Free Radical Biology and Medicine 101 (2016) 334–347

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

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

Original article

Glutaredoxins concomitant with optimal ROS activate AMPK through S-glutathionylation to improve glucose metabolism in type 2 diabetes

Kelei Dong[a,1], Meiling Wu[a,1], Xiaomin Liu[a], Yanjie Huang[a], Dongyang Zhang[a], Yiting Wang[a], Liang-Jun Yan[b], Dongyun Shi[a,*]

[a] Department of Biochemistry and Molecular Biology, Shanghai Medical College of Fudan University, Free Radical Regulation and Application Research Center of Fudan University, Shanghai 200032, People's Republic of China

[b] Department of Pharmaceutical Sciences, UNT System College of Pharmacy, University of North Texas Health Science Center, Fort Worth, TX, USA

ARTICLE INFO

Keywords:
Glutaredoxins
ROS
AMPK
Type 2 diabetes
Glucose metabolism
Glutathionylation

ABSTRACT

AMPK dysregulation contributes to the onset and development of type 2 diabetes (T2DM). AMPK is known to be activated by reactive oxygen species (ROS) and antioxidant interference. However the mechanism by which redox state mediates such contradictory result remains largely unknown. Here we used streptozotocin-high fat diet (STZ-HFD) induced-type 2 diabetic rats and cells lines (L02 and HEK 293) to explore the mechanism of redox-mediated AMPK activation. We show glutaredoxins (Grxs) concomitant with optimal ROS act as an essential mediator for AMPK activation. ROS level results in different mechanisms for AMPK activation. Under low ROS microenvironment, Grxs-mediated S-glutathionylation on AMPK-α catalytic subunit activates AMPK to improve glucose transportation and degradation while inhibiting glycogen synthesis and keeping redox balance. While, under high ROS microenvironment, AMPK is activated by an AMP-dependent mechanism, however sustained high level ROS also causes loss of AMPK protein. This finding provides evidence for a new approach to diabetes treatment by individual doses of ROS or antioxidant calibrated against the actual redox level in vivo. Moreover, the novel function of Grxs in promoting glucose metabolism may provide new target for T2DM treatment.

1. Introduction

In recent years, diabetes has been characterized as a global epidemic, with numbers expected to reach 300 million by 2025 [1]. Understanding the mechanism underlying pathogenesis of diabetes is critical to developing a cure. Many of the common key driving forces that contribute to the onset and progression of diabetes, including obesity, age and sedentary lifestyle, also contribute to the creation of an oxidizing environment. While oxidative stress has been widely recognized as an important factor in diabetogenesis, contradictory findings, in which many studies have indicated a protective role for ROS in exercise and in the prevention and treatment of diabetes [2,3], obscure its role in the disease progression.

Adenosine monophosphate-activated protein kinase (AMPK) is an important target for the treatment of diabetes [4]. The level of insulin is decreased in mice with AMPK α2-deficiency [5], and AMPK can also improve or ameliorate the symptoms of diabetes through reducing glycogen synthesis, increasing glycolysis, prompting the uptake of glucose in surrounding tissues. Therefore, many of the existing diabetes drugs including rosiglitazone [6] and metformin [7] lower blood glucose by reducing intracellular ROS via the AMPK-FOXO3 pathway [8]. Activation of the AMPK pathway has also been shown to exhibit further protective effects through preventing ROS-mediated mitochondrial division and apoptosis of endothelial cells [9,10].

