Role of microRNA-133 in rats with diabetic cardiovascular complications

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List of Abbreviations

ADA	American Diabetes association
AF	Atrial fibrillation
Ago2	Argonaute 2 protein
BM-MSCs	Bone marrow mesenchymal stem cells
CT	Cycle Threshold
CVD	Cardiovascular disease
DEPC	Diethylpyrocarbonate
DHD	Diabetic heart disease
DM	Diabetes Mellitus
DNA	Deoxy Ribonucleic Acid
dNTP	Deoxy Nucleoside Triphosphate
eIF4G	Eukaryotic initiation factor 4G
ELISA	Enzyme Linked Immunosorbent Assay
ERG	Ether related gene
ESCs	Embryonic stem cells
FBG	Fasting blood glucose
GDM	Gestational diabetes mellitus
H&E	Hematoxylin and Eosin
HLA	Human leukocyte antigen
HR	Heart rate
HSCs	Hematopoietic stem cells
IDF	International Diabetes Federation
I_{Ks}	Slowly activating delayed rectifier potassium channel
Imp8	Importin 8
IP	Intra peritoneal

List of Abbreviations (Cont.)

iPS	Induced pleuripotent stem cells
KCNQ1	Potassium voltage-gated channel subfamily Q member 1
KG	Kilo gram
LQTS	Long QT syndrome
mg/dl	Milligram/deciliter
miRNA	Micro ribonucleic acid
miRNP	microRNA ribonucleoprotein complex
mIU/L	milli International Unit/liter
mmHg	Millimeter mercury
mRNA	Messenger Ribonucleic Acid
MSCs	Mesenchymal stem cells
NK	Natural killer
OGTT	Oral glucose tolerance test
PABP	Poly(A)-binding protein
PACT	Protein activator of protein kinase R
PDAC	Pancreatic ductal adenocarcinoma
PKH26	Fluorescent dye
PTB	Polypyrimidine tract binding
QT	Distance between Q and T waves in ECG
QTc	Corrected QT interval
RISC	RNA-induced silencing complex
RNase	Ribonuclease
ROS	Reactive oxygen species
RQ	Relative Quantification
RT	Reverse Transcription

List of Abbreviations (Cont.)

RT-PCR	Reverse Transcription/ Real time Polymerase Chain Reaction
SCNT	Somatic Cell Nuclear Transfer stem cells
SD	Standard Deviation
SPSS	Statistical package for the social sciences
STZ	Streptozotocin
STZ/MSC	Streptozotocin injection followed by mesenchymal stem cells injection
T1DM	Type 1diabetes mellitus
T2DM	Type 2 diabetes mellitus
Taq DNA	Thermus Aquatica Deoxy Ribonucleic Acid
T_m	Melting Temperature
TRBP	Trans-activator RNA-binding protein
UTRs	Untranslated regions
X	Concentration Power
ΔCT	Delta Cycle Threshold
µg/ml	Microgram/Millilitre

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Role of microRNA-133 in rats with diabetic cardiovascular complications

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ABSTRACT

Background: Diabetes is one of the most health problem globally with a serious impact on morbidity, mortality & health care resources. Cardiovascular diseases are the leading cause of death in individuals with type 1 diabetes.

Aim: The purpose of this study was to evaluate the miRNA-133a expression in cardiac tissues of the diabetic rats and its relation to the cardiovascular complications.

Methods: 20 male albino rats divided into Group I (control) and group II (diabetic)
10 rats in each. Rats were made diabetic by intraperitoneal injection of streptozotocin (35mg/kg body weight). Physiological cardiovascular functions were assessed. Blood and cardiac tissue samples were taken from all rats for biochemical and histological studies. Quantitative RT-PCR for miRNA-133 expression in cardiac tissues was performed

Results: miR-133 expression was significantly increased in cardiac tissues of diabetic rats compared to control rats and was correlated to fasting blood glucose levels.

Conclusion: the present study suggests that there is a complex relationship between miR-133 expression and the cardiac functions in diabetic rats which needs more exploration.

