Activation of Ras Signaling Pathway by 8-Oxoguanine DNA Glycosylase Bound to Its Excision Product, 8-Oxoguanine

Background: 8-Oxo-7,8-dihydroguanine (8-oxoG) is an abundant DNA base lesion repaired by 8-oxoguanine glycosylase (OGG1) via the base excision repair pathway. OGG1 binds to its repair product 8-oxoG and activates canonical Ras family GTPases, causing gene activation via MAPK signaling.

Results: OGG1 complexed with 8-oxoG has guanine nucleotide exchange factor activity.

Conclusion: OGG1 complexes with 8-oxoG and has guanine nucleotide exchange factor activity.

Significance: OGG1 modulates cellular signaling via its DNA repair-independent function.

8-Oxo-7,8-dihydroguanine (8-oxoG), arguably the most abundant base lesion induced in mammalian genomes by reactive oxygen species, is repaired via the base excision repair pathway that is initiated with the excision of 8-oxoG by OGG1. Here we show that OGG1 binds the 8-oxoG base with high affinity and that the complex then interacts with canonical Ras family GTPases to catalyze replacement of GDP with GTP; thus serving as a guanine nuclear exchange factor. OGG1-mediated activation of Ras leads to phosphorylation of the mitogen-activated kinases MEK1,2/ERK1,2 and increasing downstream gene expression. These studies document for the first time that in addition to its role in repairing oxidized purines, OGG1 has an independent guanine nucleic exchange factor activity when bound to 8-oxoG.

Reactive oxygen species, generated both endogenously during respiration and by various oxidases and environmental insults, induce multiple types of damage in the genome, including strand breaks and several types of oxidized bases (1). The highly mutagenic 8-oxoG5 is a predominant base lesion (2, 3) that does not block replication or transcription (4, 5). In the genome, 8-oxoG is predominantly repaired via the base excision repair pathway, in which OGG1 recognizes and excises this lesion (1, 6). Unrepaired 8-oxoG in the genome has been linked to various pathological states, including cancer and aging processes (7, 8); however, Ogg1-null mice have a normal phenotype and longevity (9, 10), suggesting that alternative, compensatory enzymes could also repair 8-oxoG, at least in the active genes. On the other hand, Ogg1-deficient mice are resistant to inflammation, implicating OGG1 in proinflammatory signaling (11, 12). We thus hypothesized a DNA repair-independent function of OGG1 that depends on the free 8-oxoG base. We report here that the OGG1-8-oxoG complex activates canonical Ras family GTPases, cellular signaling, and gene expression. We thus document for the first time a distinct, cell-signaling function of a DNA repair enzyme.

Experimental Procedures

Reagents—8-oxoG was from Cayman Chemicals (Ann Arbor, MI); 7,8-dihydro-8-oxoadenine (8-oxoA) was from BioLog Life Science Institute, Axoxara, LLC (San Diego, CA); and guanine and 8-oxo-deoxyguanosine (8-oxodG) were from Sigma-Aldrich. 2,6-Diamino-4-hydroxy-5-formimidopyrimidine (FapyG) was a kind gift of Dr. Miral Dizdaroglu (National Institute of Standards and Technology (NIST), Gaithersburg, MD). GTP, GDP, and GTPγS were from Cytoskeleton (Denver, CO); H-Ras, N-Ras, and K-Ras proteins were from Novus Biologicals (Littleton, CO); Pan-Ras antibodies were from Millipore; nickel-nitrilotriacetic acid-agarose beads were from Qiagen; Alexa Fluor 488-conjugated antibodies were from Invitrogen. Active Ras pulldown assay kit was from Pierce Biotechnology (Thermo Fisher Scientific Inc.); and siRNAs for Ras and OGG1 depletion were from Dharmacon (Thermo Fisher Scientific Inc.).

