Published in final edited form as:

DNA Repair (Amst). 2015 February ; 26: 15–22. doi:10.1016/j.dnarep.2014.11.007.

Inactivation of a common OGG1 variant by TNF-alpha in mammalian cells

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Abstract

Reactive oxygen species threaten genomic integrity by inducing oxidative DNA damage. One common form of oxidative DNA damage is the mutagenic lesion 8-oxoguanine (8-oxodG). One driver of oxidative stress that can induce 8-oxodG is inflammation, which can be initiated by the cytokine tumor necrosis factor alpha (TNF- α). Oxidative DNA damage is primarily repaired by the base excision repair pathway, initiated by glycosylases targeting specific DNA lesions. 8-oxodG is excised by 8-oxoguanine glycosylase 1 (OGG1). A common *Ogg1* allelic variant is S326C-*Ogg1*, prevalent in Asian and Caucasian populations. S326C-*Ogg1* is associated with various forms of cancer, and S326C-OGG1 is inactivated by oxidation. However, whether oxidative stress caused by inflammatory cytokines compromises OGG1 variant repair activity remains unknown. We addressed whether TNF- α causes oxidative stress that both induces DNA damage and inactivates S326C-OGG1 via cysteine 326 oxidation. In mouse embryonic fibroblasts,

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Author Contributions: P.W.D. oversaw the entire project and edited the manuscript; E.W. provided research guidance, manuscript editing, and technical expertise on the modified comet and Amplex® Red assays; C.S. carried out some of the *in vitro* experiments; K.L. edited the manuscript; Y.W.K. provided technical expertise on the *in vitro* experiments; J.F.M. carried out the majority of the *in vitro* experiments and all the other experiments, and wrote the manuscript.

Conflict of interest statement: The authors declare no conflict of interest.

Appendix A. Supplementary data: Supplementary data associated with this article can be found in the online version.

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we found that S326C-OGG1 was inactivated only after exposure to H₂O₂ or TNF- α . Treatment with the antioxidant N-acetylcysteine prior to oxidative stress rescued S326C-OGG1 activity, demonstrated by *in vitro* and cellular repair assays. In contrast, S326C-OGG1 activity was unaffected by potassium bromate, which induces oxidative DNA damage without causing oxidative stress, and presumably cysteine oxidation. This study reveals that Cys326 is vulnerable to oxidation that inactivates S326C-OGG1. Physiologically relevant levels of TNF- α simultaneously induce 8-oxodG and inactivate S326C-OGG1. These results suggest a mechanism that could contribute to increased risk of cancer among S326C-*Ogg1* homozygous individuals.

Keywords

S326C-*Ogg1*; Cys326-*Ogg1*; H₂O₂; TNF- α ; 8-oxodG; ROS

1. Introduction

Mammalian cells undergo continuous oxidative DNA damage resulting from reactive oxygen species (ROS) that are generated endogenously from sources such as normal cellular metabolism (1) and exogenously from sources such as radiation (2). Oxidative stress occurs when the concentration of ROS exhausts the cellular antioxidant capacity, leaving ROS free to damage proteins, lipids, and nucleic acids (3). ROS-induced DNA damage can drive mutagenesis if DNA damage is left unrepaired (4). One of the major physiological contexts of oxidative stress is inflammation (5). Inflammation is one of the hallmarks of cancer (6), and is a known risk factor for cancer development (7). Inflammation in tumors is driven by cytokines such as TNF- α produced by tumor-associated cells, which induces ROS (8). Moreover, tumor cells actually produce TNF- α (9), which could cause chronic oxidative stress within the tumor microenvironment. TNF- α also promotes cancer by increasing cellular transformation, measured using a focus-formation assay (10). Similarly, stem cells treated with TNF- α underwent ROS-dependent genetic instability, and such cells injected into mice caused acute myelogenous leukemia (11).

Base excision repair (BER) is the major mechanism for repairing oxidative DNA damage. BER is initiated by *N*-glycosylases, each of which excise specific subsets of base damage (12). A common oxidative lesion is the mutagenic 8-oxoguanine (8-oxodG), which causes GC $\rightarrow$ TA transversions if left unrepaired (13). In mammalian cells, OGG1 is the major BER glycosylase that initiates repair of 8-oxodG (14). S326C-*Ogg1* (rs1052133) is an allelic variant found in 40-60% of Asian and 13-38% of Caucasian individuals, and is associated with various forms of cancer, including lung cancer (15), gastric cancer (16), orolaryngeal cancer (16), and lung adenocarcinoma (17). The contribution of S326C-OGG1 to tumorigenesis may be due to inefficient repair activity. Decreased OGG1 repair activity would explain why S326C-OGG1 is deficient in suppressing spontaneous mutagenesis (18) but the physiological conditions influencing S326C-OGG1 activity remain poorly understood and controversial.

