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Involvement of MsrB1 in the regulation of redox balance and inhibition of peroxynitrite-induced apoptosis in human lens epithelial cells

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ABSTRACT

Methionine sulfoxide reductases (Msrs) in lens cells are important for the maintenance of lens cell viability and resistance to oxidative stress damage. Peroxynitrite ($ONOO^-$), as a strong oxidizing and nitrating agent, occurred in diabetic retinopathy patients and diabetic model animal. In an attempt to shed light on the roles of MsrB1, known as selenoprotein R, in protecting human lens epithelial (HLE) cells against peroxynitrite damage, and contribution of loss of its normal activity to cataract, the influences of MsrB1 gene silencing on peroxynitrite-induced apoptosis in HLE cells were studied. The results showed that both exogenous peroxynitrite and MsrB1 gene silencing by short interfering RNA (siRNA) independently resulted in oxidative stress, endoplasmic reticulum (ER) stress, activation of caspase-3 as well as an increase of apoptosis in HLE cells; moreover, when MsrB1-gene-silenced cells were exposed to 300 μM peroxynitrite, these indexes were further aggravated at the same conditions and DNA strand breaks occurred. The results demonstrate that in HLE cells MsrB1 may play important roles in regulating redox balance and mitigating ER stress as induced by oxidative stress under physiological conditions; MsrB1 may also protect HLE cells against peroxynitrite-induced apoptosis by inhibiting the activation of caspase-3 and oxidative damage of DNA under pathological conditions. Our results imply that loss of its normal activity is likely to contribute to cataract.

1. Introduction

Cataract is one of the major complications of diabetes mellitus (Obrosova et al., 2010). Oxidative stress is believed to play a major role in cataract formation (Marchetti et al., 2005). Peroxynitrite ($ONOO^-$), a strong oxidizing and nitrating agent, can easily oxidize some amino acids such as methionine, cysteine and tryptophan (Lancellotti et al., 2010), and resulting in cellular responses such as cell signaling, oxidative injury, committing cells to necrosis or apoptosis (Radi et al., 1991a,b; Niles et al., 2006; Holthoff et al., 2010; Pacher et al., 2007). $ONOO^-$ formation was reported in diabetic retinopathy patients and diabetic experimental models (Ali et al., 2008, 2011); its formation was also demonstrated in diabetic neuropathy, nephropathy, and cardiovascular complications of diabetes (Pacher et al., 2005; Pacher and Szabo, 2006; Szabo et al., 2002), as well as in several clinical settings including diabetes, coronary artery disease, stroke, sepsis, and chronic inflammatory diseases (Lancellotti et al., 2010). Nitrotyrosine, the footprint of $ONOO^-$, was detected in the lens of streptozocin-induced diabetic animals, but not existed in the control group (Hao et al., 2006).

Methionine (Met) can undergo a reversible oxidation with the formation of methionine sulfoxide (MetO) in two diastereomeric forms S-MetO and R-MetO (Vogt, 1995). Elevated levels of MetO were detected in cataractous human lenses, crystallin and other lens proteins from human and animal eyes (Garner and Spector, 1980; Horstmann et al., 1983; Li et al., 2011). Moreover, in severe cataracts, 60% or more of Met in membrane-associated components were found to be MetO (Garner and Spector, 1980; Kantorow et al., 2004). Previous studies have proved that $ONOO^-$ can oxidize Met residues of proteins to MetO (Lancellotti et al., 2010; Berlett et al., 1996, 1997; Perrin and Koppenol, 2000; St John et al., 2001; Smallwood et al., 2003; Nakao et al., 2003; Khor et al., 2004; Botti et al., 2004). The oxidation of Met residues can affect a multitude of biological functions through corresponding direct inactivation of proteins (Marchetti et al., 2005; Davies, 2005; Weissbach et al., 2002). Fortunately, unlike most protein modifications, MetO can be converted back to Met through the action of a class of enzymes, known as methionine sulfoxide reductases (Msrs) (Kantorow et al., 2004;

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Davies, 2005). Mammals have two Msrs, MsrA and MsrB, which catalyze the thioredoxin-dependent reduction of S-MetO and R-MetO derivatives to Met (Kim and Gladyshev, 2004, 2005, 2007; Hansel et al., 2005), respectively. Both enzymes are important for lens cell function, resistance to oxidative stress, and, potentially, cataractogenesis (Marchetti et al., 2005; Hawse et al., 2004). Msr protein repair system and other redox systems play key roles in the function and maintenance of the aging eye (Brennan and Kantorow, 2009). In mammals, there is only one gene encoding MsrA, but there are three genes encoding MsrBs (MsrB1, MsrB2 and MsrB3) (Marchetti et al., 2005). All three MsrB genes are required for lens cell viability, and their silencing in lens cells caused increased oxidative stress-induced cell death (Marchetti et al., 2005). Furthermore, MsrB1 knockout mice showed increased levels of protein MetO (Fomenko et al., 2009). MsrB1 is a selenoprotein named selenoprotein R (SelR), and localized in the cell nucleus and cytosol (Marchetti et al., 2005; Picot et al., 2006; Mary et al., 2004). MsrB1 identified in mammals is widely distributed throughout different tissues (Kim and Gladyshev, 2004; Reeves and Hoffmann, 2009). Other selenoproteins, such as glutathione peroxidases, thioredoxin reductase and selenoprotein P have been shown to play a potential role in protection against peroxynitrite (Sies and Arteel, 2000; Klotz and Sies, 2003; Traulsen et al., 2004). However, the role of MsrB1 in cellular protection against peroxynitrite-induced HLE cells damage remains to be evaluated.

