Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

TSPA as a novel ATF6α translocation inducer efficiently ameliorates insulin sensitivity restoration and glucose homeostasis in *db/db* mice

Tingting Zhou [a, b, 1], Yanhua Cheng [c, 1], Wenzhong Yan [d, 1], Xiaofan Shi [b, 1], Xin Xu [b], Jinpei Zhou [c], Jian Li [d, *], Jing Chen [b, **], Xu Shen [b, e]

[a] *Wuxi School of Medicine, Jiangnan University, 1800 Lihu Road, Wuxi 214122, China*
[b] *CAS Key Laboratory of Receptor Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China*
[c] *School of Pharmacy, China Pharmaceutical University, Jiangsu, Nanjing 210009, China*
[d] *Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China*
[e] *State Key Laboratory Cultivation Base for TCM Quality and Efficacy, School of Medicine and Life Sciences, Nanjing University of Chinese Medicine, 138 Xianlin Road, Nanjing 210023, China*

ARTICLE INFO

Article history:
Received 1 April 2018
Accepted 3 April 2018
Available online xxx

Keywords:
ATF6α
ER stress
Insulin resistance
TSPA
T2DM

ABSTRACT

Activating transcription factor 6α (ATF6α) as a transducer in unfolded protein response (UPR), plays an important role in liver glucose metabolism and insulin resistance. Thus, targeting ATF6α activation has been proposed to be a potential strategy for anti-T2DM drug discovery. Here, we determined that small molecule 2-[5-[1-(4-chlorophenoxy)ethyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl]sulfanyl-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)acetamide (TSPA) functioned as an ATF6α translocation inducer effectively promoting ATF6α translocation into nucleus and ameliorating glucose homeostasis on *db/db* mice. TSPA promoted ATF6α translocation into nucleus without increing C/EBP-homologous protein (CHOP) expression. TSPA restored the tunicamycin (TM)-stimulated insulin receptor (IR) desensitization through ATF6α activation, inhibited gluconeogenesis and efficiently improved glucose homeostasis on *db/db* mice. Furthermore, TSPA protected insulin pathway involving p38/X-box binding protein 1s (Xbp1s)/ER chaperones signaling pathway. Our current study has determined that ATF6α was a promising therapeutic target and also highlighted the potential of TSPA in the treatment of type 2 diabetes mellitus (T2DM).

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease mainly characterized by hyperglycemia with insulin resistance and deficiency [1]. Although the underlying pathological basis of T2DM remains obscure, insulin resistance and hepatic glucose production impairment are accepted as two typical pathological features of T2DM [2]. Currently, a series of anti-T2DM drugs are being clinically used, but it is still urgently needed to explore novel therapeutic strategies against diabetes due to the adverse effects of known drugs [2].

Evidence has shown that the pathology of T2DM is tightly associated with endoplasmic reticulum (ER) stress [3,4]. ER as an organelle for synthesis, folding and transportation of proteins in eukaryotic cells is susceptible to physiological and pathological stimulations, such as hypoxia, starvation and protein misfolding [5,6]. At the beginning of ER stress, unfolded protein response (UPR) is activated to alleviate ER stress, but apoptosis probably

Abbreviations: Akt, protein kinase B; ATF6, activating transcription factor 6; CHOP, C/EBP-homologous protein; CNX, calnexin; CRT, calreticulin; CREB, cAMP response element binding protein; ER, Endoplasmic reticulum; ERAD, endoplasmic reticulum associated degradation; GRP94, glucose regulated protein 94; G6Pase, Glucose-6-phosphatase; IR, insulin receptor; IRE1α, ER-to-nucleus signal kinase 1α; OGTT, Oral glucose tolerance test; PEPCK, Phosphoenolpyruvate carboxykinase; PERK, PKR like ER kinase; PTT, Pyruvate tolerance test; TM, tunicamycin; TSPA, 2-[5-[1-(4-chlorophenoxy)ethyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl]sulfanyl-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)acetamide; T2DM, type 2 diabetes mellitus; UPR, unfolded protein response; Xbp1s, p38/X-box binding protein 1s.