ROS can also impair the activities of proteins by oxidizing several different amino acids. Cysteine oxidation can cause disulfide bridges that can inactivate proteins (19). *In vitro* studies suggest that S326C-OGG1 may undergo decreased activity under oxidative stress

(20-22). The mechanism by which variant Cys326 oxidation causes a loss in activity has been suggested to be S326C-OGG1 dimerization, as demonstrated with EMSA following an *in vitro* reaction (23). Importantly, Cys326 oxidation has been shown to impede glycosylase activity through cysteine disulfide bridge formation via MS analysis of clinical samples (24). S326C-OGG1 has also been shown to undergo dimerization after treatment with diamide *in vitro* (20). However, such dimerization has yet to be demonstrated under conditions of physiologically relevant oxidative stress, and thus the mechanism of Cys326 oxidation resulting in loss of activity is still unclear. In contrast, several studies indicate that S326C-OGG1 enzymatic activity is equivalent to that of WT OGG1 (25-27), while others have reported constitutively reduced activity in the variant OGG1 (23,28,29). While the methodologies employed in such studies vary, each used purified enzymes under conditions that may alter the redox status of S326C-OGG1. Therefore, a relevant question remains whether the repair activity of S326C-OGG1 is affected by physiologically relevant oxidative stressors, such as low concentrations of H₂O₂ or TNF- α , to determine whether the resulting levels of ROS are sufficient to inactivate S326C-OGG1 in mammalian cells. In contrast to previous studies, we employed physiologically relevant oxidative stressors to induce DNA damage and compare the DNA repair capability of cells expressing the wild-type and S326C-*Ogg1* variant alleles.

2. Materials & Methods

2.1. Cell Culture & Plasmid Generation

Ogg1^{-/-} and F11.1 WT mouse embryonic fibroblasts (MEFs) were a gift from Arne Klungland (University of Oslo, Norway). *Ogg1*^{-/-} and F11.1 WT MEFs were authenticated by Southern hybridization analysis (14), and by Western blot. MEFs were cultured as previously described (30), except at 5% CO₂. Immortalized *Ogg1*^{-/-} MEFs (KO) stably transfected with WT *Ogg1* or S326C-*Ogg1* and expressing similar levels of glycosylase were obtained as a gift from Nikolas J. Hodges (University of Birmingham, Birmingham, UK) and were cultured as previously described (22). WT and S326C-*Ogg1* stably transfected cells were last authenticated by sequencing, and the OGG1 expression levels in these cells as well as in *Ogg1*^{-/-} KO MEFs were tested using anti-OGG1 antibody (Abcam) (31). The WT HA-tagged *Ogg1* allele on the pRVY-Tet expression vector was a gift from Joanne Sweasy (Yale University, New Haven, CT). *Ogg1*-HA was subcloned into *pcDNA-3.1* in order to increase expression to levels detectable in our *in vitro* system. PCR was used to introduce at the *Bam*HI and *Xho*I sites to the HA *Ogg1* construct, employing OS1(F) (5'-GGATCCATGCCTGCCCCGCGCTTC-3') and OS1(R) (5'-CTCGAGTCAGAGGCTAGCGTAATCC-3'). The resulting product was sequenced (Beckman Coulter Genomics, Inc.). The S326C mutation was generated employing point mutagenesis with complementary primers SC1(F) (5'-CCGACCTGCGCCAATGCCGCCATG-3') and SC2(R) (5'-CATGGCGGCATTGGCGCAGGTCGG-3'), using the WT *Ogg1*-HA construct as a template, and verified by sequencing (Beckman Coulter Genomics, Inc.).

