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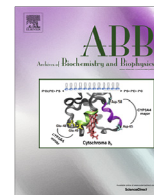

High-glucose-induced CARM1 expression regulates apoptosis of human retinal pigment epithelial cells via histone 3 arginine 17 dimethylation: Role in diabetic retinopathy

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ABSTRACT

Hyperglycemia-induced apoptosis of retinal pigment epithelial (RPE) cells is considered to be involved in the progression of diabetic retinopathy. Histone arginine methylation catalyzed by protein arginine methyltransferases (PRMTs) has emerged as an important histone modification involved in gene regulation. However, the role of PRMTs in diabetic retinopathy has not been elucidated. Here, we found that expression of coactivator-associated arginine methyltransferase 1 (CARM1; also known as PRMT4) was increased in the high-glucose treated human RPE cell line ARPE-19 and in the RPE layer of streptozotocin-treated rats. In addition, high-glucose induced apoptosis in ARPE-19 cells. To determine the function of CARM1 on RPE cell apoptosis, we performed gain- and loss-of-function studies. CARM1 overexpression increased apoptosis of RPE cells. In contrast, silencing of CARM1 expression by siRNA and pharmacological inhibition of CARM1 activity abolished high-glucose-induced RPE cell apoptosis. Furthermore, we found that inhibition of histone 3 arginine 17 (H3R17) asymmetric dimethylation attenuates both CARM1- and high-glucose-induced apoptosis in RPE cells. Together, these results show that high-glucose-induced CARM1 expression increases RPE cell apoptosis via H3R17 asymmetric dimethylation. Strategies to reduce CARM1 expression or enzymatic activity could be used to prevent apoptosis of RPE cells in the progression of diabetic retinopathy.

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Introduction

In diabetic retinopathy, visual impairment is caused by hyperglycemia-induced breakdown of the blood-retinal barrier (BRB)[2] [1,2]. Retinal pigment epithelial (RPE) cells are crucial components of the outer BRB, and RPE cellular dysfunction, including apoptosis, is considered to be related to the progression of diabetic retinopathy [3,4]. However, the molecular mechanism behind hyperglycemia-induced RPE cell apoptosis is largely unknown.

Protein arginine methyltransferases (PRMTs) catalyze the methylation of arginine residues in proteins. PRMTs are subdivided three groups following the manners of methylation. Briefly, all PRMTs convert arginine to monomethylarginine (MMA) intermediate, then type I PRMTs (PRMT1, 2, 3, 4, 6, 8) further convert MMA to asymmetric dimethylarginine (ADMA) while type II PRMTs (PRMT5, 7) convert to symmetric dimethylarginine (SDMA). [5].

PRMT4, also known as coactivator-associated arginine methyltransferase 1 (CARM1), has various substrates, including histone

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[2] *Abbreviations used:* BRB, blood-retinal barrier; RPE, retinal pigment epithelial; PRMTs, protein arginine methyltransferases; MMA, monomethylarginine; ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine; CARM1, coactivator-associated arginine methyltransferase 1; NF-κB, nuclear factor kappa B; DMEM, Dulbecco's Modified Eagle's Medium; FBS, fetal bovine serum; PBS, phosphate buffered saline; BSA, bovine serum albumin; MEF, mouse embryonic fibroblast.

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3 as well as non-histone proteins, and regulates various cellular processes, such as transcriptional regulation and RNA splicing and metabolism [6]. Among the various functions of CARM1, its regulation of the transcriptional activity of p53 has been well described [7]. A recent study also depicted the precise mechanisms by which CARM1 increases the transcription of p53 responsive genes, such as p21 and gadd45, by methylation of the KIX region of p300 [8]. Furthermore, in a previous study, we showed that plant-derived 2,3,7,8-tetrahydroxy(1)-benzopyrano(5,4,3-cde)(1)-benzopyran-5,10-dione (TBBD, ellagic acid), which is a specific inhibitor of CARM1-induced histone 3 arginine 17 (H3R17) dimethylation, represses the binding of H3R17 to the p53-responsive site within the p21 promoter [9]. As a transcriptional cofactor, CARM1 also increased the transcriptional activity of nuclear factor kappa B (NF-κB) [10,11]. Even though CARM1 can regulate p53 and NF-κB, which are closely associated with RPE cell apoptosis [12,13], its regulation of apoptosis in RPE cells has not been elucidated.

In this study, we are the first to evaluate the involvement of type I PRMTs in the development of diabetic retinopathy using human RPE cells and observed that high-glucose treatment increases CARM1 expression, which is associated with apoptosis of human RPE cells. We also found that high-glucose-induced RPE cell apoptosis is mediated by CARM1-dependent H3R17 dimethylation.