Cellular Studies—Human diploid fibroblast (MRC5) and HeLa-S cells were maintained in Earle’s minimum essential and Dulbecco’s modified Eagle’s low glucose medium, respectively. All media were supplemented with 10% fetal bovine serum, glutamine, penicillin, and streptomycin; cells were grown at

5 The abbreviations used are: 8-oxoG, 8-oxo-7,8-dihydroguanine; 8-oxoA, 7,8-dihydro-8-oxoadenine; 8-oxodG, 8-oxoguanine deoxynucleoside; GEF, guanine nucleotide exchange factor; OGG1, 8-oxoguanine DNA glycosylase 1; MRC5, human diploid lung fibroblast; GTPγS, guanosine 5′-3-O-(thio)triphosphate; PapyG, 2,6-diamino-4-hydroxy-5-formimidopyrimidine; MTH, MutT homolog.

6 H-Ras and K-Ras indicate mammalian homologs of Harvey and Kirsten sarcoma virus oncogene, respectively, and N-Ras indicates neuroblastoma RAS viral oncogene homolog.
37 °C in a 5% CO₂, 8-oxoG base (0.01–30 μM) was added to cells in serum-free media where indicated, and cell extracts were made at 0, 5, 10, 15, 20, and 30 min after 8-oxoG addition.

**Animals and Treatments**—Animal experiments were performed according to the National Institutes of Health Guidelines for Use of Experimental Animals and approved by the University of Texas Animal Care and Use Committee (Protocol number: 0807044A). Eight-week-old female BALB/c mice (The Jackson Laboratory) were challenged intranasally with 60 μl of 8-oxoG (1 μM) in saline (or with control saline) under mild anesthesia (13). The animals were sacrificed after 15 min, and lung extracts were prepared for measuring the Ras-GTP levels.

**Assessment of Ras-GTP**—Ras-GTP levels were quantified with the Active Ras pulldown assay kits. Briefly, the cells were lysed in 25 mM Tris-HCl, pH 7.5, 150 mM NaCl, 60 mM MgCl₂, 1% Nonidet P-40, and 5% glycerol, and Ras-GTP in 250-μg extracts was captured by the Ras-binding domain of Raf1 immobilized to glutathione resin (14, 15). After washing with binding buffer, the activated Ras was eluted with Laemmli buffer (0.125 M Tris-HCl, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol, pH 6.8) and quantified by Western blotting and densitometry.

**Protein Interaction Assays**—The interaction of OGG1 with H-, N-, or K-Ras was analyzed as described previously (16, 17). Briefly, individual His-Ras proteins were immobilized on nickel-nitrotetraacetic acid–agarose beads in interaction buffer (50 mM NaH₂PO₄, 300 mM NaCl, 20 mM imidazole, 0.05% Tween 20, pH 7.5) and incubated for 30 min at 4 °C. After three washes in interaction buffer, untagged OGG1 ± 8-oxoG was added in the presence or absence of GTP or GDP. The samples were incubated for 30 min at 4 °C and washed twice with interaction buffer, and the proteins eluted with Laemmli buffer were analyzed by Western blotting.

**Immunodetection of Proteins**—Cell extracts fractionated by SDS-PAGE (4–20% polyacrylamide) were transferred onto nitrocellulose membranes that were then incubated with primary antibodies and washed in Tris-buffered saline with Tween 20, and antibody binding was detected with HRP-conjugated secondary antibody and visualization by enhanced chemiluminescence detection (13). Subcellular localization of proteins was visualized in a Nikon Eclipse Ti microscope (60×) (13).

**Fluorescence Spectroscopy**—The binding of 8-oxoG to OGG1 was assessed by monitoring the decrease in intrinsic tryptophan fluorescence (18). Briefly, 0.5 μM OGG1 (100 μl) was incubated with increasing concentrations of 8-oxoG base (or 8-oxodG and FapyG as controls; 0.0, 0.0001, 0.0005, 0.001, 0.02, 0.04, 0.8, 1.2, 1.6, or 2.0 μM) for 10 min at 24 °C in 25 mM Tris-HCl (pH 7.6) containing 1 mM DTT. The tryptophan fluorescence at λem = 290–400 nm (λex = 280 nm) was analyzed in a SPEX FluoroMax spectrofluorometer (Horiba Jobin Yvon Inc., Edison, NJ). The binding constant Kd was calculated by plotting ΔF (change in fluorescence emission maximum, 336 nm) versus ligand concentration according to the equation ΔF = ΔFmax [ligand]/Kd + [ligand] (18).