2.2. Cell Lysate Preparation

MEFs were transfected as previously described (30), using 2 µg DNA per transfection. Cells were treated with 0-100 µM H₂O₂ in PBS for 15 min on ice, or 10 ng/mL TNF-α (R&D Systems) for 1 or 2 h in complete media. In some experiments, cells were pre-incubated for 2.5h with 1 mM N-acetylcysteine (Sigma-Aldrich Co. LLC). Cells were released with trypsin, washed twice with PBS and resuspended with 10 mM Tris pH 8.0, 2 mM EDTA, containing Complete™ protease inhibitor (Hoffmann-La Roche Ltd.). Glass beads were added to the cell solution, vortexed at maximum speed for 7 minutes, and centrifuged 10 min at 13,000 rpm. The supernatant protein concentration was measured using the DC™ Protein Assay (Bio-Rad Laboratories, Inc.), according to the manufacturer's instructions.

2.3. In Vitro OGG1 Activity Assays

Experimental lysates were incubated with 5'-5' ³²P-labeled 8-oxodG oligonucleotides, which was prepared as previously described (32). Lysates were brought to an equal volume by the addition of *Ogg1*^{-/-} MEFs lysate, and incubated in a reaction with 50 fmol labeled oligonucleotide, 10 mM Tris pH 8.0, 50 mM NaCl, and, 2 mM EDTA. Lysate reactions were incubated for 60 min at 37°C, and were stopped by adding an equal volume of 0.05% bromophenol blue, 0.05% xylene and 10 mM EDTA solution and heated to 100°C for 8 min. From each reaction, 7 µL were resolved in a 12.5% polyacrylamide UreaGel (National Diagnostics), which was then run at 2000V, 100mA, 125W for 90 min. The dried gel was then exposed overnight to a phosphorimager screen (GE Healthcare), which was then scanned using a Typhoon Trio+ device (GE Healthcare) (33). To calculate OGG1 activity, the 3' product and the uncut substrate band in the autoradiograms as well as OGG1 expression and beta actin levels measured by western blot were quantified by densitometry using ImageQuantTL software (GE Healthcare Life Sciences). The activity was corrected for transfection efficiency and protein loading among the different samples using the formula outlined below (34).

$$\text{Adjusted enzymatic activity} = \frac{3' \text{ fragment}}{3' \text{ fragment} + \text{uncut substrate}} / \frac{\text{OGG1-HA band}}{\text{Average OGG1-HA band}} / \frac{\beta\text{-actin band}}{\text{Average } \beta\text{-actin band}}$$

2.4. Western Blots

Western blots were carried out according to the Amersham ECL Plex western blotting system (GE Healthcare) per the manufacturer's recommendations. Resolution of 30 µg of protein from each lysate was achieved using Novex 10% NuPAGE gels (Life Technologies). Blots were probed with mouse monoclonal HA.11 16B12 (Covance Inc.) (36) overnight at 4°C with shaking, or with mouse monoclonal anti-β-actin antibody, clone AC-15 (Sigma-Aldrich) (37). Secondary antibody ECL Plex goat-α-mouse IgG Cy5 (GE Healthcare) (38) were used at a dilution of 1:2500. Laser scanning of blots was carried out using the Typhoon Trio™ (GE Healthcare Life Sciences), then quantified using ImageQuantTL software (GE Healthcare Life Sciences).

2.5. ROS & Cell Viability Assays

To measure cellular ROS, we employed the Amplex® Red Hydrogen Peroxide/Peroxidase Assay Kit (Life Technologies Corp). Cells were plated at 2.0×10^5 cells/well in 12-well plates, washed in PBS, then treated with 300 μ L 10 ng/mL TNF- α in complete media for 60 min at 5% CO₂ at 37°C. Concurrently, cells were incubated with 0.1 mM Amplex® Red (AR) + 0.1 U/mL horseradish peroxidase (HRP) with or without 131 μ g/mL bovine liver catalase (Sigma-Aldrich Co. LLC) for 30 min. Fluorescence was measured in 200 μ L of the supernatant in black special-optics Costar 96-well plates (Thermo Fisher Scientific) using a SpectraMax M5 spectrophotometer (Molecular Dynamics) and SoftMax Pro software (Molecular Dynamics), at 550 nm excitation and 590 nm emission frequency. Specific H₂O₂-induced AR oxidation was calculated by subtracting the reading from the corresponding catalase-treated samples. After ROS determination, to adjust Amplex® Red fluorescent signals for cell density, the cells remaining on the plate were fixed in 65% methanol for 5 min, then 100% methanol for 15 min., 0.1 % crystal violet was added to cells for 5 min, and 200 μ L 2% Na deoxycholate was used to solubilize cell-associated color. The crystal violet staining in 200 μ L of the supernatant was measured using the SpectraMax M5 spectrophotometer (Molecular Dynamics) and SoftMax Pro software (Molecular Dynamics) set to 595 nm. Cell viability was measured using the MTT assay (39). After treatment, 10 μ L 0.5% MTT (Invitrogen) was added to 100 μ L cell media, then incubated at 37°C at 5% CO₂ for 5h. Media was removed, then 100 μ L DMSO was added to each well, and incubated with shaking at RT in the dark until dye crystals dissolved. Cell viability was expressed as a percentage of surviving cells (average 595 nm absorption) using untreated cells as a reference.

