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Effect of methionine sulfoxide reductase B1 (SelR) gene silencing on peroxynitrite-induced F-actin disruption in human lens epithelial cells

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ABSTRACT

F-actin plays a crucial role in fundamental cellular processes, and is extremely susceptible to peroxynitrite attack due to the high abundance of tyrosine in the peptide. Methionine sulfoxide reductase (Msr) B1 is a selenium-dependent enzyme (selenoprotein R) that may act as a reactive oxygen species (ROS) scavenger. However, its function in coping with reactive nitrogen species (RNS)-mediated stress and the physiological significance remain unclear. Thus, the present study was conducted to elucidate the role and mechanism of MsrB1 in protecting human lens epithelial (hLE) cells against peroxynitrite-induced F-actin disruption. While exposure to high concentrations of peroxynitrite and gene silencing of MsrB1 by siRNA alone caused disassembly of F-actin via inactivation of extracellular signal-regulated kinase (ERK) in hLE cells, the latter substantially aggravated the disassembly of F-actin triggered by the former. This aggravation concurred with elevated nitration of F-actin and inactivation of ERK compared with that induced by the peroxynitrite treatment alone. In conclusion, MsrB1 protected hLE cells against the peroxynitrite-induced F-actin disruption, and the protection was mediated by inhibiting the resultant nitration of F-actin and inactivation of ERKs.

1. Introduction

Actin is a protein that exists in three isoforms (α, β, and γ) and in various states ranging from monomeric globular actin (G-actin) to polymeric filamentous actin (F-actin) [1,2]. F-actin plays a crucial role in fundamental cellular processes [3]. Actin also belongs to a group of proteins that are targeted for calpain mediated degradation in different types of experimental cataract [4,5]. Based on the result of actin-related globular degeneration, Mousa et al. speculated that it might be a contributing factor in cortical cataractogenesis [6].

The ERK signaling pathway is a major determinant in the control of diverse cellular processes [7]. Previous studies have indicated that F-actin may provide a scaffold for ERK signaling complexes [8,9]; and ERK1/2 activation mediates the formation of F-actin, whereas depolymerizing F-actin increased pERK1/2 expression [10]. However, Yang et al. reported that disassembly of the actin cytoskeleton inhibited phosphorylation of ERK and stabilizing actin prevented dephosphorylation of ERK [11]. Nevertheless, mechanisms for the interactions between pERK1/2 and F-actin are not well understood.

Peroxynitrite ($ONOO^-$) is the reaction product of superoxide (O_2^-) and nitric oxide (NO). The molecule rapidly breaks down at physiological pH to yield hydroxyl radical (·OH) and nitrogen dioxide radical ($\cdot NO_2$) [12]. As a potent oxidant and nitrating species, peroxynitrite is proposed to be an effector of cell damage in diabetic cataract [13], by oxidizing and/or nitrating biological molecules including proteins, lipids, and DNA [14,15]. The $ONOO^-$-induced nitration of actin protein can result in depolymerization of F-actin [16–18]. Furthermore, $ONOO^-$ oxidizes methionine (Met) residues of proteins to methionine sulfoxide (MetO) [19,20], and this oxidation may interfere a multitude of biological functions through direct inactivation of relevant proteins [21,22]. Recent evidence suggests that Mical (an actin disassembly factor) can

Abbreviations: bFGF, basic fibroblast growth factor; DMEM, Dulbecco's Modified Eagle's Medium; ECL, enhanced chemiluminescence; ERK, extracellular signal-related kinase; F-actin, filamentous actin; G-actin, globular actin; hLE, human lens epithelial; MAPK, mitogen-activated protein kinase; MetO, methionine sulfoxide; MEK, mitogen-activated protein kinase kinase; Msr, methionine sulfoxide reductase; NCS, newborn calf serum; PBS, phosphate-buffered saline; RNS, reactive nitrogen species; ROS, reactive oxygen species; RT, room temperature; SelR, selenoprotein R; siRNA, short interfering RNA; T-TBS, tris-buffered saline with Tween.

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regulate F-actin assembly and disassembly by the oxidation of Met 44 residue within the D-loop of actin [23]. Oxidation of critical Met residues can inhibit actin polymerization and distablizes F-actin [24]. 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) [25]. Mammals have two forms of Msrs: MsrA and MsrB catalyze the thioredoxin-dependent reduction of S-MetO and R-MetO derivatives to Met [26], respectively. MsrB1 is a selenoprotein named selenoprotein R (SelR), and is localized in the cell nucleus and cytosol [19,27]. MsrB1 is widely distributed throughout different tissues of mammals [11,27]. Previous studies by Marchetti et al. [21] and our group [28,29] demonstrated that MsrB1 could serve as a ROS scavenger and inhibit $ONOO^-$-induced apoptosis of human lens epithelial cells (hLEC). However, its potential role in protection against $ONOO^-$ and in protecting the F-actin assembly in hLE cells exposed to various doses of peroxynitrite remains largely unknown.