Materials and methods

Materials

Dulbecco's Modified Eagle's Medium (DMEM), Ham's nutrient mixture F-12 and fetal bovine serum (FBS) were purchased from Life Technologies (Gibco BRL, Grand Island, NY, USA). D-glucose and streptozotocin were obtained from Sigma–Aldrich (St. Louis, MO, USA). CARM1 inhibitor was purchased from Millipore (Billerica, MA, USA). CARM1 antibody (#4438; for immunofluorescence and Western blotting), PARP1 antibody (#9532) and Caspase-3 antibody (#9662) were purchased from Cell Signaling Technology (Beverly, MA, USA). β-Actin antibody (sc-1616) and α-tubulin antibody (sc-8035) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). PRMT1 antibody (ab3768), Histone 3 antibody (ab1791) and H3R17me2a antibody (ab8284) were purchased from Abcam (Cambridge, UK). H3R26me2a antibody (#07-215) was from Millipore (Billerica, MA, USA). CARM1 antibody (A300-421A; for immunohistochemistry) was from Bethyl Laboratories (TX, USA). PRMT3 antibody [14] was kindly provided by Mark T. Bedford (University of Texas, M.D. Anderson Cancer Center, Smithville, TX). All reagents were of the highest purity commercially available.

Cell culture

The human RPE cell line ARPE-19 was obtained from the American Type Culture Collection (ATCC, Rockville, MD). ARPE-19 cells were grown in DMEM/Ham's F-12 (1:1) supplemented with 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2 in air. Stock cultures of ARPE-19 cells were subcultured once a week (split ratio 1:6). Cells were grown to confluence in 60 mm dishes in DMEM/Ham's F-12 (DMEM, Gibco; F-12 Nutrient Mixture, Gibco; obtained without glucose and then supplemented by adding glucose to the appropriate concentrations) with 15 mM HEPES buffer, 10% FBS, 5.5 mM glucose, 0.35% additional sodium bicarbonate, 2.5 mM L-glutamine, and 1% penicillin/streptomycin at 37 °C. The media was changed every other day. Passaged cells were plated to yield near-confluent cultures at the end of the experiments.

Western blotting

The medium was removed and ARPE-19 cells were washed twice with ice-cold phosphate buffered saline (PBS), scraped, and then harvested by microcentrifugation (13,000 rpm for 10 min) and removed supernatant. Pellet was resuspended in M-PER Mammalian Protein Extraction Reagent (Thermo, IL, USA) containing protease inhibitor cocktail (Sigma, Missouri, USA) and phosphatase inhibitor cocktail I + II. (Sigma, Missouri, USA) The resuspended cells were lysed mechanically on ice by vortex. The protein level was quantified using the Bradford procedure. The whole cell (30 μg of protein) were separated by SDS–polyacrylamide gel electrophoresis and transferred to an enhanced nitrocellulose membrane. The blots were then washed with TBST (10 mM Tris–HCl, pH 7.6, 150 mM NaCl, 0.05% Tween-20), blocked with 5% skim milk powder in TBST for 1 h and incubated for 15 h at 4 °C with the primary antibody at the dilutions recommended by the supplier. The membrane was then washed with TBST, and the secondary antibodies conjugated to horseradish peroxidase were incubated for 1 h at room temperature. The bands were visualized with Luminescent image analyzer (ImageQuant LAS 4000, GE Healthcare, UK) using Amersham ECL™ Western Blotting Detection Reagents (GE Healthcare, UK).

mRNA extraction and quantitative RT-PCR

Total RNA was extracted from the cells using TRIzol, which is a monophasic solution of phenol and guanidine isothiocyanate purchased from (Invitrogen, Carlsbad, CA, USA). Reverse transcription was carried out with 1 μg RNA using an RT Premix reverse transcription system kit (AccuPower, Seoul, Korea) with oligo-dT18 primers. 0.5 μl of the RT products were amplified with 100 nM specific primers using a Power SYBR Green (Applied Biosystems, Warrington, UK). The primers used were 5′-CGGCTCCAAGTCCAGT AACC-3′ (sense), 5′-CGCTGCTGAGGTTGTAGGTG-3′ (antisense) for human CARM1 and 5′-AGGCCCAGAGCAAGAGAG-3′ (sense), 5′-TCA ACATGATCTGGGTCATC-3′ (antisense) for human β-actin. β-Actin was used as a control to confirm the quantity of the mRNA.

Immunofluorescence assay and confocal microscope

Cells were washed twice in PBS and fixed for 10 min with 4% paraformaldehyde in PBS. After three washes in PBS, fixed cells were permeabilized with 0.2% Triton X-100. 1% bovine serum albumin (BSA) solution was used for blocking. The cells were incubated with CARM1 antibody (dilution ratio-1:100) for 15 h at 4 °C. After three washes in PBS, the cells were incubated with anti-rabbit FITC secondary antibody. Then the cells were mounted on slides and the nuclei were visualized with 4′,6-diamidino-2-phenylindole (DAPI) present in the ProLong Gold Antifade Mounting Medium (Invitrogen, Carlsbad, CA). Immunofluorescence imaging was performed on a Leica TCS SP5 AOBS laser scanning confocal microscope (Leica Microsystems, Heidelberg, Germany) using a Leica 63× (N.A. 1.4) oil objective located at Korea Basic Science Institute Gwangju Center. Digital images were captured optical sections of 512 × 512 pixels, and were averaged four times to reduce noise. The images of cells were obtained separately with the following fluorescence excitation and emission settings: Excitation at 496 and 405 nm and emission between 500–535 and 449–461 nm for FITC-conjugated construct and DAPI, respectively. For all the experiments, exposure time was kept the same for all samples.

Animal experiments and retina isolation

Hyperglycemia was induced in overnight fasted, 10-week-old male SD rats ($n = 7$) by intraperitoneal injection of streptozotocin

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