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Activation of TGR5 promotes mitochondrial biogenesis in human aortic endothelial cells

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ABSTRACT

Impairment of mitochondrial biogenesis has been associated with vascular pathophysiology. The G-protein–coupled receptor (TGR5) is an important mediator of bile acid signaling and glucose metabolism. However, the effects of TGR5 on mitochondrial biogenesis in endothelial cells remain elusive. In this study, we found that activation of TGR5 using its specific agonist taurolithocholic acid (TLCA) promoted the expression of PGC-1α, a master regulator of mitochondrial biogenesis in human aortic endothelial cells (HAECs). Additionally, activation of TGR5 increased the expression of PGC-1α target genes, such as NRF1 and TFAM. Indeed, we found that TLCA treatment promoted mitochondrial biogenesis by increasing mitochondrial mass, mitochondrial-to-nuclear DNA (mtDNA/nDNA), COX-I expression, and cytochrome c oxidase activity in HAECs. Notably, our results displayed that activation of TGR5 resulted in a functional gain in mitochondria by increasing the rate of respiration and ATP production. Mechanistically, we found that TLCA treatment activated the transcriptional factor CREB by inducing the phosphorylation of CREB at Ser133. Using the PKA/CREB inhibitor H89 abolished the effects of TLCA on PGC-1α, NRF1 and TFAM expression as well as the increase in mtDNA/nDNA and ATP production. These findings suggest that activation of TGR5 promoted mitochondrial biogenesis in endothelial cells, which is mediated by the CREB/PGC-1α signaling pathway.

1. Introduction

Mitochondria are essential organelles found in most eukaryotic organisms. Mitochondria possess numerous physiological functions and act as the main source of adenosine triphosphate (ATP) within the cell environment [1]. Mitochondrial biogenesis plays a critical role in maintaining normal mitochondrial homeostasis. Endothelial mitochondria have contributed a lot to vascular pathophysiology [2]. Dysregulation of mitochondrial biogenesis has been found in various vascular diseases, including atherosclerosis, heart failure, and cardiac ischemia/reperfusion injury [3]. Peroxisome proliferator-activated receptor γ-coactivator-α (PGC-1α) has been considered as the master regulator of mitochondrial biogenesis [4]. In endothelial cells, PGC-1α regulates the expression of nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM). NRF1 is responsible for governing nuclear gene to encode mitochondrial proteins. TFAM plays a key role in initiating transcription and replication of mitochondrial DNA (mtDNA) [5]. Lower levels of PGC-1α are associated with the pathological development of atherosclerosis [6]. Stimulation of mitochondrial biogenesis by promoting the PGC-1α/NRF1/TFAM pathway has been considered as an important strategy for the treatment of endothelial dysfunction in vascular diseases.

The transmembrane G-protein coupled bile acid receptor (TGR5) is an important member of the G-protein-coupled receptor (GPCR) family. TGR5 is expressed in diverse tissues and organelles and mediates the intracellular signaling pathways of bile acids [7]. TGR5 has been reported to possess a variety of biological functions. For example, activation of TGR5 was found to promote energy expenditure in brown adipose tissue [8]. Also, TGR5 contributes a great deal to glucose homeostasis, potentially by increasing the secretion of GLP-1 [9]. TGR5 exerts important protective actions against gastric and liver inflammation by suppressing the NF-κB signaling pathway [10]. Interestingly, activation of TGR5 increases intracellular levels of the second messenger cyclic adenosine

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monophosphate (cAMP) and activates the cAMP response element-binding protein (CREB) transcription factor [11]. CREB plays a critical role in mediating the transcription and expression of PGC-1α [12]. However, the effects of TGR5 in mitochondrial function in endothelial cells remain unclear. The stimulatory effects of TGR5 on CREB activation lead us to speculate that activation of TGR5 might promote mitochondrial biogenesis through activation of the PGC-1α pathway in endothelial cells. We aim to verify this hypothesis in this study.

2. Materials and methods

2.1. Cell culture and treatment

Human aortic endothelial cells (HAECs), obtained from Lonza, USA, were maintained in EBM-2 media supplemented with growth factors. Cells were treated with TLCA (10, 30, and 100 μM) to determine the expressions of PGC-1α, NRF-1, and TFAM. Cells were treated with 30 μM TLCA with or without 10 μM PKA inhibitor H89 (Sigma-Aldrich, USA) to evaluate mitochondrial biogenesis.

2.2. Real-time PCR analysis and the ratio of mtDNA to nDNA

Total intracellular RNA was isolated from HAECs using the Trizol reagent (Life Technologies, USA). Complementary DNA (cDNA) was produced with an iScript-RT kit (Bio-Rad, USA) and 2 μg RNA in a reverse transcription PCR system. Expressions of target genes were determined by real-time PCR analysis on the step one ABI 7500 real-time PCR system. Primers used in this study are listed in Table 1. The ratio of mtDNA to nDNA (mtDNA/nDNA) was indexed by mtDNA to nDNA 18S [13].