2.6. Comet Assays

For comet assays, 3.0×10^6 cells were plated in 10 cm-diameter tissue culture plates the day before. Cells were collected by trypsinization, after which they were subjected to an OGG1-modified alkaline comet assay as described (40), except that 10 μ L of cells were mixed with 80 μ L 1% low melting-point agarose (Life Technologies Corp.). Damage repair was allowed by incubating in DMEM media at 37°C in 5% CO₂ for 1 h. Lysis solution included 88 μ g/mL *N-tert*-Butyl- α -phenylnitron (Calbiochem, Merck KGaA), and lysis proceeded for 2 h at 4°C. OGG1 enzyme (New England Biolabs) was incubated at 1067 U/mL in 40 mM Hepes pH 8, 100 mM KCl, 0.5 mM EDTA, and 0.02% BSA for 30 min at 37°C. DNA was separated by electrophoresis in 21 V, 0.45 mA at 4°C for 30 min in alkaline buffer supplemented with 88 μ g/mL *N-tert*-Butyl- α -phenylnitron (Calbiochem). DNA was stained with 1 μ g/mL SYBR® Green (Life Technologies Corporation) in PBS for 20 min in darkness at RT and mounted in VECTASHIELD® (Vector Laboratories, Inc.). Cells were photographed using the Hamamatsu C4742-95-12HR camera (Hamamatsu Photonics) on an Olympus IX81 microscope (Olympus Corp. of the Americas). Photographs were taken of 100 cell comets, whose average % tail DNA was then scored using CometScore (TriTek Corp.).

2.7. Cysteine Oxidation Modeling

In order to predict the likelihood that OGG1 Cys326 undergoes disulfide bridge formation, the DiANNA software program was used. This program is a neural network that predicts the likelihood of disulfide bridge formation among cysteines based on a library of secondary interaction data (41). The amino acid sequence of S326C-OGG1 was generated by downloading the 8-oxoguanine DNA-glycosylase 1 isoform 1a [Homo sapiens] NCBI accession number NP_002533.1), and substituting Cys for Ser at amino acid 326. This sequence was then entered in the DiANNA online interface. The cysteine-cysteine bond score and associated information was recorded for the highest-scoring interactions predicted.

2.8. Statistical Analysis

All statistical analysis was carried out using GraphPad Prism 6 (GraphPad Software, Inc.) In order to calculate statistical differences among discrete data points, *p* values were calculated by paired Student's t-test, while *p* values for comparing multiple values simultaneously was calculated using one-way ANOVA. All results shown are the average of three independent experiments $\pm$ standard error, unless stated otherwise.

