ARTICLE IN PRESS

Biochemical and Biophysical Research Communications xxx (2018) 1–5

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Alterations of circular RNAs in hyperglycemic human endothelial cells

Fei-Fei Shang [a], Suxin Luo [b], Xiaoxue Liang [b], Yong Xia [a, b, c, *]

[a] Institute of Life Science, Chongqing Medical University, Chongqing 400016, China
[b] Division of Cardiology, The First Affiliated Hospital, Chongqing Medical University, Chongqing 400016, China
[c] Davis Heart and Lung Research Institute, Division of Cardiovascular Medicine, Department of Biological Chemistry and Pharmacology, The Ohio State University College of Medicine, 473 West 12th Avenue, Columbus, OH 43210, USA

ARTICLE INFO

Article history:
Received 21 March 2018
Accepted 25 March 2018
Available online xxx

Keywords:
Circular RNA
Hyperglycemia
Endothelial cell
microRNA

ABSTRACT

Circular RNA (circRNA), a family of RNA generated by RNA circularization, is ubiquitously expressed in tissues and possesses increasingly important biological functions. Hyperglycemia-induced endothelial dysfunction is an initiating event in the pathogenesis of diabetes-associated cardiovascular complications. How high glucose may affect circRNAs is unknown. To address this issue, human endothelial cells were exposed to high glucose treatment and the changes of circRNAs were measured by RNA sequencing. A total 3686 circRNAs, including 1040 previously unrecorded circRNAs, were detected; and 95 different expression (DE) circRNAs were observed. The host genes of these DE circRNAs were further studied by function enrichment analyses. These analyses revealed genes of phosphoproteins, transferases, and zine finger proteins. Since circRNAs can function as a microRNA (miRNA) sponge, circRNAs-miRNAs interaction networks were explored by bioinformatics. These analyses identified a number of miRNAs, which might interact with DE circRNAs and play roles in the actions of high glucose on endothelial cells. These results demonstrate that high glucose exposure profoundly changes circRNA expression in endothelial cells. Altered circRNA expression may contribute to the effects of high glucose on endothelial function in diabetes.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

Diabetes is becoming one of the paradigmatic disorders of this century. The primary character of diabetes is the recurrent hyperglycemia—high blood glucose levels. Elevated blood glucose impairs vascular endothelial cells and this leads to the development of major cardiovascular complications including atherosclerosis, hypertension, etc. [1,2]. Thus, protecting endothelium is a critical approach to prevent cardiovascular diseases in diabetes. To develop effective approaches to protect endothelium, it is needed to first understand how high glucose causes endothelial injury. Current understandings on diabetic endothelial injury include oxidative stress, inflammation, etc. Yet these have not given rise to therapies. Overall, the mechanistic understanding on how high glucose impairs vascular endothelial cells remains incomplete [3].

Recent studies reveal that noncoding RNAs play important roles in cardiovascular regulation and diseases [4]. Among them, circular RNAs (circRNAs) form a covalently closed continuous loop with the 3′ and 5′ ends joined together. This feature confers numerous properties to circRNAs, many of which have only recently been identified. Studies show that circRNAs play important roles in nervous system, cancer, and cardiomyocytes [5–7]. For example, it was reported that circRNA_010567 contributed to myocardial fibrosis through acting on miR-141 and TGF-β1 [7]. CircRNAs can regulate downstream molecules via multiple mechanisms. A main mechanism is referred as "competing endogenous RNAs" or "miRNA sponges". This mechanism shows that circRNA could bind to miRNAs to sequester them [8]. A number of miRNAs have been reported to play important roles in the pathogenesis of endothelial dysfunction in diabetes [9–11].

How high glucose levels affect circRNAs in endothelial cells is unknown. Here, we used RNA sequencing technique to measure the global changes of circRNAs in human endothelial cells exposed to high glucose. Possible targets of differentially expressed circRNAs under high glucose condition are explored.

* Corresponding author. The Ohio State University College of Medicine, Room 2130G, 333 West 10th Avenue, Columbus, OH 43210, USA.
E-mail address: yong.xia@osumc.edu (Y. Xia).

https://doi.org/10.1016/j.bbrc.2018.03.187
0006-291X/© 2018 Elsevier Inc. All rights reserved.

Please cite this article in press as: F.-F. Shang, et al., Alterations of circular RNAs in hyperglycemic human endothelial cells, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.187

ARTICLE IN PRESS

2. Materials and methods

2.1. Cell culture

Human umbilical cord was harvested from a healthy caesarean pregnancy. The individual was given informed written consent and ethical permission was obtained from the Ethics Committee of Chongqing Medical University. Preheated sterile PBS was used to rinse surface and endovascular blood. 0.2% collagenase I (Sigma, USA) was injected in closed umbilical veins. Umbilical cord was incubated at 37 °C for 7 min. Digestive liquid was collected and mixed with equal volumes of M199 media contained 10% FBS. After centrifugation, human umbilical vein endothelial cells (HUVECs) were cultured in ordinary medium. The medium comprised of M199 basic medium (Gibco, USA), 10% FBS, 20 mg/L ECGS, 2 mM L-Glutamine and 100 mg/L heparin sodium.

The HUVECs of passage 6 were subjected to high glucose (HG) treatment. Normal group was cultured with ordinary medium contained 1 g/L glucose. HG group was suffered 5.4 g/L glucose. And control group was adjusted osmotic pressure to equal with HG group by mannitol. Medium was changed every 8 h to maintain constant concentration of the glucose. After 24 h of treatment, the cells were harvested for further analyzed.