**Guanine Nucleotide Exchange Assay**—Nucleotide-free H-Ras (6 pmol) was loaded with an equimolar amount of GDP or GTP in a buffer containing 20 mM Tris (pH 7.5), 150 mM NaCl, 3 μM MgCl₂, 1 mM dithiothreitol, and 50 μg of bovine serum albumin at 24 °C (16, 19). Guanine nucleotide exchange assays were initiated by the addition of OGG1 ± 8-oxoG in the presence of a 10-fold excess of GTPyS or GDP. The molecular ratio of Ras and OGG1:8-oxoG was 1:1 or 10:1. After 0, 0.5, 1, 2, 4, 8, 16, or 32 min, nucleotide exchange reactions were terminated by adding 60 mM MgCl₂. Ras-GTP levels were determined using Active Ras pulldown assays. Changes in Ras levels were analyzed by Western blotting.

**Down-regulation of Gene Expression**—Cells were transfected with control siRNA (siGENOME nontargeting siRNA) or target-specific siRNAs for Harvey (H)-Ras (catalog number M-004142), Kirsten (K)-Ras (catalog number M-005692), neuroblastoma RAS viral oncogene homolog (N)-Ras (catalog number M-003919), and/or OGG1 (catalog number M-010957) (siGENOME SMARTpools, Thermo Scientific) using INTERFERin™ reagent (Polyplus Transfection Inc.), and incubated in growth medium for 72 h.

**Statistical Analysis**—Results were analyzed for significant differences using analysis of variance and Student’s t test. Differences were considered significant at p < 0.05. Data are expressed as the mean ± S.D.

**RESULTS AND DISCUSSION**

The free 8-oxoG base is generated exclusively during the repair of 8-oxoG in DNA, initiated by OGG1 (1). To mimic a transient increase in its intracellular level, we added 8-oxoG base to OGG1-proficient cells (MRC5) and analyzed the impact on the transcriptome using Affymetrix GeneChip. Ingenuity Pathways Analysis of microarray gene expression data (National Center for Biotechnology Information (NCBI), GEO accession number GSE26813) showed that 8 of the top 10 pathways that responded to 8-oxoG involved the small G protein Ras (supplemental Fig. S1). Ras GTPases activate a variety of cellular signaling pathways (20, 21).

To confirm these observations, we showed that the addition of 8-oxoG increased GTP-bound Ras levels in a time- (Fig. 1A) and dose-dependent (Fig. 1B) manner. The lowest dose of 8-oxoG base that increased Ras-GTP to a detectable level was 100 nM in MRC5 cells (Fig. 1B). The time course of Ras activation was consistent with rapid cellular uptake of 8-oxoG base. For example, at 1 min after the addition, ~70% of 8-oxoG was taken up by the cells, as shown by GS/MS analysis (supplemental Fig. S2A). Importantly, after intranasal challenge of mice (13) with 8-oxoG (60 μl of 1 μM 8-oxoG), we observed increased Ras-GTP levels in the lungs (Fig. 1E).

We hypothesized that 8-oxoG bound to OGG1 mediates guanine nucleotide exchange in Ras. In support of this idea, adding 8-oxoG to OGG1-depleted MRC5 cells (Fig. 1C; supplemental Fig. S2, B and D) or HeLa-S cells (Fig. 1D; supplemental Fig. S2, C and D) did not cause an increase in Ras-GTP levels. Free 8-oxoG was unique in increasing the Ras-GTP level as neither 8-oxoD nor other oxidized bases (FapyG or 8-oxoA) nor the original guanine base displayed this activity (Fig. 1F).