3. Results & Conclusions

3.1. H₂O₂ Exposure Decreases S326C-OGG1 Cleavage Activity In Vitro

In order to determine the intrinsic activity of S326C-OGG1 in the absence of exogenous oxidative stress, *Ogg1*^{-/-} MEFs were transfected with WT or S326C-*Ogg1* (see Materials & Methods). Cell lysates were incubated with ³²P-labeled oligonucleotides containing 8-oxodG for 1h, sufficient to allow the oligonucleotide cleavage to proceed to the maximum extent possible (Fig. S1). The amount of cleaved versus uncleaved oligonucleotide was used to measure the intrinsic N-glycosylase/AP lyase activity of S326C-OGG1 compared to WT OGG1, using reactions without lysate as a control (Fig. 1A). No significant difference was observed between the enzymatic activity of S326C-OGG1 and WT OGG1 in non-treated cells (both with ~30% oligonucleotide cleavage, Fig. 1B). The expression of MTH1 was not an issue in these experiments because MTH1 hydrolyzes 8-oxo-dGTP, and the 1h time course for the experiment was insufficient for the occurrence of DNA replication necessary for dNTP incorporation. The expression level of OGG1 was used to normalize the measurement of 8-oxodG glycosylase activity. The expression of MUTYH, another BER enzyme, was not of interest because it excises A paired with 8-oxodG, while the oligonucleotide in these experiments was paired with C (35). To assess whether S326C-OGG1 undergoes a loss of activity under oxidative stress, *Ogg1*^{-/-} MEFs were transfected with WT or S326C-*Ogg1* and exposed to a dose range of 0-100 μ M of H₂O₂ for 15 min, which caused no cytotoxicity (Fig. S2A). S326C-OGG1 activity decreased following treatment with increasing doses of H₂O₂. Treatment with 100 μ M H₂O₂ caused a significant loss of 8-oxoguanine cleavage activity in S326C-OGG1 compared to WT OGG1, while treatment with 25 μ M H₂O₂ did not (*p* = 0.055) (Fig. 1C). Oxidative stress resulting from exposure to H₂O₂ therefore causes inactivation of the enzymatic activity of S326C-OGG1, but not WT OGG1.

3.2. S326C-OGG1 Activity Decreases after Cellular Exposure to TNF- α Due to Oxidative Stress

Because of the substantial loss in activity of S326C-OGG1 following exposure to H₂O₂, we tested whether intracellular ROS induced by an inflammatory cytokine, such as TNF- α , are also capable of inactivating S326C-OGG1. We employed *Ogg1* knockout (KO) MEFs stably expressing wild-type (42) or S326C-OGG1 at comparable levels (Fig. S3A) that express receptors for TNF- α and respond with increased intracellular ROS levels as part of the TNF- α -mediated signal transduction cascade (43). Cellular ROS increases following treatment with TNF- α were measured by employing Amplex Red (AR), which is a reporter for H₂O₂ and added catalase to duplicate wells to ensure that the fluorescent signal in the assay specifically reported levels of H₂O₂ (Fig. 2A). We exposed cells to 10 ng/mL TNF- α because it was the lowest dose at which maximal cleavage activity loss was observed (Fig. S4A), is not cytotoxic (Fig. S2B), and approximates reported plasma concentrations under pathological pro-inflammatory states (5 ng/mL) (44). Importantly, neither H₂O₂ (Fig. S5A) nor TNF- α (Fig. S5B) cause OGG1 degradation after the exposure conditions tested. TNF- α treatment causes an increase in cellular H₂O₂ levels, which is abolished in N-acetylcysteine (NAC) -treated cells (Fig. 2A). Cells were exposed to TNF- α in the presence or absence of the antioxidant NAC (45) to measure the degree to which ROS induced by TNF- α could be scavenged and reduced. These results are consistent with previously reported measurements of increased ROS following TNF- α treatment (8,46). TNF- α also caused inactivation of S326C-OGG1 after just 1h of treatment, as measured *in vitro* (Fig. 2B). The inactivation of S326C-OGG1 was prevented by treatment with NAC, while NAC treatment alone does not affect activity (Fig. 2B). WT OGG1 displayed no loss in activity after treatment with TNF- α , indicating that the activity of S326C-OGG1 is sensitive to cellular oxidative stress induced by TNF- α (Fig. 2B). The distinct sensitivity of the S326C-OGG1 variant to the redox environment may explain the disparities among measurements of S326C-OGG1 activity under different conditions previously reported in the literature (26-28).

3.3. TNF- α Exposure Causes Increases in 8-oxodG and Inactivates S326C-OGG1 Variant via Oxidation

After establishing the inactivation of S326C-OGG1 following cellular exposure to TNF- α , we tested next whether such inactivation would lead to the accumulation of 8-oxodG in nuclear DNA. We measured the 8-oxodG repair activity of S326C-OGG1 variant under oxidative stress employing the comet assay. After exposing stably-transfected *Ogg1* KO, WT, and S326C-*Ogg1* MEFs to 10 ng/mL TNF- α for 1h, the accumulation of 8-oxodG was determined by incubating the cells embedded in the agarose with recombinant OGG1. OGG1 treatment increases the number of single strand breaks detected in the alkaline comet assay by its associated lyase activity, as it primarily recognizes 8-oxodG in the nuclear DNA (40). TNF- α exposure resulted in a significant increase in 8-oxodG levels in each *Ogg1* genotype detected immediately following treatment (Fig. 3A). However, S326C-*Ogg1* variant cells and *Ogg1* KO cells were unable to repair 8-oxodG within a 60-minute recovery period following TNF- α exposure, while WT *Ogg1* cells were able to completely repair the damage. In order to exclude the possibility that S326C-OGG1 variant is constitutively inactive in this cell system, we addressed whether S326C-OGG1 possesses repair activity,