These findings contradict with studies that suggest that AMPK is activated by oxidative stress [11,12]. In a study examining embryos of

Abbreviations: Allo, allopurinol; AMPK, adenosine monophosphate-activated protein kinase; Apo, apocynin; Cu-Zn-SOD, Cu-Zn-superoxide dismutase; DTT, dithiothreitol; ECAR, extracellular acidification rate; GK, glucokinase; GLUT4, glucose transporter 4; GPxs, glutathione peroxidases; GR, glutathione reductase; Grx-1, glutaredoxin 1, cytosolic; Grx-2, glutaredoxin 2, mitochondrial; Grxs, glutaredoxins; GYS-1, glycogen synthase-1; HPLC, high performance liquid chromatography; LDH, lactate dehydrogenase; Mn-SOD, Mn-superoxide dismutase; NAC, N-acetyl cysteine; NOX, NADPH oxidase; OCR, oxygen consumption rate; OGTT, oral glucose tolerance test; p-AMPK, phospho-AMPK α1/α2; PFK-1, phosphofructokinase-1; p-PFK-2/PFK-2, phospho-PFK-2/PFK-2; p-GYS, phospho-glycogen synthase; PK, pyruvate kinase; Prxs, peroxiredoxins; ROS, reactive oxygen species; R-SOH, sulfenic acid; R-SO_2H, sulfinic acid; R-SO_3H, sulfonic acid; SD, Sprague-Dawley; STZ-HFD, Streptozotocin-high fat diet; T2DM, type 2 diabetes mellitus; TEM, transmission electron microscope; Trxs, thioredoxins; XO, xanthine oxidase

* Corresponding author.
E-mail address: dyshi@fudan.edu.cn (D. Shi).

[1] These authors contribute equally to this work.

http://dx.doi.org/10.1016/j.freeradbiomed.2016.10.007
Received 13 September 2016; Received in revised form 9 October 2016; Accepted 11 October 2016
Available online 13 October 2016

pregnant mice, AMPK activation was observed in hypoxia-induced oxidative stress. However, these effects were abolished in the presence of antioxidants glutathione ethyl ester and vitamin E. Therefore, it seems that both oxidative stress and antioxidant interventions can lead to AMPK activation. These contradictory mechanisms of AMPK activation highlight a need for further investigation.

Hence, the present study was designed to understand the mechanism underlying ROS/antioxidant regulation of AMPK function in diabetes. We hypothesized that different redox states in vivo impact AMPK activation in different mechanisms, thus affecting downstream metabolic pathways. We used streptozotocin-high fat diet (STZ-HFD) induced diabetic rats and liver cells to explore the impact of different redox states on AMPK and its downstream glucose pathways. Our results show that AMPK is activated by either oxidative stress or ROS inhibition. The different amount of cellular ROS could result in different mechanisms for AMPK activation: either by Grx-dependent redox modification or by AMP-dependent allosteric activation. We demonstrated that under low ROS microenvironment Grxs act as an essential mediator to allow S-glutathionylation on AMPK-α catalytic subunit and AMPK activation, thus improving glucose metabolism while keeping redox balance in T2DM.

2. Methods

2.1. Diabetic animal models

All animal-related procedures were approved by the Fudan University Institutional Laboratory Animal Ethics Committee. Male SD rats (150–160 g body weight) were obtained from Fudan University Animal Center (Shanghai, China). Induction of type 2 diabetes by STZ (Sigma Aldrich, USA)-high fat diet (STZ-HFD) was performed as previously described [13–15] and more details were shown in supplementary materials. Normal chow and high-fat diet (HFD) were purchased from Shanghai SLRC laboratory animal Company Ltd (Shanghai, China) and the nutritional composition was shown in supplementary Table S1. Measurement of OGTT was performed according to the procedure previously described [16]. Accu-Chek Performa (Roche Diagnostics, Germany) was used to measure blood glucose levels in rats. Alternatively, after a 12 h fasting period, rats were administered with glucose of 2.5 g/kg body weight. Insulin secretion was detected using an Iodine [^{125}I] Insulin Radioimmunoassay kit (Shanghai Radioimmunoassay Technology Research Co Ltd, Shanghai, China). This study was approved by the Research Ethics Committees of Fudan University and the methods were carried out in accordance with the approved guidelines.