2.3. Western blot analysis

HAECs were lysed to extract proteins. Protein concentration was measured by the BCA method (Thermo Fisher Scientific, USA). Protein samples were separated by 12% SDS-PAGE. Blots were then transferred to PVDF membranes, followed by blocking with 5% non-fat milk at RT for 2 h. Then membranes were sequentially incubated with PGC-1α, NRF-1, TFAM, COX-1, p-CREB, total CREB, and β-actin primary antibodies at 4 °C overnight and HRP-conjugated secondary antibodies (1:5000) for 1 h at RT. Blots were developed by enhanced chemiluminescence (Thermo Fisher Scientific, USA).

2.4. Mitochondria mass determination

Mitochondria mass in HAECs was determined by staining with the mitochondria-specific dye Mitotracker red. Briefly, HAECs (5×10^5) were seeded onto cover-slips in 6-well plates and incubated for 12 h. Then cells were treated with 30 μM TLCA for 48 h. After washing 3 times with HBST, HAECs were loaded with 20 nM Mitotracker red (Life Technologies, USA) for 15 min. Nuclei of HAECs were stained with DAPI. Fluorescence signals were visualized and recorded at 100 × oil immersion using a Zeiss fluorescence microscope. One hundred random individual cells were selected to calculate average integrated optical density (IOD) of fluorescence with Image-Pro Plus software (Version 5.0) to index mitochondrial mass.

2.5. Determination of cytochrome c oxidase activity

Cytochrome c oxidase activity in HAECs was measured by a colorimetric assay kit (Sigma-Aldrich, USA) in 96-well plates. Briefly, 10 μl cell lysate was added to assay buffer. A kinetic program for 30–45 min at 30 s intervals was run to measure cytochrome c oxidase activity.

2.6. Measurement of intracellular ATP

Intracellular levels of ATP were determined by a commercial ATP detection kit (Life Technologies, USA). Briefly, HAECs were lysed, followed by centrifugation at 12,000 × g for 15 min at 4 °C. Supernatant was collected to be mixed with an equal volume (50 μl) of the luciferin/luciferase reagent. Chemiluminescence signals were evaluated with a microplate luminometer.

2.7. Measurement of mitochondrial respiration rate

O_2 consumption of HAECs was evaluated using a commercial respirometer equipped with a Peltier thermostat and electromagnetic stirrer. After the indicated treatment, 5×10^6 cells were collected and put in a glass chamber equilibrated in ambient room air with continuous stirring (800 r.p.m.) for 10 min. The oxygen consumption was detected at 2 S intervals and the recording was stopped after stabilization of the O_2 consumption.

2.8. Statistical analysis

Experimental data are presented as means ± S.E.M. The statistical significance of differences was determined by one-way analysis of variance (ANOVA). A P value less than 0.05 was considered statistically significant.

3. Results

3.1. Activation of TGR5 increased the expression of PGC-1α and its target genes NRF1 and TFAM

PGC-1α is a master modulator of mitochondrial biogenesis. TLCA is a well-known agonist of TGR5. Our findings showed that TLCA treatment significantly increased the expression of PGC-1α at both the mRNA level (Fig. 1A) and the protein level (Fig. 1B) in a dose-dependent manner (10, 30, and 100 μM) in HAECs. NRF1 and TFAM are two important target genes of PGC-1α and the executors of mitochondrial biogenesis. Indeed, real-time PCR analysis revealed that the gene expressions of NRF1 and TFAM were markedly increased in response to treatment with 30 μM TLCA (Fig. 1C). Accordingly, western blot results displayed that 30 μM TLCA treatment significantly increased the protein expressions of

Table 1
Real-time PCR primers.

Gene name	Forward	Reverse
PGC-1α	5′-CAATGAATGCAGCGGTCTTA-3′	5′-ACGTCTTTGTGGCTTTTGCT-3′
NRF-1	5′-CTAGTG TGGGACAGCAA-3′	5′-AATTCCGTCGATGGTGAGA-3′
TFAM	5′-GGCACAGGAAACCAGTTAGG-3′	5′-CAGAACACCGTGGCTTCTAC-3′
GAPDH	5′-CCACGCTCAGACACCAT-3′	5′-CCAGGCGCCCAATACG-3′
mtDNA	5′-CAAACCTACGCCAAAATCCA-3′	5′-GAAATGAATGAGCCTACAGA-3′
nDNA (18S)	5′-ACGGACCAGAGCGAAAGCA-3′	5′-GACATCTAAGGGCATCACAGAC-3′

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