employing KBrO_3 as a DNA damaging agent which causes oxidative DNA damage without increasing cellular ROS levels (Fig. 3B) and therefore without affecting the oxidation status of cysteine residues (47). S326C-OGG1 variant was able to repair 8-oxodG arising from treatment with KBrO_3 (Fig. 3C), indicating that S326C-OGG1 is functional, but it is inactivated by cysteine oxidation during oxidative stress.

3.4. Cys326 Oxidation May Cause S326C-OGG1 Dimerization through Disulfide Bridge Formation

To address the mechanism by which S326C-OGG1 variant oxidation causes loss of activity, the role of Cys326 disulfide bridge formation was investigated by employing the DiANNA software program that predicts the likelihood of disulfide bridge formation from cysteine oxidation states using a predictive pattern recognition network based upon known secondary structure interaction data (41). DiANNA predicted that S326C-OGG1 undergoes disulfide bond formation, either inter- or intra-molecularly, between Cys326 and Cys140, Cys241, and Cys146 (Fig. S6A). While the software predicts S326C-OGG1 will form a total of six possible disulfide bonds, WT OGG1 has the potential to form only three predicted disulfide bonds (Fig. S6B), suggesting that the presence of Cys326 substantially increases the likelihood of disulfide bond formation. These results are based on the score calculated from the likelihood of oxidation of a given cysteine (compared to a database of known oxidized cysteines), the likelihood of secondary interactions between any two cysteines, and the likelihood of disulfide bridge formations between the two cysteines.

4. Discussion

We have shown for the first time that oxidative stress caused by exposure to $\text{TNF-}\alpha$ or H_2O_2 not only generates potentially mutagenic 8-oxodG lesions, but also inactivates S326C-OGG1 variant in the repair of 8-oxodG. The impact of cellular oxidative damage is therefore predicted to be greater in individuals harboring this oxidation-sensitive glycosylase variant, because they may be at a greater risk for persistent DNA damage as they remain unrepaired for extended periods of time, which may result in mutations. When S326C-*Ogg1* cells are exposed to oxidative stressors, the Cys326 residues will undergo oxidation, which may cause the S326C-OGG1 enzymes to dimerize. After undergoing oxidation, S326C-OGG1 enzymes carry out inefficient repair of 8-oxodG lesions induced by oxidative stress. Because of this increased DNA damage and decreased repair, S326C-*Ogg1* carriers may have an increased potential for cancer development under oxidative stress by a mechanism such as the one proposed in Figure 4. Our findings stand in contrast to studies showing that the S326C-OGG1 variant has constitutively reduced activity compared to the WT protein (23,28,29). The results of this study do not contradict recent findings that S326C-OGG1 undergoes aggregation *in vivo* under conditions of oxidative stress (48). However, since S326C-OGG1 dimerization has been shown in previous studies (20,23,24) and is predicted *in silico* (Fig. S6), dimerization is believed to underpin the S326C-OGG1 repair activity loss seen under oxidative stress in this study.

Consistent with this model, although only S326C-*Ogg1* homozygosity is associated with cancer among people not exposed to a particular oxidative stressor (15), both S326C-*Ogg1* variant heterozygotic and homozygotic individuals are at a significantly increased risk of