The binding of free 8-oxoG base to OGG1 was analyzed by changes in the intrinsic Trp fluorescence of OGG1 (18). A concentration-dependent decrease in Trp fluorescence (Fig. 2A) indicated the OGG1 conformational change as a result of the interaction. The binding constant (Kd) 0.56 ± 0.19 nM calcu-
lated from the binding isotherms (Fig. 2B) indicates its high affinity for 8-oxoG, which was unexpected, and predicted that the product binding would inhibit the enzyme. However, we observed the exact opposite in that 8-oxoG stimulated the activity of OGG1 in a concentration-dependent manner (supplemental Fig. S3, A–F). This suggests that 8-oxoG serves as a cofactor for OGG1 by binding to an independent site and not to the active site pocket in OGG1. This was further supported by our observation that the free FapyG base, with abundance similar to that of 8-oxoG in oxidatively damaged DNA, and which is an equally good OGG1 substrate (22, 23), did not affect the fluorescence of OGG1. Furthermore, OGG1 did not bind 8-oxoG equally good OGG1 substrate (22, 23), did not affect the activity of OGG1 in a concentration-dependent manner (supplemental Fig. S3, A–F). This suggests that 8-oxoG serves as a cofactor for OGG1 by binding to an independent site and not to the active site pocket in OGG1. This was further supported by our observation that the free FapyG base, with abundance similar to that of 8-oxoG in oxidatively damaged DNA, and which is an equally good OGG1 substrate (22, 23), did not affect the fluorescence of OGG1. Furthermore, OGG1 did not bind 8-oxoG (supplemental Fig. S3, D and C), demonstrating the specificity of the binding of OGG1 to free 8-oxoG base.

Activation of GTases involves displacement of GDP by GTP, a process mediated by GEFs (24). GEFs accelerate the exchange of GDP for GTP in Ras-GTPase, and active GTP-bound Ras releases GEF (24, 25). We then explored a possible interaction between OGG1 and Ras (16, 17, 26) and observed that in the presence of 8-oxoG, OGG1 specifically was bound to H-Ras (Fig. 2C, lane 2). However, OGG1 alone did not interact with the binding isotherms shown in A (18). Interaction of OGG1 with Ras protein requires 8-oxoG base. H-Ras (2.7 pmol) was bound to nickel-agarose beads (see “Experimental Procedures”), and OGG1 complexed with 8-oxoG has guanine nucleotide exchange factor activity. H-Ras (2.7 pmol) was bound to nickel-agarose beads (2.7 pmol) of His-H-Ras were immobilized on nickel-agarose beads before incubation with OGG1 (6 pmol) plus 8-oxoG (2.7 pmol) for 30 min at 24 °C. Levels of Ras-GTP were quantified using pulldown immunoblot assays as in Fig. 1.

Activation of Ras GTPases involves displacement of GDP by GTP, a process mediated by GEFs (24). GEFs accelerate the exchange of GDP for GTP in Ras-GTPase, and active GTP-bound Ras releases GEF (24, 25). We then explored a possible interaction between OGG1 and Ras (16, 17, 26) and observed that in the presence of 8-oxoG, OGG1 specifically was bound to H-Ras (Fig. 2C, lane 2). However, OGG1 alone did not interact with the binding isotherms shown in A (18). Interaction of OGG1 with Ras protein requires 8-oxoG base. H-Ras (2.7 pmol) was bound to nickel-agarose beads (see “Experimental Procedures”), and OGG1 complexed with 8-oxoG has guanine nucleotide exchange factor activity. H-Ras (2.7 pmol) was bound to nickel-agarose beads (2.7 pmol) of His-H-Ras were immobilized on nickel-agarose beads before incubation with OGG1 (6 pmol) plus 8-oxoG (2.7 pmol) for 30 min at 24 °C. Levels of Ras-GTP were quantified using pulldown immunoblot assays as in Fig. 1.

