expression in diabetic rats. Dab2 siRNA significantly decreased Dab2 protein expression and significantly reversed the Wnt3a and ß-catenin protein expression as compared with diabetic rats at 28 days after diabetic induction. Overexpression of mutant type miR-145 and antagomir-145 did not significantly affect the Dab2, Wnt3a and ß-catenin protein expression as compared with diabetic rats at 28 days. The in situ hybridization also demonstrated less miR-145 signal in diabetic rats as compared with sham group (Figure S3). Diabetic rats showed more Dab2 signals in left ventricular myocardium as compared with sham group. Overexpression of miR-145, pretreatment with Dab2 siRNA and valsartan decreased Dab2 signal expression (Figure S4). The Dab2 signal in the ventricular myocytes was similar in the groups of diabetic rats, overexpression of mutant type miR-145 and administration of antagomir-145.

4 | DISCUSSION

A miR is a small, 20-25-nucleotide nonprotein-coding RNA that transcriptionally or post-transcriptionally modulates the expression of its target genes by interacting with the 3'untranslated regions of target mRNA and promoting target mRNA degradation (gene silencing).[4] Many miRs have been investigated in diabetic complications.[22] However, the effect of hyperglycaemia on the regulation of miR-145 expression in cardiomyocytes is currently
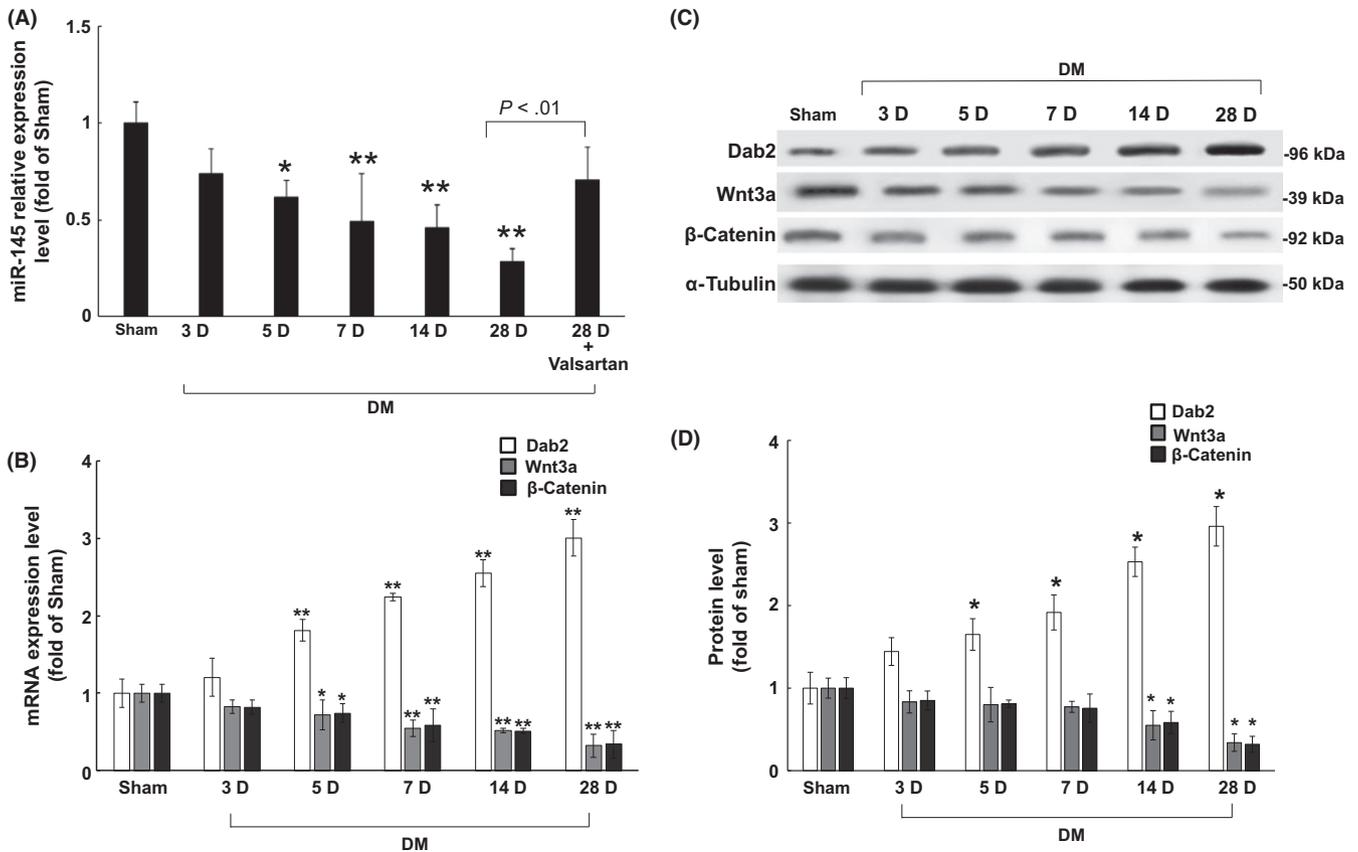
FIGURE 5 Expression of miR-145, Dab2, wnt3a/ß-catenin in left ventricular myocardium of diabetic rats. (A) Quantitative analysis of miR-145 mRNA for various periods of time. (B) Quantitative analysis of Dab2, wnt3a and ß-catenin mRNA for various periods of time after diabetic induction. The values from diabetic rats have been normalized to be matched GAPDH measurement and then expressed as a ratio of normalized values to mRNA in sham group. (C) Representative western blots for Dab2 and wnt3a/ß-catenin for various periods of time after diabetic induction. (D) Quantitative analysis of Dab2 and wnt3a/ß-catenin protein levels. The values from diabetic rats have been normalized to matched α-tubulin measurement and then expressed as a ratio of normalized values to protein in sham group.*$P < .05$ vs. sham. **$P < .01$ vs. sham. N = 5 per group

