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Original Contribution

8-Oxoguanine DNA glycosylase-1-mediated DNA repair is associated with Rho GTPase activation and α-smooth muscle actin polymerization

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ABSTRACT

Reactive oxygen species (ROS) are activators of cell signaling and modify cellular molecules, including DNA. 8-Oxo-7,8-dihydroguanine (8-oxoG) is one of the prominent lesions in oxidatively damaged DNA, whose accumulation is causally linked to various diseases and aging processes, whereas its etiological relevance is unclear. 8-OxoG is repaired by the 8-oxoguanine DNA glycosylase-1 (OGG1)-initiated DNA base excision repair (BER) pathway. OGG1 binds free 8-oxoG and this complex functions as an activator of Ras family GTPases. Here we examined whether OGG1-initiated BER is associated with the activation of Rho GTPase and mediates changes in the cytoskeleton. To test this possibility, we induced OGG1-initiated BER in cultured cells and mouse lungs and used molecular approaches such as active Rho pull-down assays, siRNA ablation of gene expression, immune blotting, and microscopic imaging. We found that OGG1 physically interacts with Rho GTPase and, in the presence of 8-oxoG base, increases Rho–GTP levels in cultured cells and lungs, which mediates α-smooth muscle actin (α-SMA) polymerization into stress fibers and increases the level of α-SMA in insoluble cellular/tissue fractions. These changes were absent in cells lacking OGG1. These unexpected data and those showing that 8-oxoG repair is a lifetime process suggest that, via Rho GTPase, OGG1 could be involved in the cytoskeletal changes and organ remodeling observed in various chronic diseases.

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Oxidative stress is generated by multiple exogenous agents and endogenous sources in mammalian cells. The resulting reactive oxygen species (ROS) cause damage to cellular molecules, including DNA [1]. In DNA guanine is a primary ROS target because it has the lowest reduction potential among the nucleic acid bases [2]; 8-oxo-7,8-dihydroguanine (8-oxoG) is thus one of the most abundant base lesions. Because of its potential to pair with adenine, 8-oxoG is also one of the most mutagenic DNA lesions among over 20 identified oxidative modifications to guanine [3,4]. Accumulation of 8-oxoG in DNA has been linked to various inflammatory diseases, as well as aging processes [5]. The oxidatively damaged bases are preferentially repaired by the base excision repair (BER) pathway [6,7], which utilizes glycosylases to excise the lesion via cleaving its N-glycosidic bond, followed by endonucleolytic cleavage and subsequent gap-filling [6,8].

8-Oxoguanine DNA glycosylase-1 (OGG1) is the key enzyme in removing 8-oxoG and 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) from DNA with equal specificity and kinetics during BER to prevent mutations and maintain genomic integrity [6,9,10]. Surprisingly, *Ogg1*-knockout (KO) mice have no particular phenotype; their life span is unaltered, showing only a moderate

Abbreviations: 8-oxoG, 8-oxo-7,8-dihydroguanine; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; α-SMA, α-smooth muscle actin; Ab, antibody; BER, base excision repair; FU, fluorescence unit; FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; GEF, guanine nucleotide exchange factor; GOx, glucose oxidase; GST, glutathione *S*-transferase; ^{Mant}GTP, 2′-(or-3′)-*O*-(*N*-methylanthraniloyl)guanosine 5′-triphosphate; ^{Mant}GDP, 2′-(or-3′)-*O*-(*N*-methylanthraniloyl)guanosine 5′-diphosphate; OGG1, 8-oxoguanine DNA glycosylase-1; ROS, reactive oxygen species

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predisposition to tumorigenesis, despite increased genomic 8-oxoG levels [11–13]. A lack of OGG1 activity in KO mice resulted in increased resistance to inflammation [14], changes in whole-body energy homeostasis, increased susceptibility to obesity, and metabolic dysfunction [15]. These results suggest additional, not yet defined functions of OGG1 protein and/or its repair product, 8-oxoG. In support of this hypothesis, accumulating data suggest that OGG1 may play roles in multiple cellular processes. For example, it has been shown that OGG1 colocalizes with centrioles (microtubule organizing centers), microtubule networks, and mitotic chromosomes [16,17]. It has also been shown that OGG1-generated free 8-oxoG base binds to OGG1 and increases its β-lyase activity, mediating product-assisted catalysis in an enzyme-catalyzed reaction [18]. Our recent studies also showed that 8-oxoG binds cytoplasmic OGG1, and the OGG1 · 8-oxoG complex acts as a guanine nucleotide exchange factor (GEF) catalyzing the exchange of GDP with GTP to promote activation of Ras and Rac1 small GTPases [19–21].

In response to DNA damage, or in DNA damage-induced senescent cells, reorganization of the cytoskeleton, actin filaments (stress fibers), and filopodia has been observed [22], although the molecular events directly responsible for these cytoskeletal morphological changes are not yet well defined. Among the small GTPase family members, activation of the Ras homology (Rho) proteins RhoA, RhoB, and RhoC has been shown to regulate many aspects of intracellular actin dynamics, including stress fiber formation [23]. Taking into account that OGG1 functions as a GEF when complexed with 8-oxoG [19–21], we hypothesized that OGG1-BER is associated with Rho activation and transient morphological changes in ROS-exposed cells. Here we show that OGG1-initiated repair of oxidative DNA damage was followed by an increase in Rho–GTP levels and stress fiber formation in cultured cells and lung tissues. Thus our studies establish a link between repair of oxidatively altered guanine and changes in cellular architecture, placing OGG1 at the center of a complex signaling network.