Activation of Ras GTPases by 8-oxoG-bound OGG1

**FIGURE 1.** Activation of Ras GTPases in OGG1-expressing cells by 8-oxoG base. A–F, parallel cell cultures were exposed to 1 μM (A, C, D, and F) or increasing concentration of 8-oxoG (B). Cell extracts were made at the times points indicated (in A) or 15 min after challenge (B, C, D, E, and F). Ras-GTP levels were determined by GST pulldown assays (15, 16). A and B, activation of Ras GTases in a time-dependent (A) and dose-dependent manner (B) in MRC5 cells upon 8-oxoG exposure. C and D, siRNA-mediated OGG1 depletion causes decreased Ras activation. E and F, MRC5 cells upon 8-oxoG exposure.

**FIGURE 2.** OGG1 complexed with 8-oxoG has guanine nucleotide exchange factor activity. A, fluorescent spectroscopic analysis of 8-oxoG binding to OGG1. FL indicates fluorescence. B, the Kd was calculated from the binding isotherms shown in A (18). Interaction of OGG1 with Ras protein requires 8-oxoG base. H-Ras (2.7 pmol) was bound to nickel-agarose beads (see “Experimental Procedures”), and OGG1 complexed with 8-oxoG has guanine nucleotide exchange factor activity. H-Ras (2.7 pmol) was bound to nickel-agarose beads (2.7 pmol) of His-H-Ras were immobilized on nickel-agarose beads before incubation with OGG1 (2.7 pmol) plus 8-oxoG (2.7 pmol) for 1 h at 4 °C. The proteins were stripped from the beads for immunoblotting. Upper panel, His-H-Ras bound to nickel-agarose beads; lower panel, OGG1 protein eluted from His-H-Ras. E, guanine nucleotides decrease the OGG1 interaction with Ras. His-H-Ras (2.7 pmol) bound to agaoroase beads was incubated with untagged OGG1 (2.7, 1.3, 0.67, and 0 pmol) and equimolar 8-oxoG for 30 min, and a 10-fold molar excess of GTP or GDP was then added for 30 min at 24 °C. Levels of eluted proteins were determined by Western blotting. F, exchange of Ras-bound GDP for GTP. H-Ras protein (6 pmol) was loaded with GDP (6 pmol), and nucleotide exchange was initiated by adding OGG1 (6 pmol) plus 8-oxoG (Φ) or 0.6 pmol of OGG1 plus 8-oxoG (◇), OGG1 (□), or 8-oxoG (◇) alone, together with GTP (6 pmol). G, GDP-GTP exchange by OGG1. H-Ras protein (6 pmol) was loaded with GDP, and guanine nucleotide exchange was initiated by adding 6 pmol of OGG1 plus 8-oxoG (Φ) or 0.6 pmol of OGG1 plus 8-oxoG (◇), OGG1 (□), or 8-oxoG (◇) alone, together with 10-fold excess GDP. Ras-GTP was quantified using pulldown immunoblot assays as in Fig. 1. G and F, right panels, bands in left panels were quantitated by densitometry (ImageJ), and the time course of nucleotide exchange is depicted graphically. n = 3–4.
with H-Ras under identical conditions (Fig. 2C, lane 1), suggesting that an 8-oxoG-induced conformational change in OGG1 (Fig. 2A) allows its binding to Ras. Quantitation of eluted OGG1 and comparison with input Ras indicated a nearly equimolar binding of OGG1 to H-Ras (Fig. 2D). Furthermore, GTP was more effective than GDP in inhibiting the interaction between Ras and OGG1-8-oxoG (Fig. 2E). These data strongly suggest that the conformation of nucleotide-free Ras allows the most stable interaction with OGG1-8-oxoG, which is weakened in the presence of guanine nucleotides. Similar interactions of K-Ras and N-Ras with OGG1-8-oxoG were also observed, and guanine nucleotides, especially GTP, decreased these interactions (supplemental Fig. S4, A and B). Our observations are consistent with those showing high affinity binding between nucleotide-free Ras and GEF (e.g. CDC25), which is decreased to an undetectable level by guanine nucleotides, especially GTP, due to nucleotide-induced conformational changes in the Ras protein (16, 27).

