High Glucose Induces Mitochondrial Dysfunction in Retinal Muller Cells: Implications for Diabetic Retinopathy

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Abstract

Objective: Muller cell loss is associated with the development of early stage diabetic retinopathy. However, the high glucose (HG) induces mitochondrial dysfunction and promotes apoptosis in retinal Muller cells. In this study, we examined whether HG promotes apoptosis in rat retinal Muller cells (rMC-1) by inducing mitochondrial changes.

Methods: rMC-1 were grown in normal (N, 5mM glucose) or HG (30mM) medium for 6 days. Cells were stained with MitoTracker Red, imaged with confocal microscopy, and analyzed for mitochondrial morphology change. Mitochondrial metabolic function was assessed by measuring oxygen consumption rate (OCR) using XF24 bioenergetic assay. TUNEL assay was performed and cyt c levels were assessed using Western blot analysis to examine apoptosis.

Results: Cells grown in HG medium exhibited significantly increased mitochondrial fragmentation compared to those grown in N medium. OCR was significantly reduced in rMC-1 grown in HG medium compared to those grown in N medium. The number of TUNEL positive cells was significantly increased in cells grown in HG medium compared to those grown in N medium with concomitant increase in cyt c levels.

Conclusions: Findings from this study indicate that HG promotes mitochondrial dysfunction in retinal Muller cells by inducing mitochondrial morphology changes with concomitant increase in cyt c and apoptosis. HG-induced mitochondrial morphology changes and subsequent mitochondrial dysfunction may play a role in retinal Muller cell loss associated with diabetic retinopathy.

Materials & Methods

Cell Culture: rMC-1 were grown on poly-D-lysine-coated, glass slide-bottomed dishes (MatTek, Ashland, MA) in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum (Sigma-Aldrich), antimycotics, and antibiotics. To determine the sustained effect of HG on mitochondrial morphology and membrane potential in rMC-1, cells were grown for 6 days in normal (5 mM) or HG (30 mM) medium. Before imaging, the cells were subjected to various stains and examined by confocal microscopy.

Confocal Microscopy: Cells were imaged live by confocal microscopy (LSM 710 Meta; Carl Zeiss Meditec, Oberkochen, Germany) with a 60x oil immersion objective. The cells were kept at 37°C in a 5% CO2 humidified microscope stage chamber. MitoTracker Red (Invitrogen-Molecular Probes, Eugene, OR) was used to stain cellular mitochondria and subjected to 543-nm helium-neon laser excitation, and emission was recorded through a band-pass 650-710-nm filter.

Mitochondrial Morphology Analysis: Quantitative analysis of mitochondrial morphology was conducted for calculation of form factor (FF) and aspect ratio (AR) values derived from lengths of major and minor axes. Images of mitochondria were analyzed using NIH-developed Image J software (National Institutes of Health, Bethesda, MD). An AR value of 1 indicates a perfect circle, and as mitochondria elongate and become more elliptical, AR increases. An FF value of 1 corresponds to an unbranched mitochondrion, and higher FF values indicate a longer, more branched mitochondrion.

Cellular Oxygen Consumption: The oxygen consumption rates of rMC-1 grown in normal or HG medium for 6 days was measured by a bioenergetic assay (XF24, Seahorse Bioscience, Billerica, MA). Briefly, rMC-1 were plated and grown on 24-well microplate for 6 days in normal or HG medium to assess cellular oxygen consumption rate. The microplate was then assayed (XF24 Extracellular Flux Analyzer; Seahorse Bioscience), to measure extracellular flux changes of oxygen and pH in the medium immediately surrounding the adherent cells.

Western Blot Analysis: To identify levels of mitochondrial and cytosolic cytochrome c, WB analysis was performed as previously described [7]. B-actin and VDAC-1 were used as cytoplasmic and mitochondrial protein loading control, respectively.

Terminal dUTP Nick-End Labeling (TUNEL): To determine apoptosis, a TUNEL assay was performed on BRPs grown in normal or HG medium (ApopTag In Situ Apoptosis Detection kit; Chemicon, Temecula, CA), as previously described [7]. Briefly, cells grown on coverslips were fixed with 4% PFA and incubated with TdT enzyme at 37°C for 1 hour, then incubated with antidigoxigenin peroxidase. Cells were then stained with DAPI, and images from 10 random fields were captured for analysis.

Results

Figure 1. Muller cells grown in HG condition show mitochondria that are fragmented and punctate as compared to the long, tubular networks of normal mitochondria, with concomitant reduction in FF and AR.

Figure 2. Muller cells grown in HG condition show decreased steady state and maximal oxygen consumption rates compared to cells grown in normal condition.

Figure 3. rMC-1 grown in HG medium showed increased levels of cytochrome c in the cytosol, a marker for apoptosis.

References


Summary

Results suggest that HG induces morphological and functional changes in the mitochondria. These changes are evidenced by mitochondrial fragmentation, decreased oxygen consumption, elevated levels of cytochrome c, and increased TUNEL positive cells under HG condition.

Conclusion

HG-induced mitochondrial morphology changes and subsequent mitochondrial dysfunction may play a key role in retinal Muller cell loss associated with DR.

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