HLE cell apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals (Li et al., 1995). Peroxynitrite-induced apoptosis was reported in various types of cells (Lin et al., 1998; Dickhout et al., 2005; Guner et al., 2009). Although the full apoptotic pathway induced by peroxynitrite is not well understood, the activation of a caspase-3 family protease is essential for initiating the process of peroxynitrite-induced apoptosis in HL-60 cells (Lin et al., 1998); peroxynitrite triggers apoptosis in cardiomyocytes in vitro and in the myocardium in vivo, through a pathway involving caspase-3 activation (Levrand et al., 2006). However, the influence mechanism of MsrB1 gene silencing on peroxynitrite-induced HLE cell apoptosis remains virtually unknown.

In this study, peroxynitrite (a strong oxidant)-induced cell injury and the effect of MsrB1 gene silencing on peroxynitrite-mediated ER stress, cell apoptosis and apoptotic pathway in HLE cells were investigated.

2. Materials and methods

2.1. Reagents

Propidium iodide (PI), Hochest 33258 and protease inhibitor cocktail were purchased from Sigma Co. Dulbecco's Modified Eagle's Medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylte-trazolium bromide (MTT), and newborn calf serum (NCS) were obtained from Gibco BRL (Gaithersburg, MD, USA); Penicillin G and streptomycin sulfate were purchased from Amersco. Lipofectamine 2000 was obtained from Invitrogen. All other reagents were of analytical or biochemical reagents.

$ONOO^-$ was synthesized from sodium nitrite and acidic hydrogen peroxide as previously described (Klotz et al., 2000), and excess hydrogen peroxide was removed by treatment with MnO_2. Concentration of $ONOO^-$ was determined spectrophotometrically at 302 nm ($\varepsilon = 1670\ M^{-1}\ cm^{-1}$).

2.2. Cell culture and treatment with peroxynitrite

HLE cells (SRA01/04, Sciencell, USA) (Marchetti et al., 2005) were cultured in DMEM medium supplemented with 10% (v/v) heat-inactivated NCS and antibiotics (penicillin, 100 U/mL; streptomycin, 100 μg/mL) at 37 °C in the present of 5% CO_2. Before experimentation, cells at approximately 80% confluence were serum starved for 24 h, washed twice with phosphate-buffered saline (PBS), and subsequently placed in modified PBS (50 mM Na_2HPO_4, 90 mM NaCl, 5 mM KCl, 0.8 mM $MgCl_2$, 1 mM $CaCl_2$, and 5 mM glucose, pH 7.4). This buffer was used to avoid interfering reactions of $ONOO^-$ and other oxidant systems used in our experiments with media constituents and to provide sufficient buffering capacity to avoid pH changes after the addition of alkaline solutions of $ONOO^-$ (Van der Vliet et al., 1998).

Various stock concentrations of $ONOO^-$ were freshly prepared in 0.5 N NaOH. HLE cells were washed twice with PBS and then placed in modified PBS at room temperature. After adding $ONOO^-$, the cells were immediately mixed with the incubation buffer by rapid swirling, in order to assure optimal exposure of the cells to $ONOO^-$ before decomposition. To avoid increases in pH, $ONOO^-$ was added in volumes that were a maximum of 1% of the culture volume to yield the given final concentrations. The cells were incubated for 20 min at 37 °C, then were washed, incubated with culture medium, and maintained for the additional time required for each experiment. $ONOO^-$ decomposition solution was used as vehicle control (Zhou et al., 2009).

2.3. MTT assay for cellular growth and survival

Cell growth and survival was measured by MTT assay (Ohguro et al., 1999). Briefly, HLE cells were plated at a density of $\sim 2 \times 10^4$ cells per well in 24-well plates and grown until subconfluent in DMEM containing 10% newborn calf serum. Cells were serum starved for 24 h and treated with $ONOO^-$ for 12 h and then the cells were incubated with MTT (0.5 mg/mL) at 37 °C for 4 h, after that the media was carefully removed, formazan salts were dissolved in dimethyl sulphoxide, and the solution was determined spectrophotometrically at 570 nm (Ohguro et al., 1999).

2.4. MsrB1 RNA interference

Double-stranded short interfering (si) RNAs specific for MsrB1 were 5′-GCGUCCGGAGCACAAUAGATT-3′ (sense) and 5′-UCUAUUGUGCUCCGGACGCTT-3′ (antisense) (Marchetti et al., 2005), and 5′-UUCUCCGAACGUGUCACGUTT-3′ (sense) and 5′-ACGUGACACGUUCGGAGAATT-3′ (antisense) for negative control. siRNAs were synthesized by Shanghai GenePharma (Shanghai, China).

HLE cells were plated in 24-well plates, 6-well plates or 60 mm plates, and grown until 70%–80% confluence, then transfected with siRNA and Lipofectamine 2000 according to the manufacturer's instructions. After transfection for approximately 24 h, the cells were treated with or without $ONOO^-$ and incubated with fresh serum-free media for 12 h or 24 h and harvested for analysis.

2.5. Observation of cell morphology

HLE cells treated with $ONOO^-$ (300 μM) for 12 h were collected and washed with PBS, then fixed with methanol and acetic acid (3:1, v/v) for 20 min at room temperature. After washing, the cells were incubated in Hoechst 33258 at a final concentration of 5 μg/mL at room temperature for 30 min. Nuclear morphology was then observed under a fluorescence microscope (IX71, Olympus, Tokyo, Japan).

After HLE cells were treated with $ONOO^-$ (300 μM) for 24 h, the cells were washed twice with PBS and then fixed in 2.5% glutaraldehyde in 0.1 M PBS (pH 7.2) at 4 °C for 4 h. Then the cells were washed with PBS and then post-fixed in 1% osmium tetroxide at room temperature for another 2 h. After dehydration with gradient