Increases in the Ras-GTP level upon exposure of cells to 8-oxoG (Fig. 1) and physical interaction between Ras and OGG1 (Fig. 2, C, D, and E) could cause guanine nucleotide exchange. Indeed, in the presence of 8-oxoG, OGG1 caused replacement of GDP-bound to Ras with GTP (Fig. 2F) at equimolar or higher molar ratios of H-Ras:OGG1. We subsequently showed that OGG1 also catalyzed the release of H-Ras-bound GTP replacement with GDP (Fig. 2G). OGG1 or 8-oxoG alone did not induce guanine nucleotide exchange (Fig. 2F and G). Densitometric analysis of the bands in Fig. 2, F and G (left panels) shows striking similarities between the kinetics of GTP-GTP and GDP-GTP exchange on Ras (Fig. 2, F and G, right panels), suggesting that OGG1 indiscriminately releases the nucleotide in vitro and allows rebinding; thus its activity is similar to that of other Ras-GEFs (16, 27). In the intracellular environment, due to the high GTP/GDP ratio (~10-fold higher GTP than GDP; (25)), the released GDP is exchanged for GTP in the Ras protein. OGG1-8-oxoG induced similar guanine nucleotide exchange with N-Ras and K-Ras (supplemental Fig. S5, A–D).

Ras-GTP binds to the Ras-binding domain (RBD) of the Raf1 serine/threonine kinase (14), and its subsequent phosphorylation is necessary, but not sufficient, for mediating the mitogen-activated protein kinase (MAPK) activity of Raf1 as phosphorylated Raf1 requires additional protein-protein and membrane-lipid interactions (28). Increasing the cellular 8-oxoG level in MRC5 cells induced rapid phosphorylation of the MAPK kinase (MEK1/2) and extracellular signal-regulated kinase (ERK1/2) and the nuclear translocation of the latter (Fig. 3, A and B). To verify that ERK1/2 phosphorylation is Ras-dependent, H-, K-, and N-Ras were depleted with siRNA (Fig. 3C, upper panel). After 8-oxoG addition, ERK1/2 phosphorylation was significantly decreased in N-Ras-ablated MRC5 cells (Fig. 3C, middle panel, last lane).

Immunoblotting analysis showed abundant expression of N-Ras and K-Ras in MRC5 cells, whereas H-Ras was barely detectable (Fig. 3D, middle panel). Importantly, the addition of 8-oxoG to these cells resulted almost exclusively in N-Ras activation (Fig. 3D, upper panels), demonstrating selectivity to the activation process. These data are consistent with those showing that siRNA to N-Ras decreased ERK1/2 phosphorylation, whereas only a marginal effect of K-Ras depletion was seen (Fig. 3C, middle panel). To test whether 8-oxoG activates only N-Ras or other isoforms as well, we examined HeLa-S cells, which express H-Ras, K-Ras, and N-Ras (supplemental Fig. 6A). After 8-oxoG addition, H-, K-, and N-Ras were all activated (supplemental Fig. 6B), which implies that the 8-oxoG base activates Ras isoforms in a cell type-specific manner in OGG1-proficient cells.

In conclusion, we document for the first time that OGG1 binds the free 8-oxoG base at a nonsubstrate site with high affinity and that this complex interacts with the canonical Ras family GTPases to increase their GTP-bound forms by facilitating guanine nucleotide exchange. OGG1-mediated Ras activation initiates signal transduction for transcriptional activation of downstream genes. We propose that 8-oxoG released from DNA by OGG1 binds back to the enzyme to activate signaling of downstream genes. We propose that 8-oxoG released from DNA by OGG1 binds back to the enzyme to activate signaling of downstream genes. We propose that 8-oxoG released from DNA by OGG1 binds back to the enzyme to activate signaling of downstream genes.
8-oxoG similarly activates DNA repair in vivo has not yet been investigated. In any case, our results so far suggest an unusual, complex signaling network activated by small GTPases and triggered by the generation and repair of 8-oxoG in the genome.