unknown. In this study, we found that high glucose concentration swiftly increased the miR-145 expression (within 1 hour) and that sustained hyperglycaemia stimulation could inhibit miR-145 expression in rat cardiomyocytes. The upregulation of miR-145 expression immediately after hyperglycaemia (0.5 hour) may reflect a protective response because miR-145 could protect cardiomyocyte apoptosis against oxidative stress.[8] Hyperglycaemia for 0.5 hour could increase reactive oxidative species formation in cardiomyocytes, and antioxidant with N-acetylcysteine could reduce reactive oxidative species formation and decrease miR-145 expression in cardiomyocytes (Figure S5).

Ang II has been reported to be enhanced by high glucose concentration.[23] In this study, we found that high glucose concentration from 0.5 to 6 hour enhanced Ang II secretion from cardiomyocytes, and Ang II mRNA expression was also increased after high glucose concentration. MiR-145 was significantly inhibited by Ang II. Addition of valsartan, an Ang II receptor antagonist, significantly upregulated miR-145 expression in high glucose concentration for 4 hour. This finding indicates that Ang II via angiotensin receptor downregulates miR-145 in cardiomyocytes under high glucose concentration. Valsartan treatment in cardiomyocytes not treated with high glucose has no effect on miR-145 expression, indicating that miR-145 expression in cardiomyocytes not treated with high glucose is not dependent on Ang II receptor. Our study is the first one to demonstrate that valsartan has a direct effect on mediating the expression of miR-145 in cardiomyocytes under hyperglycaemic conditions. Hyperglycaemia has been demonstrated to upregulate AT1 receptor expression in vascular smooth muscle cells and renal mesangial cells.[24,25] The increased AT1 receptor in hyperglycaemia condition is through oxidative stress.[25]