developing lung cancer after exposure to the oxidative stressors polycyclic aromatic hydrocarbons (PAHs) (49). PAHs (components of coal and tobacco smoke) cause both oxidative damage to DNA as well as the accumulation of ROS and are examples of environmental oxidative stressors that could inactivate S326C-OGG1 (49) in addition to their role as mutagens (50). Decreased activity of S326C-OGG1 may contribute to the development of lung cancer, as proposed in a study that found that low OGG1 activity is associated with lung cancer among patients who smoke tobacco (51). Such studies suggest that oxidative stress is a mechanism by which cellular microenvironments inactivate S326C-OGG1 and thereby contribute to tumorigenesis. In S326C-*Ogg1* heterozygous blood cells, increased DNA damage has been measured (52), suggesting that even a single allele of S326C-*Ogg1* may be sufficient to cause decreased 8-oxodG repair capacity. One contributor to the association between S326C-*Ogg1* and tumorigenesis may be loss of heterozygosity (LOH) (53), which is one of the most common DNA alterations in cancer (54). Although only S326C-*Ogg1* homozygosity is associated with cancer (15), S326C-*Ogg1* cells that underwent LOH would be expected to be at increased tumorigenic risk. Moreover, oxidative lesions such as 8-oxodG arise in non-random patterns that are consistent among individuals (55), suggesting there may be hotspots for oxidative damage. If such a hotspot exists in an oncogene, TNF- α could induce 8-oxodG formation, while simultaneously inactivating S326C-OGG1 via Cys326 oxidation, increasing the likelihood for the emergence of a mutation with functional consequences. Exposure to TNF- α can cause oncogenic transformation *ex vivo* in repair-compromised cells (11), suggesting that TNF- α could drive oncogenic transformation via the induction of DNA damage and through repair enzyme inactivation as shown in this study.

Collectively, the results in this study show that oxidative stress caused by TNF- α not only induces DNA damage, but for the first time that TNF- α exposure causes inefficient repair in S326C-*Ogg1* variant cells. Chronic oxidative stress, such as that caused by exposure to TNF- α (56) or by additional mechanisms involved in cell transformation (57) could cause persistent dysfunction of S326C-OGG1 and continuous induction of 8-oxodG that results in increased mutation rates. Tumor cells secrete TNF- α themselves, illustrated in a model of ovarian cancer (9), which could cause chronic oxidative stress in a signaling loop leading to tumorigenesis. Cytokine signaling contributes to the chronic oxidative stress associated with carcinogenesis, caused by oxidative damage to both proteins and DNA (58,59). S326C-OGG1 inactivation similar to the findings in these studies (~50%) was observed after S326C-*Ogg1* variant cells were treated by the inflammatory modulator nitric oxide (60). Nitric oxide can be upregulated in response to TNF- α signaling, which could contribute to a chronic oxidative stress phenotype (61). Likewise, the decreased repair capacity of BER glycosylase NEIL1 variant S82C may involve cysteine dimerization (62). Modulating the redox environment of S326C-*Ogg1* tumors could cause increased sensitivity to DNA damage-dependent cancer therapies, such as radiation therapy and cisplatin chemotherapy, which has been demonstrated to exert toxicity partly through inducing oxidative stress (58,59). Decreased S326C-OGG1 activity is likely due to cysteine disulfide bond formation and future studies should address the mechanism of S326C-OGG1 oxidation, as well as measuring the predicted increase in mutagenesis expected under such conditions. By modeling the redox regulation of S326C-OGG1 under oxidative stress, a better

understanding of the induction and management of dysfunctional DNA repair phenotypes that can contribute to tumorigenesis is likely to emerge. Ultimately, biomarker screening for S326C-*Ogg1* may be a valuable predictor of cancer risk, guiding S326C-*Ogg1* individuals toward limiting oxidative stress and inflammation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank Joanne Sweasy for the *Ogg1*-HA construct, Arne Klungland (Oslo Univ. Hospital, Oslo, Norway) for the *Ogg1*^{-/-} MEFs, and Nik Hodges (Univ. of Birmingham, Birmingham, UK) for the WT, *Ogg1* KO, and S326C-*Ogg1* MEFs. We would like to thank the Doetsch lab for their helpful input and support. We would also like to thank Xiaohui Brotzge for her guidance in *in vitro* experimental conditions. This work was supported by NIH Grant CA120288 (to P.W.D.).

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Highlights

- Physiological TNF- α levels induce 8-oxodG and inactivate S326C-OGG1.
- S326C-OGG1 variant inactivation is mediated by ROS induced by TNF- α exposure.
- The activity of S326C-OGG1 and WT OGG1 is equivalent without oxidative stress.

Fig. 1.