Acknowledgments—We thank Dr. David Konkel (Biochemistry and Molecular Biology) for careful editing of the manuscript. We thank Drs. Miral Dizdaroglu and Pawel Jaruga (Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD) for assessment of 8-oxoG base levels. We also thank the anonymous reviewer for suggesting examination of the effect of 8-oxoG addition on MTH expression.

REFERENCES
1. Mitra, S., Hazra, T. K., Roy, R., Ikeda, S., Biswas, T., Lock, J., Boldogh, I., and Izumi, T. (1997) Complexities of DNA base excision repair in mammalian cells. Mol. Cells 7, 305–312
2. Shibutani, S., Takeda, M., and Grillman, A. P. (1991) Insertion of specific bases during DNA synthesis past the oxidation-damaged base 8-oxoG. Nature 349, 431–434
3. Cheng, K. C., Cahill, D. S., Kasai, H., Nishimura, S., and Loeb, L. A. (1992) 8-Hydroxyguanine, an abundant form of oxidative DNA damage, causes G → T and A → C substitutions. J. Biol. Chem. 267, 166–172
4. Maga, G., Villani, G., Crespan, E., Wimmer, U., Ferrari, E., Bertocci, B., and Hübscher, U. (2007) 8-Oxo-guanine bypass by human DNA polymerases in the presence of auxiliary proteins. Nature 447, 606–608
5. Haracska, L., Yu, S. L., Johnson, R. E., Prakash, L., and Prakash, S. (2000) Efficient and accurate replication in the presence of 7,8-dihydro-8-oxo-8-deoxyguanine by DNA polymerase η. Nat. Genet. 25, 458–461
6. Rosenquist, T. A., Zharkov, D. O., and grillman, A. P. (1997) Cloning and characterization of a mammalian 8-oxoguanine DNA glycosylase. Proc. Natl. Acad. Sci. U.S.A. 94, 7429–7434
7. Shigenaga, M. K., Hagen, T. M., and Ames, B. N. (1994) Oxidative damage and mitochondrial decay in aging. Proc. Natl. Acad. Sci. U.S.A. 91, 10771–10778
8. David, S. S., O'Shea, V. L., and Kundu, S. (2007) Base excision repair of oxidative DNA damage. Nature 447, 941–950
9. Klungland, A., Rosewell, I., Hollenbach, S., Larsen, E., Daly, G., Epe, B., Seeberg, E., Lindahl, T., and Barnes, D. E. (1999) Accumulation of mutagenic DNA lesions in mouse defective in removal of oxidative base damage. Proc. Natl. Acad. Sci. U.S.A. 96, 13300–13305
10. Minowa, O., Arai, T., Hirano, M., Monden, Y., Nakai, S., Fukuda, M., Itoh, M., Takano, H., Hippiou, Y., Aburatani, H., Masumura, K., Nohmi, T., Nishimura, S., and Noda, T. (2000) Min/OGG1 gene inactivation results in accumulation of 8-hydroxyguanine in mice. Proc. Natl. Acad. Sci. U.S.A. 97, 4156–4161
11. Touati, E., Michel, V., Thibierge, J. M., Avé, P., Huereau, M., Bourgade, F., Klungland, A., and Labigne, A. (2006) Deficiency in OGG1 protects against inflammation and mutagenic effects associated with H. pylori infection in mouse. Helicobacter 11, 494–505
12. Mabley, J. G., Pacher, P., Deb, A., Wallace, R., Elder, R. H., and Szabó, C. (2005) Potential role for 8-oxoguanine DNA glycosylase in regulating inflammation. FASEB J. 19, 290–292
