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Methionine sulfoxide reductase B2 is highly expressed in the retina and protects retinal pigmented epithelium cells from oxidative damage

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ABSTRACT

Methionine sulfoxide reductase B2 (MSRB2) is a mitochondrial enzyme that converts methionine sulfoxide (R) enantiomer back to methionine. This enzyme is suspected of functioning to protect mitochondrial proteins from oxidative damage. In this study we report that the retina is one of the human tissues with highest levels of MSRB2 mRNA expression. Other tissues with high expression were heart, kidney and skeletal muscle. Overexpression of an MSRB2-GFP fusion protein increased the MSR enzymatic activity three-fold in stably transfected cultured RPE cells. This overexpression augmented the resistance of these cells to the toxicity induced by 7-ketocholesterol, tert-butyl hydroperoxide and all-trans retinoic acid. By contrast, knockdown of MSRB2 by a miRNA in stably transfected cells did not convey increased sensitivity to the oxidative stress. In the monkey retina MSRB2 localized to the ganglion cell layer (GLC), the outer plexiform layer (OPL) and the retinal pigment epithelium (RPE). MSRB2 expression is most pronounced in the OPL of the macula and foveal regions suggesting an association with the cone synaptic mitochondria. Our data suggests that MSRB2 plays an important function in protecting cones from multiple type of oxidative stress and may be critical in preserving central vision.
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1. Introduction

The methionine sulfoxide reductases (MSRs) are a family of enzymes capable of converting methionine sulfoxides back to methionine (Brot and Weissbach, 1983). This process plays a decisive role in recovering protein functionality and in protection against oxidative stress (Weissbach et al., 2002). Methionine sulfoxidation produces two diastereoisomers: Met(S)O and Met(R)O. MSRAs are responsible for reducing the S form (Brot et al., 1981) and MSRBs the R form (Grimaud et al., 2001) of the sulfoxides.

In mammals, MSRAs are coded by one gene regulated by two distinct promoters (Lee et al., 2006; Pascual et al., 2009). Their protecting role against oxidative stress has been well established in multiple cell lines (Moskovitz et al., 1998; Yermolaieva et al., 2004; Kantorow et al., 2004; Picot et al., 2005). MSRAs may also play a role in senescence. Age-related decreases in MSRA expression and activity has been shown in rat tissues (Petropoulos et al., 2001) and the overexpression of MSRA in the fruit fly increases lifespan (Ruan et al., 2002). MSRA knockout mice exhibit abnormal behavior and neurodegeneration (Moskovitz et al., 2001; Pal et al., 2007; Oien et al., 2008; Salmon et al., 2009). In addition, MSRAs have been implicated in the pathogenesis of aging diseases (Moskovitz, 2005) including Alzheimer's (Gabbita et al., 1999) and Parkinson's (Wassef et al., 2007 Liu et al., 2008). By contrast, the functions of the MSRBs in providing resistance to oxidative stress and in the aging process are not as well understood. The MSRBs are coded by three different genes: MSRB1, MSRB2 and MSRB3 (Kim and Gladyshev, 2004). MSRB2, also known as CBS-1 (Jung et al., 2002), is a mitochondrial protein of 182 amino acids long (Huang et al., 1999). Overexpression of MSRB2 has been reported to protect leukemia cells from H_2O_2 (Cabreiro et al., 2008) and from zinc induced oxidative stress (Cabreiro et al., 2009). Down regulation of MSRB2 with siRNAs has been reported to increase oxidative stress-induced cell death in lens cells (Marchetti et al., 2005).

Abbreviations: RPE, retinal pigment epithelium; CH, choroid or choriocapillaris; ROS, rod outer segments; RIS, rod inner segments; ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; GCL, ganglion cell layer; MSRB2, methionine sulfoxide reductase B2; MNR, monkey neural retina; MPEC, monkey RPE-choroid; TBHP, tertiary-butyl hydroperoxide; ATRA, all-trans retinoic acid; 7KCh, 7-ketocholesterol; L-MetO-DABS, dabsyl L-methionine sulfoxide; Met-DABS, dabsyl methionine; Trp-DABS, dabsyl tryptophan.

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In the retina more than 60% of the total MSR activity is due to the MSRBs (Lee et al., 2006) but little is known about their expression and function. In this study we determined the localization of MSRB2 in the monkey retina and examined its protective function in cultured RPE cells. Our data suggests that MSRB2 may play a decisive role in protecting the retina, (especially macula and fovea), from oxidative stress.

2. Materials and methods

2.1. Materials

7-Ketocholesterol (7KCh) was purchased from Steraloids, Inc. (Newport, RI). All-trans retinoic acid (ATRA), tert-butyl hydroperoxide (TBHP), Dabsyl Chloride, Methionine, Methionine sulfoxide, Tryptophan, hydroxypropyl-β-cyclodextrin (HPBCD) were purchased from Sigma–Aldrich Co. (St Louis, MO). DMEM and DMEM/F12 media were purchased from Atlanta Biologicals (Atlanta, GA). DNAse I, TRIzol®, and SuperScript III reverse transcriptase were purchased from Invitrogen Corp. (Carlsbad, CA). Monkeys (Macacca mulatta) eyes were provided by the Pathology Department of the Division of Veterinary Resource after completion of approved protocols. All animal studies were performed in accordance to the guidelines for animal research at NIH and in adherence to the ARVO statement for the use of animals in ophthalmic and vision research.

