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Deletion of the Basigin gene results in reduced mitochondria in the neural retina

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ABSTRACT

Basigin-null mice are characterized as blind from the time of eye opening, with degeneration of the retina beginning at 8 weeks of age, and progressing until the entire photoreceptor cell layer is destroyed. It is likely that a metabolic deficiency underlies the blindness and degeneration phenotypes, as it has been determined that Basigin-null mice do not express the transporter protein monocarboxylate transporter one on the membrane of photoreceptor cells and inner segments, nor Müller cells of the neural retina, as is observed in normal mice. The purpose of the present study was to assess the health of mitochondria in normal and Basigin-null mice, specifically to determine if mitochondria within the Basigin-null mouse neural retina are metabolically active. This was achieved via a measurement of cytochrome C concentration and the expression of autophagy-specific proteins via ELISA analyses. Additionally, Mitotracker dyes were used to assess the number and relative activity of mitochondria. It was determined that cytochrome C concentrations and expression of autophagy-specific proteins were not increased in Basigin-null animals, as compared to control animals. Also, while Basigin-null mice do have metabolically active mitochondria, the amount of mitochondria was greatly reduced, when compared to control animals. The results suggest that a reduction in mitochondria is a result, rather than the cause, of the metabolic deficiency observed in Basigin-null mice, and likely occurs because of reduced metabolic activity in the absence of MCT1 expression.

1. Introduction

Basigin gene products are intriguing members of the Immunoglobulin (Ig) superfamily that have been implicated in myriad cellular and physiological processes (reviewed in [1]). The Basigin gene is located on chromosome 10 in mice and produces two main products in the neural retina via differential splicing, originally named Basigin [2] and Basigin-2 [3]. The Basigin transcript is ~1.5 kb and the protein is 27 kDa, which becomes a 45 kDa integral membrane glycoprotein upon glycosylation [2]. Basigin-2 is a transcript of ~1.8 kb and the glycosylated protein is 55 kDa [3]. The two proteins differ in the number of extracellular Ig domains, with Basigin possessing two and Basigin-2 possessing three [3]. It should be noted that the Basigin nomenclature has recently been amended such that Basigin is now Basigin variant-2 and Basigin-2 is now Basigin variant-1 [4]. The shorter protein is expressed throughout the body, including Müller glial cells, the apical and basolateral surfaces of the retinal pigmented epithelium (RPE), and blood vessel endothelial cells within the mammalian eye, and whereas the longer protein is only expressed on photoreceptor neurons [3].

More than a decade ago, a strain of mice was generated in which the gene for Basigin was deleted by molecular means [5]. Basigin null animals have several abnormalities, including infertility in both males and females [5], lower sensitivity to irritating odors [5], reduced response to foot shock [6] and blindness [7,8].

Studies indicate that Basigin-null animals are visually impaired at the time of eye opening (2 weeks of age), despite normal retina architecture at that age [8]. At visual maturity (3 weeks of age), the photoreceptor outer segments appear less dense and shorter than those of control animals, and at 8 weeks, retinal degeneration is observed [9]. Degeneration of the photoreceptors continues such that by eight months of age, the entire photoreceptor layer is missing [7,9].

Philp et al. [10] demonstrated that Basigin gene products are necessary for the targeting of monocarboxylate transporters (MCTs) to the plasma membrane and are essential for normal retinal function. MCT1 is the primary lactate carrier found in Müller cells and the inner segments of photoreceptors, as well as on the apical membrane of the RPE [10]. When Basigin is absent, MCT1 expression is not observed at the plasma membrane, but rather

Abbreviations: RPE, retinal pigmented epithelium; MCT, monocarboxylate transporter; PBS, phosphate-buffered saline; ELISA, enzyme-linked immunosorbant assay.

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in intracellular vesicles [10]. It was hypothesized that a lactate metabolon exists in the retina in which Basigin gene products adhere Müller cells to photoreceptors so that MCT1 can efficiently transfer lactate from the Müller cells to the photoreceptors, which then use lactate as an energy source (reviewed in [1]). In the absence of MCT1, lactate from Müller cell metabolism is no longer available for energy metabolism in the photoreceptors, which could lead to the blindness phenotype [10].

The purpose of this present study was to determine whether mitochondrial metabolism or overall health is compromised in the Basigin-null mouse retina. The authors hypothesized that the mitochondria in Basigin-null mice photoreceptors are less active than those of littermate controls due to the lack of lactate presentation to those cells. This was assessed via a series of biochemical analyses.

2. Materials and methods

2.1. Generation of mouse retina cell extracts

Basigin-null mice and heterozygous littermate controls were euthanized according to an accepted protocol (UNF IACUC #10–009) and the eyes were immediately removed. Neural retinas were isolated from individual eye cups in Phosphate-buffered Saline (PBS; 50 mM potassium phosphate, 150 nM NaCl, pH 7.2) and the tissue was lysed in Cell Extraction Solution (10 mM Tris, pH 7.4, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% deoxycholate; Invitrogen/Life Technologies, Carlsbad, CA, USA) containing 1 mM phenylmethanesulfonyl fluoride (PMSF; Sigma–Aldrich, St. Louis, MO, USA) and protease inhibitor cocktail (Pierce/Thermo Scientific, Rockford, IL, USA). The tissue was incubated on ice for 30 min with vortex mixing every 10 min, followed by centrifugation at 13,000 rpm for 10 min at 4 °C. The protein concentrations of the cleared lysates were determined via a protein assay using Coomassie (Bradford) stain (Pierce/Thermo Scientific).