13. Boldogh, I., Basci, A., Choudhury, B. K., Dhariajya, N., Alam, R., Hazra, T. K., Mitra, S., Goldblum, R. M., and Sur, S. (2005) ROS generated by pollen NADPH oxidase provide a signal that augments antigen-induced allergic airway inflammation. J. Clin. Invest. 115, 2169–2179
14. Block, C., Janknecht, R., Herrmann, C., Nassar, N., and Wittinghofer, A. (1996) Quantitative structure-activity analysis correlating Ras/Raf interaction in vitro to Raf activation in vivo. Nat. Struct. Biol. 3, 244–251
15. Taylor, S. J., Resnick, R. J., and Shalloway, D. (2001) Nonradioactive determination of Ras-GTP levels using activated Ras interaction assay. Methods Enzymol. 333, 333–342
16. Lai, C. C., Boguski, M., Broek, D., and Powers, S. (1993) Influence of guanine nucleotides on complex formation between Ras and CDC25 proteins. Mol. Cell. Biol. 13, 1345–1352
17. Chataway, T. K., and Barratt, G. J. (1995) Purification of histidine-tagged Ras and its use in the detection of Ras-binding proteins. Mol. Cell. Biochem. 144, 167–173
18. Hegde, M. L., Theriot, C. A., Das, A., Hegde, P. M., Guo, Z., Gary, R. K., Hazra, T. K., Shen, B., and Mitra, S. (2008) Physical and functional interaction between human oxidized base-specific DNA glycosylase NEIL1 and flap endonuclease 1. J. Biol. Chem. 283, 27028–27037
19. Field, J., Broek, D., Kataoka, T., and Wigler, M. (1987) Guanine nucleotide activation of, and competition between, RAS proteins from Saccharomyces cerevisiae. Mol. Cell. Biol. 7, 2128–2133
20. Vetter, I. R., and Wittinghofer, A. (2001) The guanine nucleotide-binding switch in three dimensions. Science 294, 1299–1304
21. Wittinghofer, F. (1998) Ras signaling: caught in the act of the switch-on. Nature 394, 337–343
22. Hu, J., de Souza-Pinto, N. C., Haraguchi, K., Hogue, B. A., Jaruga, P., Greenberg, M. M., Dizdaroglu, M., and Bohr, V. A. (2005) Repair of formamidopyrimidines in DNA involves different glycosylases: role of the OGG1, NTH1, and NEIL1 enzymes. J. Biol. Chem. 280, 40544–40551
23. Bourne, H. R., Sanders, D. A., and McCormick, F. (1990) The GTPase superfamily: a conserved switch for diverse cell functions. Nature 348, 125–132
24. Boriack-Sjodin, P. A., Margarit, S. M., Bar-Sagi, D., and Kuriyan, J. (1998) The structural basis of the activation of Ras by Sos. Nature 394, 337–343
25. Meller, N., Irani-Tehrani, M., Kiosses, W. B., Del Pozo, M. A., and Schwartz, M. A. (2002) Zizimin1, a novel Cdc42 activator, reveals a new GEF domain for Rho proteins. Nat. Cell Biol. 4, 639–647
26. Mosteller, R. D., Han, J., and Broek, D. (1994) Identification of residues of the H-ras protein critical for functional interaction with guanine nucleotide-exchange factors. Mol. Cell. Biol. 14, 1104–1112
27. Kyriakis, J. M., App, H., Zhang, X. F., Banerjee, P., Brautigan, D. L., Rapp, U. R., and Avruch, J. (1992) Raf-1 activates MEK kinase-kinase. Nature 358, 417–421
28. Nakabeyu, Y., Oka, S., Sheng, Z., Tsuchimoto, D., and Sakumi, K. (2010) Programmed cell death triggered by nucleotide pool damage and its prevention by MTH1 with oxidized purine nucleoside triphosphatase. Mutat. Res. 703, 51–58