* Corresponding author. Shanghai Key Laboratory of New Drug Design, School of Pharmacy, East China University of Science and Technology, Shanghai 200237, China.
** Corresponding author. Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China.
E-mail addresses: jianli@ecust.edu.cn (J. Li), jingchen@simm.ac.cn (J. Chen).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.04.025
0006-291X/© 2018 Elsevier Inc. All rights reserved.

Please cite this article in press as: T. Zhou, et al., TSPA as a novel ATF6α translocation inducer efficiently ameliorates insulin sensitivity restoration and glucose homeostasis in *db/db* mice, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.025

ARTICLE IN PRESS

initiates when such mitigation fails to ameliorate ER stress [7]. There are three ER-transmembrane transducers of UPR, such as inositol requiring ER-to-nucleus signal kinase 1α (IRE1α), PKR like ER kinase (PERK) and activating transcription factor 6 (ATF6). These signaling pathways increase the expression of ER chaperones, inhibit protein entry into ER and accelerate the degradation of misfolded proteins, finally leading to the amelioration of accumulated misfolded proteins in ER [8]. Among which ATF6 is a critical regulator of ER quality control proteins in mammalian cells [7,9]. As a member of ATF/cAMP response element binding protein (CREB) family, ATF6 contains two subtypes (ATF6α and ATF6β) [10,11]. ATF6α as an ER-membrane-bound transcription factor is retained in ER through interactions between its luminal tail and the ER chaperone immunoglobulin protein/GRP78 [11] and is activated by protein misfolding in the ER [9]. After stimulation, ATF6α (90 kDa) dissociates from ER chaperone immunoglobulin protein to expose its Golgi localization sequences, and transfers to Golgi apparatus, where it is cleaved by site-1 and site-2 proteases into active 50 kDa fragment [12]. Subsequently, cytosolic ATF6α (50 kDa) translocates into nucleus binding to ER stress response element, UPR element or cAMP response element and activates the transcription of ER stress response-related genes, including ER chaperone immunoglobulin protein, GRP78, glucose-regulated protein 94 (GRP94) and protein disulfide isomerase [6].

It has been identified that ATF6α is closely related to glucose metabolism and ATF6α inhibits gluconeogenic gene expression by disrupting the interaction between CREB and CREB regulated transcription coactivator 2 [13]. It is reported that ATF6α decreases fasting glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK) mRNA transcription and reduces blood glucose level in mice [14] and ATF6α over-expression reverses the tunicamycin (TM)-induced insulin receptor (IR) desensitization [10]. Therefore, all these findings have addressed the tight association of ATF6α with liver glucose metabolism and insulin resistance, thus highlighting the potential of ATF6α as a therapeutic target for anti-T2DM drug development [2].

In the current work, we discovered that the small molecular compound, 2-[5-[1-(4-chlorophenoxy)ethyl]-4-phenyl-4*H*-1,2,4-triazol-3-yl]sulfanyl-*N*-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazol-4-yl)acetamide (TSPA, Fig. 1A), induced ATF6α translocation and reversed the TM-stimulated IR and protein kinase B (Akt) desensitization involving p38/X-box binding protein 1s (Xbp1s)/ER chaperones signaling pathway. Moreover, the assay on *db/db* male mice indicated that TSPA efficiently improved glucose homeostasis *in vivo*. Our results have expounded the ATF6α-mediated signaling pathway in response to insulin sensitivity restoration and highlighted the potential of TSPA in the treatment of T2DM.

2. Materials and methods

2.1. Materials

G418 was purchased from Beyotime (China). Tunicamycin (TM) was purchased from Sigma (USA). Anti-ATF6α antibody was obtained from Imgenex Corp (USA). Anti-insulin receptor β, anti-phospho-IGF-I receptor β (Tyr1135/1136)/insulin receptor β (Tyr1150/1151), anti-Akt, anti-phospho-Akt (Ser473), anti-p38, anti-phospho-p38 (Thr180/Tyr182), anti-GAPDH were purchased from Cell Signaling Technology (USA). Anti-spliced Xbp1 and anti-CHOP were purchased from Santa Cruz Biotechnology (USA).

