Biochemical and Biophysical Research Communications xxx (2018) 1–8

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Optineurin E50K triggers BDNF deficiency-mediated mitochondrial dysfunction in retinal photoreceptor cell line

Myoung Sup Shim [a, 1], Keun-Young Kim [b], Mark Noh [a], Ji Yoon Ko [a], Sangphil Ahn [a], Michelle A. An [a], Takeshi Iwata [c], Guy A. Perkins [b], Robert N. Weinreb [a], Won-Kyu Ju [a, *]

[a] Hamilton Glaucoma Center, Shiley Eye Institute and Department of Ophthalmology, University of California San Diego, La Jolla, CA, 92039, USA
[b] National Center for Microscopy and Imaging Research and Department of Neuroscience, University of California San Diego, La Jolla, CA, 92093, USA
[c] Division of Molecular and Cellular Biology, National Institute of Sensory Organs, National Hospital Organization Tokyo Medical Center, Tokyo, Japan

ARTICLE INFO

Article history:
Received 26 July 2018
Accepted 3 August 2018
Available online xxx

Keywords:
Glaucoma
Optineurin
E50K mutation
BDNF
Mitochondrial dysfunction
RGC-5 cell

ABSTRACT

Optineurin (OPTN) mutations are linked to glaucoma pathology and E50K mutation shows massive cell death in photoreceptor cells and retinal ganglion cells. However, little is known about E50K-mediated mitochondrial dysfunction in photoreceptor cell degeneration. We here show that overexpression of E50K expression triggered BDNF deficiency, leading to Bax activation in RGC-5 cells. BDNF deficiency induced mitochondrial dysfunction by decreasing mitochondrial maximal respiration and reducing intracellular ATP level in RGC-5 cells. However, BDNF deficiency did not alter mitochondrial dynamics. Also, BDNF deficiency resulted in LC3-mediated mitophagosome formation in RGC-5 cells. These results strongly suggest that E50K-mediated BDNF deficiency plays a critical role in compromised mitochondrial function in glaucomatous photoreceptor cell degeneration.

1. Introduction

Primary open angle glaucoma (POAG) is characterized by a slow and progressive degeneration of retinal ganglion cells (RGCs) and optic nerve damage that lead to vision loss [1]. Although controversial [2,3], there are several studies that have demonstrated that loss of photoreceptors is associated with patients with POAG and experimental glaucoma [4–6]. Nevertheless, the pathophysiological mechanisms of glaucomatous photoreceptor cell degeneration are currently not well characterized.

Among the various mutations of *optineurin (OPTN)*, the E50K is the most prevalent mutant form that is associated with POAG [7]. OPTN is a highly expressed protein in the retina and has ubiquitous effects. In particular, it has been shown to be involved in the maintenance of Golgi organization [8], regulation of nuclear factor kappa B (NF-κB) signaling [9] and induction of autophagy and/or mitophagy [10,11]. Recent studies, including those from our laboratory, have demonstrated that E50K mutation triggers not only age-related RGC loss but also photoreceptor cell degeneration, resulting in functional visual impairment in transgenic mice [12–15].

There is accumulating evidence demonstrates that BDNF protects not only RGCs but also photoreceptor cells against glaucomatous damage [16–18] and rescues retinal function in a mouse model of glaucoma [19]. Our recent study has demonstrated that both retina of E50K transgenic ($E50K^{-tg}$) mice and RGCs overexpressing E50K *in vitro* induced BDNF deficiency [14]. Of note, BDNF regulates mitochondrial function by changing respiratory efficiency [20]. However, the mitochondrial pathogenic mechanism underlying the E50K mutation-mediated BDNF alteration in glaucomatous photoreceptor cell degeneration remains unclear.

We here report that E50K mutation triggers BDNF deficiency-mediated mitochondrial bioenergetic dysfunction and mitophagosome formation in a photoreceptor cell line, RGC-5 cells.

2. Materials and methods

2.1. Cell culture

RGC-5 cell culture was performed as described previously [21]. Briefly, the cells were cultured in Dulbecco's modified Eagle's

* Corresponding author. Hamilton Glaucoma Center, Department of Ophthalmology, University of California San Diego, 9415 Campus Point Drive, La Jolla, CA, 92037, USA.
E-mail address: wju@ucsd.edu (W.-K. Ju).
[1] Current address is Department of Ophthalmology, Duke University, Durham, North Carolina, USA.

https://doi.org/10.1016/j.bbrc.2018.08.025
0006-291X/

medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin (Invitrogen, US) at 5% CO_2 and 37 °C.

2.2. Transfection of RGC-5 cells

Transfection of RGC-5 cells was performed as described previously [22] with some modifications. Briefly, 100 μl of Amaxa™ Basic Glial cells Nucleofector™ Solution (Lonza, US) was mixed with 8×10^6 cells and 2–4 μg of each plasmid or siRNAs, and then the cells were electroporated using an Amaxa™ Nucleofector II Device (Lonza).

2.3. Cell viability assay and cellular ATP measurement

RGC-5 cells (0.5×10^4 per well) were plated on a 96-well plate. Cell viability was measured using 3-[4, 5-dimethylthiazol-2yl]-2, 5-diphenyl tetrazolium bromide (MTT) and a microplate reader (Spectra MAX; Molecular Devices Corp., US) [23]. The cellular ATP level was measured using a luciferase-based assay kit (Promega Corp., US) and a microplate luminometer (GMI Inc., US) [23].