2.2. Immunohistochemistry in monkey retina

A fresh monkey eye from a 7 year old female Rhesus was collected immediately after euthanasia and immersed overnight in ice-cold, freshly prepared 4% formaldehyde (Polysciences, Inc., Warrington, PA) in 1× PBS. After fixation, vibrotome sections of 100 μm were prepared as previously described (Lee et al., 2006). Sections were incubated overnight at 4 °C with the mouse anti-MSRB2 monoclonal antibody (1:100, Abnova Corp, Taipei, Taiwan). The sections were developed using a goat anti-mouse Alexa Fluor-633 secondary antibody (1:300, Invitrogen Corp, Carlsbad, CA). Nuclei were stained with DAPI and capillary endothelium cells were stained with Isolectin GS-IB4 Alexa Fluor-488 (Invitrogen Corp). The sections were imaged by confocal microscopy (model SP2, Leica mycrosystems, Exton, PA).

2.3. Subcellular localization of MSRB2 in D407 cells

D407 cells were co-transfected (2 μg each) with MSRB2-GFP fusion construct and the pDsRed2-Mito plasmid (Clontech, PaloAlto, CA) as a mitochondrial marker. The cells were plated in two-well Lab-Tek chamber slides (Nalge Nunc International, Naperville, IL). The nuclei were stained with DAPI. The cells were imaged by confocal microscopy.

2.4. RNA isolation and real time RT-PCR

Total RNA was isolated from monkey retinal tissues and D407 human RPE cell line using TRIzol® (Invitrogen). Total RNAs from neural retina and other human tissues were purchased from BD Biosciences (San Jose, CA). RNA (2 μg) was treated with DNase I and cDNA was synthesized with SuperScript III reverse transcriptase. Real-time PCR was performed using SYBR Green in an ABI 7500 instrument (Applied Biosystems, Foster City, CA). Values were calculated according to a standard curve. Primers for MSRB2 and the ribosomal 18S rRNA are represented in Table 1.

Table 1
Primers used for the preparations of expression constructs and qRT-PCR.

Primers for Real time		
MSRB2	F	CACAAGAGAAAAGGGAACGGAACC
	R	GTCGCAGCACACGCAATGATAC
18S	F	ATGCTCTTAGCTGAGTGTCCCG
	R	ATTCCTAGCTGCGGTATCCAGG
Primers to clone MSRB2 ORF		
	F	ATGGCGCGGCTCCTCTGGTT
GFP construct	R	CGTGTTTCCTTGGTTTGAACTTCAAAG
With stop codon	R	TCAGTGTTTCCTTGGTTTGAACTTCAA
Primers to clone MSRB2-GFP into PiggyBac vector		
	F	AGAATTCGCGATGTACGGGCCAGATATA
	R	TCGTACGGGTTTAATTCATTATTTGTAGAGC
miRNA primers cloned into Piggybac vector		
	F	TGCTGATACATTCCTGCTTCCTTGTTGTTTTGGCC ACTGACTGACAACAAGGACAGGAATGTAT
	R	CCTGATACATTCCTGTCCTTGTTGTCAGTCAGTGG CCAAAACAACAAGGAAGCAGGAATGTATC

2.5. Construction of the pMSRB2 and pMSRB2-GFP expression plasmids

Two MSRB2 expression constructs were generated by cloning the MSRB2 open reading frame with and without the natural stop codon into the pcDNA 3.1 CT-GFP-TOPO vector (Invitrogen Corp). Thus, one construct (pMSRB2) generates the normal MSRB2 and the other (pMSRB2-GFP) generates the MSRB2 fused to green fluorescent protein (GFP). The MSRB2 open reading frame was amplified from human retina cDNA using specific primers (see Table 1 for sequences) and Platinum Taq polymerase (Invitrogen Corp).

2.6. DNA sequencing

All plasmid constructs were verified by direct DNA sequencing. Sequencing of the different plasmid constructs was performed using the BigDye terminator v3.1 cycle sequencing kit and an ABI 3130 Genetic Analyzer instrument (Applied Biosystems, Inc.) following the manufacturer's protocol.

2.7. Cell cultures

D407 cells were a kind gift from Richard Hunt (Department of Pharmacology and Microbiology, University of South Carolina, Columbia, SC). Cells were grown in DMEM medium supplemented with 4% fetal bovine serum (FBS). Penicillin 10 U/ml, streptomycin 100 μg/μl and 2 mM of L-glutamine were added to both mediums.

2.8. Cell transfection

Transfections were performed on 2 × 10^6 cells by electroporation using the Cell Line nucleofector V Kit and the Nucleofector ™ II instrument (Amaxa Biosystems Inc., Gaithersburg, MD) according to the manufacturer's protocol.

2.9. Construction of stable D407 cell lines overexpressing MSRB2

Stably transfected D407 cells overexpressing MSRB2 were generated using the lepidopteran transposon piggyBac vector (Ding et al., 2005). The initial plasmid PB [Act-RFP] (Ding et al., 2005) was modified by removing the actin promoter (Act) and the red fluorescent protein (RFP) and inserting the CMV promoter with the MSRB2-GFP coding region followed by the Blasticidin resistance gene (controlled by the EM7 promoter) for selection. The transposase (PB) recognitions sites were preserved in the flanking