2.2. Cell culture

ATF6α-U2OS cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplied with 10% fetal bovine serum, 100 unit/mL penicillin/streptomycin and 0.5 mg/mL G418. Mouse primary hepatocytes were isolated from 9-week-old C57BL/6J mice fasted overnight with water ad libitum according to the previous report [15]. All cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere.

2.3. ATF6α distribution assay

ATF6α-U2OS cells were seeded into 96-well plates overnight and then incubated with positive control or compounds for 5 h. Then the cells were stained with Hoechst and fixed with formaldehyde solution for 20min. Finally, the cells were imaged by In Cell Analyzer 2000 with filters of Hoechst and GFP. Translocation of EGFP-ATF6α was analyzed with the GFP fluorescence intensity in nucleus and cell.

2.4. Western blot assay

Mouse primary hepatocytes were harvested by lysate buffer (4% SDS, 62.5 mmol/L Tris-HCl, 25% glycrol, and bromophenol blue, PH = 6.8). Liver tissue was homogenated with RIPA containing protease and phosphatase inhibitor (Thermo, USA). Protein samples was separated by SDS-PAGE, and transferred to the nitrocellulose filter membrane (GE, USA). Membranes were incubated with primary antibodies at 4 °C overnight, and secondary antibodies for 2 h at room temperature. Blots were visualized by Super Signal West

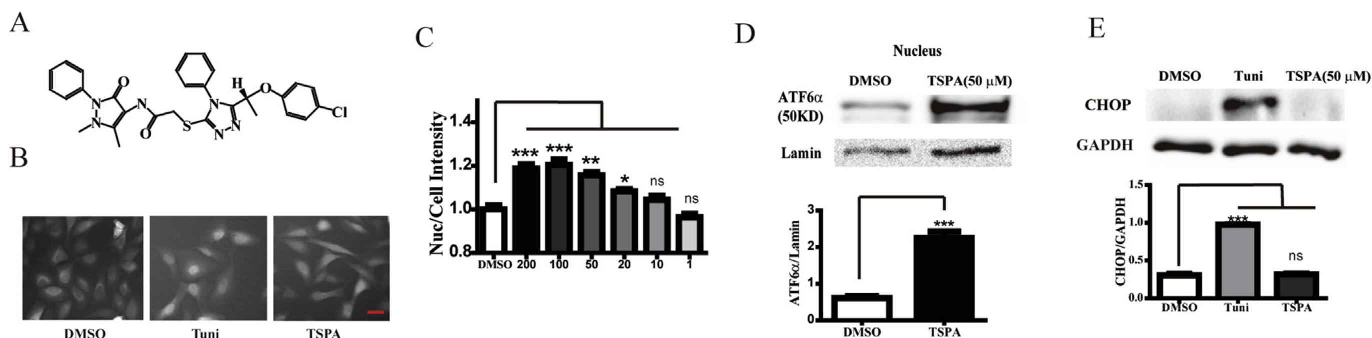

Fig. 1. TSPA promoted ATF6α nuclear translocation without increasing CHOP expression. (A) Chemical structure of TSPA. (B) Translocation of ATF6α in response to TSPA. Cells were treated with TSPA (50 μM) or TM (Tuni, 2 μg/mL) for 5 h and the nuclear accumulation of ATF6α was detected. Scale bar, 10 μm. (C) Quantitative analysis of the ratio of ATF6α in nuclear and cell in response to different concentrations of TSPA. (D) Mouse primary hepatocytes were treated with 50 μM TSPA for 24 h. Then the cells were harvested to determine the level of ATF6α in nucleus. (E) Mouse primary hepatocytes were treated with TM (5 μg/ml) and TSPA (50 μM) for 24 h. Then the cells were collected to determine the level of CHOP. All data were obtained from three independent experiments, and were presented as mean ± S.E.M, *P < 0.05, **P < 0.01, ***P < 0.001, One-way ANOVA or *t*-test.

Please cite this article in press as: T. Zhou, et al., TSPA as a novel ATF6α translocation inducer efficiently ameliorates insulin sensitivity restoration and glucose homeostasis in *db/db* mice, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.025

Download English Version:

https://daneshyari.com/en/article/8293013

Download Persian Version:

https://daneshyari.com/article/8293013

Daneshyari.com