To explore the cytoprotection of MsrB1 against $ONOO^-$-induced damage in hLE cells, here, we preliminarily examined the effect of $ONOO^-$ on ERK phosphorylation and the regulation of pERK1/2 expression on F-actin assembly in cultured hLE cells before and after MsrB1 gene silencing by RNAi.

2. Materials and methods

2.1. Reagents

Dulbecco's Modified Eagle's Medium (DMEM) and newborn calf serum (NCS) were obtained from Gibco BRL (Gaithersburg, MD). Penicillin G and streptomycinsulfate were purchased from Amersco. Lipofectamine 2000 was obtained from Invitrogen. Protease inhibitor cocktail was purchased from Sigma Co. (St. Louis, MO). Recombinant human basic fibroblast growth factor (bFGF) was obtained from Peprotech (Rocky Hill, CT). All other reagents were of analytical or biochemical reagents.

$ONOO^-$ was synthesized from sodium nitrite and acidic hydrogen peroxide as previously described [30], 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 $ONOO^-$

The hLE cells (SRA01/04, Sciencell, Carlsbad, CA) [25] 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 presence 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). To avoid increases in pH, $ONOO^-$ was added at a maximum volume of 1% of the culture 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. In control experiments, cells were treated with aliquots of $ONOO^-$ stock solutions that were allowed to decompose completely by overnight (vehicle control) [31,32].

2.3. MsrB1 RNA interference

Double-stranded short interfering (si) RNAs specifically designed for MsrB1 were 5′-GCGUCCGGAGCACAAUAGATT-3′ (sense) and 5′-UCUAUUGUGCUCCGGACGCTT-3′ (antisense) [21], and for the negative control were 5′-UUCUCCGAACGUGUCACGUTT-3′ (sense) and 5′-ACGUGACACGUUCGGAGAATT-3′ (antisense). The siRNA fragments were synthesized by Shanghai GenePharma (Shanghai, China).

The hLE cells were plated in 6-well plates or 60 mm plates, grown until 70–80% confluence, then transfected with siRNA and Lipofectamine 2000 according to the manufacturer's instructions. At 24 h after the transfection, the cells were treated with various concentrations of $ONOO^-$ and incubated with fresh serum-free media for 1 h or 24 h before being harvested for analysis.

2.4. Inhibition test

U0126 (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylmercapto]butadiene; Beyotime, China) was used as a highly selective inhibitor of both mitogen-activated protein kinase kinase (MEK1/2) and ERK1/2. In all inhibition test, 25 μM U0126 was added at 1 h before the addition of bFGF or $ONOO^-$. The inhibitor was added to the culture one more time during the culture period. The control dishes were supplemented with an equivalent volume of solvent dimethylsulfoxide [7].

2.5. Immunofluorescence and confocal microscopy

The hLE cells were plated on 20-mm glass bottom cell culture dish, and grown to confluence at 37 °C. After treatment with $ONOO^-$, the cells were treated as reference [2], washed with PBS, fixed with 3.7% formaldehyde solution at room temperature (RT) for 20 min and then permeabilized with 0.1% Triton X-100 in PBS at RT for 15 min. The cells were washed with PBS and then blocked in 10% NCS at RT for 1 h. F-actin was stained with FITC-Phalloidin (Enzo Life Sciences, Farmingdale, NY) at RT for 1 h and washed sufficiently. The cells were imaged with a Zeiss LSM 510 laser scanning confocal microscope (Carl zeiss, Jena, Germany).

2.6. Immunoprecipitation and Western blot analysis

The cells were lysed in RIPA buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.5% sodium deoxycholate, 0.1% SDS, 1% Triton X-100, 1 mM NaF, 1 mM Na_3VO_4, 1 mM EDTA, 10 uL/mL protease inhibitor cocktail P8340). Soluble proteins were separated on 10% polyacrylamide gels and blotted onto a nitrocellulose membrane. Subsequently, the membranes were incubated with specific antibodies against target protein (GAPDH, ERK1/2 and phosphor-ERK1/2 (Tyr 204) (Santa Cruz Biotechnology, Dallas, TX); F-actin (Xing Xing Tang Biotechnology, Beijing, China); nitrotyrosine antibody (Millipore, Billerica, MA)), and antigen–antibody complexes were visualized by enhanced chemiluminescence.

For immunoprecipitation experiments, hLE cell lysates containing 0.5 mg of total protein were incubated with 4 μg of anti-F-actin for 1 h at 4 °C. After collection on protein A/G plus-Agarose, the immune complexes were washed three times with lysis buffer, boiled in Laemmli buffer for 5 min, and thereafter subjected to Western blotting analysis.

2.7. Statistical analysis

All data were expressed as mean ± SD. The statistical significance of difference between groups was evaluated using analysis of variance (ANOVA) followed by Tukey test for multiple comparisons, and a P value less than 0.05 was considered statistically significant.

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