Dab2 is the target of miR-145.[9] The miR-145 binding sites in the 3'untranslated region of Dab2 in cardiomyocytes could mediate Dab2 expression. MiR-145 downregulates Dab2 expression in cardiomyocytes. In this study, we found that brief exposure to high glucose concentration
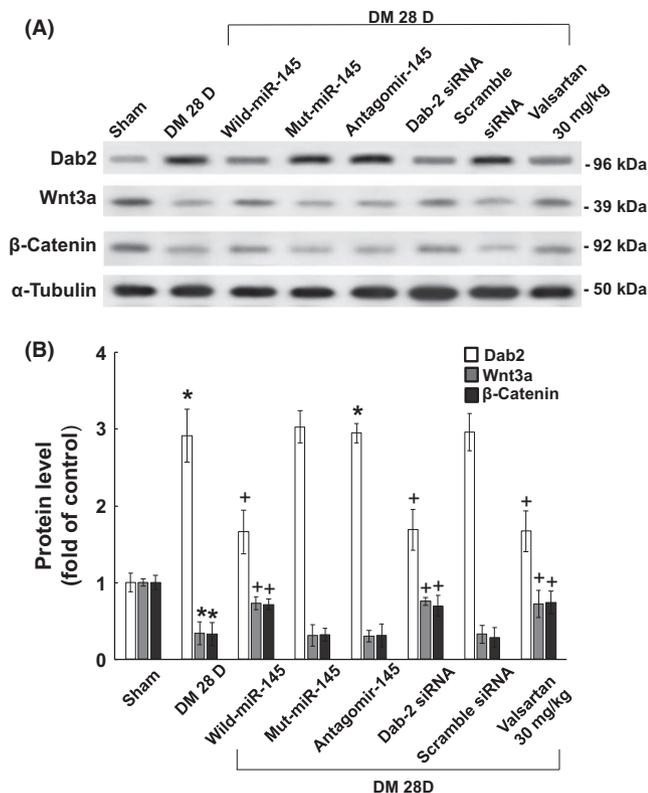
FIGURE 6 Effect of miR-145 overexpression, antagomir-145 and valsartan on Dab2, wnt3 and ß-catenin protein expression in diabetic rats. (A) Representative western blots for Dab2 and wnt3a/ß-catenin in diabetic rats of 28 days under various kinds of treatment. (B) Quantitative analysis of Dab2 and wnt3a/ß-catenin protein levels. The values from diabetic rats have been normalized to matched α-tubulin measurement and then expressed as a ratio of normalized values to protein in sham group. $*P < .01$ vs. sham. $+P < .01$ vs. DM 28 D. n = 5 per group

decreased Dab2 mRNA and protein expression while sustained high glucose concentration increased Dab2 mRNA and protein expression. Brief exposure to high glucose concentration increased Wnt3a and ß-catenin mRNA and protein expression but sustained high glucose concentration inhibited Wnt3a and ß-catenin mRNA and protein expression. Valsartan can reverse the effect of high glucose concentration on Dab2, Wnt3a and ß-catenin expression. These results indicate that Dab2 and Wnt3a expression in high glucose concentration is mediated by Ang II in cardiomyocytes. Diabetic mellitus in practical medicine is not only the hyperglycaemic condition but also the insulin resistance condition. The insulin resistance is an important mechanism to influence energy metabolism in cardiomyocytes. MiR-145 has been shown to inhibit insulin action in cardiomyocytes.[26]

The expression of miR-145 in left ventricular myocardium gradually declined the in diabetic rats from 3 days to 28 days. Dab2 mRNA and protein expression was gradually increased while Wnt3a and ß-catenin mRNA and protein

expression was gradually declined from 3 to 28 days in diabetic condition. Immunohistochemical stain and in situ hybridization assay confirmed the protein expression of miR-145 and Dab2 in cardiomyocytes. This in vivo result was consistent with that in the in vitro study. There is a trend that when the expression of Dab2 increased after induction of diabetic condition, the expression of miR-145 decreased. Overexpression of miR-145 and treatment with valsartan could reverse the Dab2, Wnt3a and ß-catenin protein expression induced by diabetic condition. Our results are consistent with previous studies which showed miR-145 decreased in patients with coronary artery disease and in animal model of acute myocardial infarction.[10,11] Therefore, emphasis could be laid upon the targeting of miR-145 as a novel therapeutic strategy for the management of diabetic heart diseases as supposed from their functions in repair of infarcted myocardium or protective roles against oxidative stress-induced apoptosis.[8,9]

In summary, this study reveals the molecular regulation of miR-145 in the downstream gene of Dab2 expression of cardiomyocytes under hyperglycaemic condition. Ang II plays a critical role in the regulation of miR-145 under hyperglycaemic conditions. The decreased miR-145 will increase the expression of its target gene Dab2. Increased Dab2 will then decrease Wnt3a and ß-catenin expression in cardiomyocytes and left ventricular myocardium. MiR-145 has been considered to be a therapeutic target to reduce atherosclerosis in apolipoprotein E knockout mice.[27] The better understanding of the detailed mechanisms of therapeutic miR-145 under hyperglycaemia condition will provide us a new insight into therapeutic development for cardiovascular diseases that frequently encountered in patients suffering from diabetes mellitus.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.