Materials and methods

Materials

8-OxoG (Cat. No. 89290) was from Cayman Chemical Co. (Ann Arbor, MI, USA); FapyG was a kind gift from Dr. Miral Dizdaroglu (National Institute of Standards and Technology, Gaithersburg, MD, USA); glucose oxidase from *Aspergillus niger* (GOx; Cat. No. G7141-10KU), FITC-conjugated phalloidin (Cat. No. P5282-.1MG), triethanolamine (TEA; Cat. No. 90279-100ML), and N-TER Nanoparticle siRNA Transfection System (Cat. No. N2913) were from Sigma–Aldrich (St. Louis, MO, USA); rabbit polyclonal antibody (Ab) to α-smooth muscle actin (anti-α-SMA; Cat. No. ab5694) was from Abcam (Cambridge, MA, USA); monoclonal Abs to glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Cat. No. 2118S) and α-tubulin (Cat. No. 3873S) were from Cell Signaling Technology (Danvers, MA, USA); active Rho pull-down and detection kit (Cat. No. 16116Y) was from Thermo Scientific Pierce Biotechnology (Rockford, IL, USA); OGG1 rabbit monoclonal Ab (Cat. No. 5104-1) was from the Abcam subsidiary Epitomics (Burlingame, CA, USA). siGENOME SMARTpool for human OGG1 (Cat. No. M-005147-03) was from Dharmacon Thermo Scientific (Pittsburgh, PA, USA). Rho activator (calpeptin, Cat. No. CN01) and Rho inhibitor (C3 transferase, Cat. No. CT04) were from Cytoskeleton (Denver, CO, USA); His-tagged RhoA protein (Cat. No. NBP1-50933) was from Novus Biologicals (Littleton, CO, USA); and OGG1 protein was a kind gift from Dr. Hazra (Department of Biochemistry & Molecular Biology, UTMB, Galveston, TX, USA). 2′-(or-3′)-*O*-(*N*-methylanthraniloyl) guanosine 5′-triphosphate (^{Mant}GTP) and 2′-(or-3′)-*O*-(*N*-methylanthraniloyl)guanosine 5′-diphosphate (^{Mant}GDP) were from Cytoskeleton.

Cell culture

Human diploid fibroblast (MRC5) and mouse embryonic fibroblast (MEF) cells were maintained in Earle's minimum essential medium and Dulbecco's modified Eagle's medium/F-12 (Ham) supplemented with 10% fetal bovine serum (FBS), glutamine, penicillin, and streptomycin; cells were grown at 37 °C in 5% CO_2, starved in 0.5% FBS medium for 24 h, and starved in FBS-free medium for 16 h before Rho activation. 8-OxoG (10 μM) or GOx (100 ng/ml) was added to cells in serum-free medium, and cell extracts were made at the indicated times after 8-oxoG or GOx addition.

Animals

Animal experiments were performed according to the National Institutes of Health guidelines for the use of experimental animals and approved by the University of Texas Animal Care and Use Committee (Protocol 0807044A). Eight-week-old female BALB/c mice (The Jackson Laboratory, Bar Harbor, ME, USA) were challenged intranasally with 1 μM 8-oxoG (60 μl) or an equal volume of vehicle and sacrificed, and the lungs were excised [24]. In other experiments, mice were directly sacrificed without treatment.

Histology and immunohistochemistry

For immunochemistry histology staining of α-SMA or F-actin, mice were challenged with 1 μM 8-oxoG (in 60 μl phosphate-buffered saline; PBS) and sacrificed after 30 min. Mouse lungs were fixed, sectioned (4 μm), and stained with α-SMA Ab and/or FITC–phalloidin. Cells on chambers slides were challenged with the OGG1-initiated BER by-product 8-oxoG for 20 min and after treatment fixed with 4% paraformaldehyde for 20 min at room temperature, then washed twice with wash buffer (0.05% Tween 20 in PBS), and permeabilized with 0.1% Triton X-100 for 3 min. After the wash, blocking solution (1% bovine serum albumin in 0.05% Tween 20 in PBS) was added for 30 min. Mouse anti-rabbit α-SMA monoclonal Ab and FITC–phalloidin were diluted in the blocking solution. After 1 h incubation with the primary Ab (1:300) followed by three washes with washing buffer, the secondary Ab (goat anti-mouse, FITC-conjugated, dilution 1:500) was added for 30–60 min. Cells were washed with 0.05% Tween 20 in PBS three times, dried, and mounted in antifade reagent (Cat. No. S3023 from Dako North America, Carpinteria, CA, USA). Stress fibers were visualized under a microscope using FITC-conjugated phalloidin (50 μg/ml) per the manufacturer's protocol, at 600 × magnification. Each image shown was a typical one in the field of view.

Assessment of active Rho levels

Assessment of Rho–GTP levels in cells was conducted using the active Rho pull-down assay kit as recommended by the manufacturer and described previously [19,20]. Briefly, cells were challenged with 100 ng/ml GOx or 10 μM 8-oxoG at 37 °C for the indicated times and lysed in 25 mM Tris–HCl, pH 7.5, 150 mM NaCl, 60 mM $MgCl_2$, 1% Nonidet P-40, and 5% glycerol, and Rho–GTP in 500 μg extracts was captured by the GST–Rho-binding domain of Rhotekin immobilized to glutathione resin [25]. After being washed with binding buffer, the activated Rho (Rho–GTP) was eluted with Laemmli buffer (0.125 M Tris–HCl, 4% SDS, 20%

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