Keywords: diabetes mellitus • cardiovascular complications• miR-133 expression

Introduction

Diabetes mellitus (DM) is one of the top 10 leading causes of morbidity and mortality, affecting nearly 350 million people worldwide ***(Xi and Bu, 2014).*** Diabetes is a serious condition with potentially devastating complications that affects all age groups worldwide ***(International Diabetes Federation, 2013).*** The prevalence of diabetes, has been increasing at alarming rates all over the world and is estimated to rise to 552 million adults by 2030 ***(Chen et al., 2011, Whiting et al., 2011).***The associated increase in mortality and morbidity makes it one of the major health and socio-economic problems in our society ***(Chen et al., 2011, Moura et al., 2013).***

Type 1diabetes mellitus (T1DM) is a chronic disease in which pancreatic β cells are destroyed by self-autoimmune attack ***(Wu et al., 2014).*** It is a complex multifactorial disorder which involves a loss of self-tolerance leading to the autoimmune destruction of pancreatic β-cells ***(Ezquer et al., 2014).*** Clinical studies showed that transplantation of islets is a sufficient cure for aiding patient to relieve from diabetes-related symptoms. However, the lack of applicable donor cells limits the treatment ***(Wu et al., 2014).***The scientific community and diabetic patients are thus, still waiting for an effective therapy which could preserve the remaining β-cells, replenish islet mass and protect newly generated β-cells from auto-immune destruction ***(Ezquer et al., 2014).***

Diabetic heart disease (DHD) is the leading cause of morbidity and mortality among people with diabetes, being responsible for approximately 80% of the deaths in diabetics ***(Rawal et al., 2014).*** Importantly, cardiovascular complications in the diabetics develop at a much earlier stage, although remaining asymptomatic till the later stage of the disease, thereby restricting its early detection and active therapeutic management ***(Katare et al., 2010).*** Thus, a better understanding of the modulators involved in the pathophysiology of DHD is necessary for the early diagnosis and development of novel therapeutic implications for diabetes-associated cardiovascular complications ***(Rawal et al., 2014).***

Kv7.1 is a potassium channel causing a voltage gated repolarization current. It is encoded by KCNQ1 and expressed both in β-cells ***(Rosengren et al., 2012)*** and in cardiomyocytes ***(Moss et al., 2007)***.

Many studies reported that functional mutations, or down-regulation of KCNQ1 lead to long QT syndrome (LQTS) which is associated with prolonged ventricular repolarization predisposes to life-threatening arrhythmias. However, the clinical and physiological significance of functional KCNQ1 mutations in β-cells of pancreas is still unknown ***(Torekov et al., 2014).***

MicroRNAs (miRNAs) are small (~ 22 nucleotides) noncoding RNAs which post-transcriptionally regulate gene expression by binding complementary sequences within messenger RNAs (mRNAs). The human genome encodes over 1800 miRNAs, which target about 60% of human genes ***(Hedley et al., 2014).***

MicroRNAs participate in many essential biological processes, such as cell proliferation, differentiation, apoptosis and stress ***(Mathieu and Ruohola-Baker, 2013).*** They are novel regulators involved in cardiac physiology and pathophysiology, including the regulation of cardiac physiological function and participation in the genesis of cardiac diseases. Many recent studies suggest that miRNAs play a crucial role in the pathogenesis of diabetes and many cardiovascular complications by regulating the expression of multiple genes ***(Zampetaki et al., 2010, Cardin et al., 2012)***.

It was found that miR-133a -induced by hyperglycemia- leads to suppression of insulin biosynthesis. miR-133a decreased polypyrimidine tract binding protein expression which is required for stabilization of insulin mRNA, leading to decrease insulin secretion. This may contributes as one of the mechanisms by which hyperglycemia causes beta-cell dysfunction ***(Fred et al., 2010)***.

MiR-133a is the most abundant miRNAs in the heart and has been reported to regulate cardiac ion channels ***(Hedley et al., 2014).*** It is selectively expressed in heart and have been used as early markers of cardiac tissue damage ***(Chen et al., 2014).*** The three forms of miR-133 (namely: miR-133a-1**,** miR-133a-2 and miR-133b) are expressed in cardiac muscles ***(Topkara and Mann, 2011).*** Several studies have demonstrated the important role of miR-133 in various cardiovascular diseases. However, their exact role in DHD is still unrecognized ***(Rawal et al., 2014).***