2.2. RNA isolation and sequencing

Total RNA was extracted using the TRIZOL reagent (Thermo, USA) following the manufacturer's protocol. The libraries were constructed using TruSeq Stranded Total RNA with Ribo-Zero Gold according to the manufacturer's instructions. Briefly, rRNA was removed from RNA simples. After fragment, reverse transcriptase was used to synthesize cDNA. Then the purified samples were ligated 3′ end AMP and sequencing adapters. At last, enriched fragments were evaluated using the Agilent 2100 Bioanalyzer (Agilent Technologies, USA). These libraries were sequenced on the Illumina sequencing platform (HiSeqTM 2500) and 150 bp/125bp paired-end reads were generated.

2.3. Bioinformatics analysis

CIRI software was used to process sequencing results [12]. According to BLAST analysis, we identified circRNAs that were recorded by CircBase [13]. CIRI also predicted new circRNAs that were not reported before.

We further calculate the RPM (spliced reads permillonmapping) of each circRNA to quantitate their expression level. Compared with normal and control group, the circRNAs that fold change is greater than 2 were selected in HG group. Then the heatmap of different

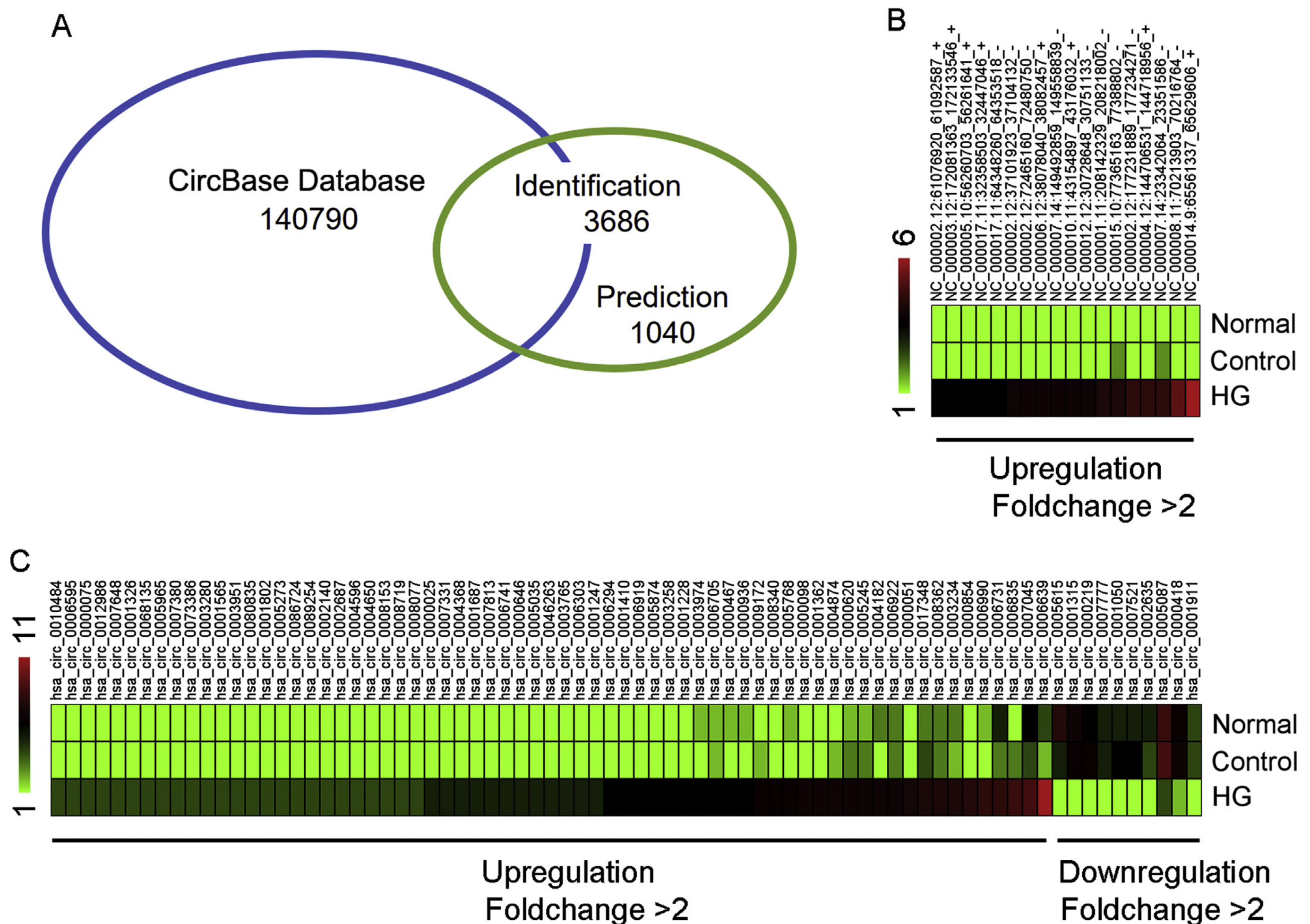

Fig. 1. RNA sequencing identified circRNA expression of HUVECs between high glucose group and normal/control group. A, The CircBase has recorded 140790 circRNAs. And we identified 3686 circRNAs. Among these circRNAs, 1040 circRNAs were new that were not record in CircBase. B, Heatmap showed 2-fold changing of predicted circRNA after 30 mM glucose treatment. C, Heatmap showed 2-fold changing of CircBase recorded circRNA after 30 mM glucose treatment.

Please cite this article in press as: F.-F. Shang, et al., Alterations of circular RNAs in hyperglycemic human endothelial cells, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.03.187

Download English Version:

https://daneshyari.com/en/article/8293060

Download Persian Version:

https://daneshyari.com/article/8293060

Daneshyari.com