The activity of S326C-OGG1 and WT OGG1 is equivalent in the absence of oxidative stress, and only S326C-OGG1 activity decreases following treatment with an oxidative stressor. **A.** ³²P-labeled 8-oxodG-containing oligonucleotide fragments were resolved in a polyacrylamide urea gel generated after incubation with whole-cell lysates from *Ogg1*^{-/-} MEFs subjected to mock transfection (control), or transfected with WT *Ogg1* or S326C-*Ogg1*. Only the portion of the gel showing uncut oligo, 3' product, and 5' product is shown. **B.** Cleavage activity from three experiments was averaged following adjustment for OGG1 expression as described in **Materials & Methods**. S326C-OGG1 and WT OGG1 have similar levels of activity in the absence of oxidative stress. The statistical comparison between the activity of WT and S326C-*Ogg1* samples was carried out using a paired Student's t-test. **C.** Treatment with 100 μM H₂O₂ inhibits S326C-OGG1, but not WT OGG1 activity. Activity was measured by exposing cells to 0-100 μM H₂O₂ for 15 min, then measuring cleavage activity as described in **A**, except expressed as a percentage of the mean activity of the untreated samples. Values shown represent the average of 3 independent experiments ± SE. Significant differences are represented by (*) $p < 0.05$ according to one-way ANOVA.

Fig. 2. S326C-OGG1 variant is inactivated by cellular exposure to TNF- α . **A.** TNF- α exposure increases H_2O_2 levels in mouse embryonic fibroblasts. WT, S326C-*Ogg1*, and *Ogg1* KO MEFs were treated with 10 ng/mL of TNF- α in media for 1h, then subjected to treatment with Amplex Red for 30 min. Results shown are the average of 3 independent experiments, all $\pm$ SE. **B.** TNF- α inhibits S326C-OGG1 through oxidation. Activity assay results indicate that N-acetylcysteine (NAC) treatment reverses the activity inhibition caused by TNF- α treatment in S326C-*Ogg1* cells. *Ogg1*^{-/-} MEFs were transfected with WT or S326C-*Ogg1*, treated with 10 ng/mL TNF- α for 1h $\pm$ pre-treatment with 1 mM NAC for 2.5h or 100 μ M H_2O_2 for 15 min, then subjected to the oligonucleotide cleavage assay described previously. All values represent the average of 3 independent experiments $\pm$ SE. Significant differences are represented by (*) $p < 0.05$ according to one-way ANOVA. "N.S." represents a non-significant difference.

Fig. 3. S326C-Ogg1 variant cells display a loss in DNA repair activity under oxidative stress. **A.** S326C-Ogg1 variant cells display delayed repair activity following exposure to TNF-α. Cells were immobilized in agarose, treated with 10 ng/mL TNF-α for 1h, lysed, the DNA was digested with OGG1, then subjected to alkaline electrophoresis. The percentage of DNA that migrated beyond the nuclear area was quantified after fluorescent microscopy in 100 cells per cell line per treatment. **B.** KBrO₃ treatment causes oxidative DNA damage without increasing cellular H₂O₂. WT MEFs were treated with 2 mM KBrO₃ or 10 ng/mL TNF-α for 1h. MEFs were then subjected to Amplex Red (AR) treatment, and the fluorescent signal was measured. The results shown are the average of three independent experiments ± SE. **C.** S326C-Ogg1 repair activity of nuclear DNA damage is comparable to WT OGG1 when oxidative stress is not imposed. Cells were subjected to OGG1-modified alkaline comet assay as in **A**, except that cells were treated with 2mM KBrO₃ for 1h instead of TNF-α. All values represent the average of 3 independent experiments ± SE. Significant differences are represented by (*) $p < 0.05$ according to one-way ANOVA. "N.S." represents a non-significant difference.

Fig. 4.

Proposed biological model for increased tumorigenic potential in S326C-*Ogg1* variant cells. **(1a & 1b)** H₂O₂ and TNF-α cause cellular oxidative stress that induces DNA damage in WT and S326C-*Ogg1* cells. **(2a)** In WT *Ogg1* cells, 8-oxodG damage is efficiently repaired. **(2b)** However, H₂O₂ and TNF-α exposure diminishes S326C-OGG1 activity *in vivo* (presumably via Cys326 oxidation that results in dimerization), causing accumulation of mutagenic 8-oxodG that is not repaired efficiently. **(3a)** Repaired 8-oxodG is bypassed nonmutagenically by DNA polymerase (DNAP) during replication. **(3b)** Unrepaired 8-oxodG is also bypassed by DNAP during replication, which can establish a permanent mutation. **(4b)** Increased levels of 8-oxodG and a decreased 8-oxodG repair capacity may cause an increase in mutation frequency underlying the association between S326C-*Ogg1* and tumorigenesis, particularly under oxidizing conditions.