2.2. Cell culture

Human liver cell line L02 and human emborynic kidney cell line 293T were obtained from Chinese Academy of Sciences (China). Culture of cells was performed as previously described [17]. Cells were maintained in Dulbecco's modified Eagle's medium (GIBCO, USA), low glucose culture medium and were incubated in 5% CO_2 cell incubator at 37 °C. The medium was supplemented with 10% fetal bovine serum (Solarbio, Beijing Solarbio Science & Technology Company Ltd, Beijing, China), 10^5 unit/L Penicillin and Streptomycin (North China Pharmaceutical Company Ltd, China).

2.3. Transmission electron microscope analysis

After rats were anesthetized, part of their liver tissues were cut and fixed by glutaraldehyde. Ultra-structures of liver cells were then further analyzed by transmission electron microscope at Fudan University (Shanghai, China).

2.4. Assay for GK, PFK-1, PK, LDH and aconitase activity

Liver glucokinase (GK) and PFK-1 activities were measured as described previously [18]. PK activity was measured as previously described [18]. LDH activity was measured as previously described [19]. Aconitase activity was determined spectrophotometrically [20]. Enzyme activity was standardized using protein quantification.

2.5. GSH/GSSG ratio measurement

Mouse liver tissues were homogenized into 50 µl 1 M HPO_3 for GSH detection. The suspension was centrifuged with 12,000 rpm for 10 min at 4 °C and then assayed according to the manufacturer's instructions (Changzhou Redox Biological Technology Corporation, CN) as previously described [22].

2.6. ATP and AMP content analysis

ATP and AMP were measured using high performance liquid chromatography (HPLC) as previously described [23]. About 20–30 mg liver tissues were homogenized on ice, the homogenate was treated with perchloric acid. Homogenized samples were centrifuged for 12,000 rpm at 4 °C for 30 min. Supernatant was then collected and mixed with potassium carbonate, followed by further centrifugation for 12,000 rpm at 4 °C for 20 min. Supernatant was obtained for the determination of ATP, and AMP content by HPLC. The detection wavelength was 254 nm.

2.7. S-Glutathionylation of AMPK and detection of GSS-AMPK adduct formation

GSS-AMPK adduct were measured as described previously [24]. L02 cells (2×10^6/ml) were incubated with ethyl ester GSH-biotin (6 mM) for 1.5 h. Cells were then washed twice with culture buffer to remove the excess of GSH and treated with H_2O_2 for 20 min. Cell lysates were prepared in the presence of N-ethylmaleimide (5 mM) and then passed through Bio-Gel P10 to remove free GSH-biotin and N-ethylmaleimide. The level of GSS-protein conjugates was determined using non-reducing Western blot analysis with streptavidin-HRP, whereas GSS-AMPK subunit levels were measured after pull-down with streptavidin-agarose (60 min at 4 °C), followed by reducing SDS-PAGE and Western blot analysis with antibodies against AMPK α subunit.

2.8. Metabolic assays

Respirometry (oxygen consumption rate, OCR, indicative of mitochondrial OXPHOS) and the extracellular acidification rate (ECAR, indicative of glycolysis) of cells were measured using an XF24 Extracellular Flux Analyzer (Seahorse Bioscience) as described previously [25]. For OCR analysis, 10^4cells per well were seeded in complete growth medium in 96-well plates designed for the XF24. Grx-1/2 siRNA (500 nM) were transfected to L02 cells for 48 h in a CO_2-free incubator for measurement. A program with a typical 8-min cycle of mix (3 min), dwell (2 min), and measurement (3 min) was used. During measurement, oligomycin (OM), carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) and antimycin A (AA) were added to a final concentration of 10 µm, 1 µm and 10 µm, respectively. For ECAR analysis, Grx-1/2 siRNA-treated cells (10^4 cells per well) were used for the measurement. The default standard glycolysis stress-test program was selected. Measurements were conducted using final concentrations of 10 mM glucose, 1 µm oligomycin and 50 mM 2-deoxyglucose, respectively.