2.2. Cytochrome C assay

Cytochrome C standards were generated at 1:2 serial dilutions in Standard Diluent Buffer (Invitrogen/Life Technologies). The normal and Basigin-null mouse retina cell extracts were diluted 1:10 in Standard Diluent Buffer (100 µl total volume). The standards and retina cell extracts were subjected to a cytochrome C ELISA assay (Invitrogen/Life Technologies) such that the concentrations of cytochrome C for each retina cell extract could be determined via a standard curve.

2.3. Autophagy assay

For detection of autophagy, ELISA was performed using the mouse retina cell extracts from normal and Basigin-null animals. The extracts (diluted to 100 µg/mL in PBS) were transferred to individual wells of a 96-well plate and incubated overnight at 4 °C. The extracts were removed and antibodies specific for key components of autophagy induction, including RAB 24, APG1, APG16L, BECN1 and APG5L (Abgent Antibodies, San Diego, CA, USA), diluted 1:100 in PBS, were added to appropriate wells (100 µl total volume) and incubated for 30 min at 37 °C. The solutions were removed and the wells were washed three times with PBST (PBS containing 0.05% Tween 20). Alkaline phosphatase-conjugated goat-anti-rabbit secondary antibody (Pierce/Thermo Scientific) was diluted 1:1000 in PBS, delivered to appropriate wells (100 µl total volume), and incubated for 30 min at 37 °C. The solutions were removed and the wells were washed three times with PBST. Alkaline phosphatase substrate (PNPP; Pierce/Thermo Scientific) and was added to all the wells (100 µl total volume) and color development was allowed to proceed. All reactions were stopped at the same time by adding 50 µl of 2 N NaOH to each well. The absorbance within each well was measured at 405 nm using a Bio-Tek Powerwave XS plate reader (Biotek Instruments, Winooski, VT, USA) and correlated to the relative concentration of autophagy target protein present in the retina cell extracts. All runs were performed in duplicate.

2.4. Mitotracker assay

Basigin-null mice and heterozygous littermate controls were sacrificed according to an accepted protocol (UNF IACUC #10–009) and the eyes were immediately removed. Neural retinas were isolated from individual eye cups in PBS and then transferred to solutions containing both Mitotracker Red FM and Mitotracker Green FM (Molecular Probes/Invitrogen/Life Technologies), each diluted 1:1000 in PBS. The tissues were disrupted by passing through a micropipette tip and incubated for 2 h at 37 °C. The tissues were pelleted by centrifugation at 13,000 rpm for 10 min and resuspended in PBS. Fluorescence was measured using a Bio-Tek Fluorescence FLx800 plate reader at 550–650 nm for Mitotracker Red FM and 400 nm for Mitotracker Green FM. Relative values for total (Mitotracker Green FM) and active (Mitotracker Red FM) mitochondria within each sample were determined.

3. Results and discussion

It has been proposed that a lactate shuttle exists within the retina, in which Basigin gene products interact with MCT1 to deliver lactate from the Müller cells to the photoreceptor cells. Lactate is a monocarboxylate that serves as a source of pyruvate for metabolism in vertebrate nervous tissues, which is subsequently converted to acetyl CoA and fuels oxidative phosphorylation [11]. Basigin-null mice do not express MCT1 on the plasma membranes of Müller cells or photoreceptor cells [10]. It has therefore been proposed that in the absence of the lactate shuttle complex, as in the Basigin-null mouse retina, the photoreceptor cells are not sufficiently nourished by the Müller glial cells and they never function [1,10]. Since mitochondria are the organelles responsible for cellular respiration, the aim of this project was to assess mitochondrial health in the Basigin-null mouse retina.

Initially, mitochondrial health was assessed via a cytochrome C expression assay. Mitochondrial cytochrome C is a water soluble protein that functions as an electron carrier in the respiratory chain. The release of cytochrome C from the mitochondria, the organelle that regulates apoptosis, precedes caspase activation and is considered an early event in the apoptotic process. These characteristics make the detection of this protein an effective tool in determining the health of mitochondria in Basigin-null animals. Basigin-null and littermate control retinas were harvested at three weeks and three months of age and protein lysates were isolated. The assay uses an ELISA format to compare cytochrome C expression in samples relative to a standard curve of cytochrome C. It was determined that the concentration of cytochrome C was reduced in the Basigin-null mouse retinas compared to the concentrations observed in retinas of littermate controls (Fig. 1). These data suggest that apoptosis is not activated in the Basigin-null mouse retina, as compared to those of littermate controls. This is a surprising result, as it is assumed that the retinal degeneration observed in Basigin-null mice proceeds via apoptosis [9].

Autophagy, like apoptosis, is a natural and tightly regulated pathway, but involves the lysosomal degradation of whole cytoplasmic organelles and their components. Several neurodegenerative