2.4. Measurement of mitochondrial respiration

RGC-5 cells (2×10^4 per well) following 48 h transfection were seeded into Seahorse XF24-well plates approximately 6 h before the measurement. Oxygen consumption rate was measured using an XF24 analyzer (Agilent, US). After measuring the basal respiration, oligomycin (2 μg/ml), an inhibitor of ATP synthesis and carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP; 0.3 μM), the uncoupler, were sequentially added to measure maximal respiration.

2.5. Quantitative real-time RT-PCR

cDNAs were amplified using MX3000P (Stratagene, US) real-time PCR system with iQ™ SYBR Green super-mix (Bio-Rad, US) and BDNF (Forward; 5′-gcggcagataaaaagactgc-3′ and Reverse; 5′-cttatgaatcgccagccaat-3′) primers for 40 cycles [initial incubation at 50 °C for 2 min and then at 95 °C for 10 min, and 40 cycles (95 °C for 15 s, 55 °C for 1 min and 72 °C for 1 min)]. GAPDH mRNA, an internal control, was amplified along with the target genes, and the *Ct* value of GAPDH was used for normalization [14].

2.6. Western blot analysis

Western blot analysis was performed as described previously [23]. Briefly, RGC-5 cells were harvested and lysed for 30 min on ice with a modified RIPA lysis buffer (150 mM NaCl, 1 mM EDTA, 1% NP-40, 0.1% SDS, 1 mM DTT, 0.5% sodium deoxycholate and 50 mM Tris-Cl, pH 7.6), containing the complete protease inhibitors. Primary antibodies included Bax (6A7; 1:1000; Santa Cruz Biotechnology, US), BDNF (1:1000; Santa Cruz Biotechnology), phospho-cyclic adenosine monophosphate response element-binding protein (CREB, Serine 133) (1:1000; Life Technologies, Grand Island, US), cyclophilin D (CypD) antibody (1:1000; Life Technologies), dynamin-related protein 1 (DRP1) (1:5,000, BD Transduction Laboratories, US), phospho-DRP1 (Serine 616) (1:1,000, Cell Signaling, US), microtubule-associated protein 1A/1B-light chain 3 (LC3) (1:3000; MBL International, US), OPTN (1:1000; Santa Cruz Biotechnology), total oxidative phosphorylation (OXPHOS) complex (Cx) (containing a mixture of antibodies to CxI-IV and ATP synthase, 1:4000; Life Technologies), optic atrophy type 1 (OPA1) (1:5000; BD Transduction Laboratories), mitofusin (Mfn)1 and 2 (1:3000; Abcam), huntingtin (HTT) (1:2,000, Millipore) and actin (1:10,000; Millipore, US). The images were captured and quantified by using ImageQuant™ LAS 4000 system and Image Quant TL 8.1 Software Package (GE Healthcare Bio-Science, US) and the band densities were normalized to the band densities for actin.

2.7. Immunocytochemistry

Cells were fixed with 4% paraformaldehyde (Sigma, US) as previously described [24]. Briefly, cells were incubated with LC3 (1:500; MBL International) for 16 h at 4 °C and then images were acquired with confocal microscopy (Olympus FluoView1000; Olympus, Japan).

2.8. Transmission electron microscopy

Cells were fixed with 2% paraformaldehyde, 2.5% glutaraldehyde (Ted Pella, US) in 0.15 M sodium cacodylate (pH 7.4) and were prepared as previously described [23,25]. Ultrathin (70 nm) sections were evaluated by a FEI spirit transmission EM operated at 120 kV equipped with 2048 × 2048 pixel CCD camera.

2.9. Electron microscope tomography

For quantitative analysis, the number of mitochondria was normalized to the total area occupied by axons in each image, which was measured using ImageJ (NIH; http://rsb.info.nih.gov/ij/) [23,25]. For each reconstruction, a double-tilt series of images at 1-degree tilt increments was collected with a FEI titan intermediate-voltage electron microscope operated at 300 kV and equipped with a 4096 × 4096 pixel CCD camera. The IMOD package was used for rough alignment with the fine alignment and reconstruction performed using the TxBR package.

2.10. Statistical analysis

Data were presented as the mean ± SD. Comparison of two conditions was evaluated using the unpaired, two-tailed Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Overexpression of OPTN E50K triggers BDNF deficiency in RGC-5 cells

We found that overexpression of E50K significantly decreased *BDNF* gene expression in RGCs by 44.71 ± 3.29% and RGC-5 cells by 12.66 ± 4.59% (Fig. 1A). Consistently, overexpression of E50K significantly decreased BDNF by 0.48 ± 0.03-fold in RGC-5 cells compared with control (Fig. 1B). Since endogenous BDNF can be regulated by CREB [26] and wild-type HTT [27], we also found that overexpression of E50K mutation significantly decreased HTT protein expression by 0.82 ± 0.02-fold and phosphorylation of CREB at serine 133 (p-CREB S133) by 0.81 ± 0.03-fold in RGC-5 cells (Fig. 1B).

3.2. BDNF deficiency activates Bax but does not increase CypD expression in RGC-5 cells

We found that BDNF deficiency significantly increased active Bax protein expression by 1.80 ± 0.25-fold in RGC-5 cells but did not affect the expression levels of total Bax and p-CREB S133 (Fig. 1C), suggesting that p-CREB S133 is an upstream regulator of BDNF. In addition, BDNF deficiency did not alter the expression level of CypD protein, which has a critical role in mitochondrial permeability transition pore opening-mediated apoptosis [28], in

Please cite this article in press as: M.S. Shim, et al., Optineurin E50K triggers BDNF deficiency-mediated mitochondrial dysfunction in retinal photoreceptor cell line, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.08.025