Tumor Necrosis Factor-α Increases Reactive Oxygen Species by Inducing Spermine Oxidase in Human Lung Epithelial Cells: A Potential Mechanism for Inflammation-Induced Carcinogenesis

Naveen Babbar and Robert A. Casero, Jr.

The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland

Abstract

Inflammation has been implicated in the development of many human epithelial cancers, including those of the stomach, lung, colon, and prostate. Tumor necrosis factor-α (TNF-α) is a potent pleiotropic, proinflammatory cytokine produced by many cells in response to injury and inflammation. Here, we show that TNF-α exposure results in increased production of reactive oxygen species (ROS), with a concomitant increase in the production of 8-oxo-deoxyguanosine, a marker for oxidative DNA damage, in human lung bronchial epithelial cells. The source of the ROS in TNF-α–treated cells was determined by both pharmacologic and small interfering RNA (siRNA) strategies to be spermine oxidase (SMO/PAOh1). SMO/PAOh1 oxidizes spermine into spermidine, 3-aminopropanal, and H_2O_2. Inhibition of TNF-α–induced SMO/PAOh1 activity with MDL 72,527 or with a targeted siRNA prevented ROS production and oxidative DNA damage. Further, similar induction in SMO/PAOh1 is observed with treatment of another inflammatory cytokine, interleukin-6. The data are consistent with a model that directly links inflammation and DNA damage through the production of H_2O_2 by SMO/PAOh1. Further, these results suggest a common mechanism by which inflammation from multiple sources can lead to the mutagenic changes necessary for the development and progression of epithelial cancers. (Cancer Res 2006; 66(23): 11125-30)

Introduction

Carcinogen exposure and chronic inflammation are two important events in tumor development, with the latter accounting for ~20% of human cancers (1). Whereas the causal relationship between carcinogen exposure and cancer has been intensely investigated, the molecular and cellular mechanisms linking chronic inflammation to tumorigenesis remain largely unresolved. Acute inflammation starts a cascade of cytokines and chemokines that attract immune and nonimmune cells to infiltrate disrupted and damaged tissue. The process of acute inflammation is usually self-limiting because the production of proinflammatory cytokines gives way to the anti-inflammatory cytokines as healing progresses (2, 3). Tumor necrosis factor-α (TNF-α) is a potent pleiotropic cytokine and is a major mediator of inflammation with multiple biological functions (4). TNF-α plays a critical role in both tissue destruction and damage recovery and may initiate an inflammatory cascade consisting of other inflammatory cytokines (5). Low-dose, chronic TNF-α production is a feature of many tumor cells. TNF-α acts as a growth factor in certain tumor types, increasing levels of positive cell cycle regulators, as well as components of growth factor receptor signaling pathways, such as ras and c-*myc* (6). TNF-α has also been shown to have roles in inducing chemoresistance in several cancers, mediating androgen independence in prostate cancer and both promoting DNA damage and inhibiting DNA repair by up-regulating nitric oxide–dependent pathways (7). Further, TNF-α has been shown to lead to the production of reactive oxygen species (ROS) in many different cell types (8, 9).

Helicobacter pylori infection and its attendant inflammation have been associated with gastric cancer. It has been shown recently that *H. pylori* induces spermine oxidase (SMO/PAOh1) in exposed gastric epithelial cells resulting in the production of sufficient H_2O_2 to result in DNA damage (10). SMO/PAOh1 is an inducible FAD-dependent polyamine oxidase, which oxidizes spermine, to produce spermidine, H_2O_2, and 3-aminopropanal (11). These results showed a link between bacterial infection and a cellular mechanism to produce some of the necessary genetic changes that are required for the initiation and progression of cancer (10).

As several cancers are associated with inflammation, with or without associated infectious agents, we sought to determine if exposure to TNF-α, a general mediator of inflammation, induced SMO/PAOh1, H_2O_2 production, and DNA damage in a human lung epithelial cell model. The results indicate that TNF-α exposure leads to the induction of SMO/PAOh1, which produces sufficient H_2O_2 to result in potentially mutagenic DNA damage and presents a molecular mechanism by which general inflammation can contribute directly to the development of cancer.

Materials and Methods

Materials. All cell culture reagents, DNA-modifying enzymes, Trizol reagent (total RNA isolation reagent), and LipofectAMINE reagent were purchased from Life Technologies, Inc. (Rockville, MD). All primers and oligonucleotides were custom made by Invitrogen Corp. (Carlsbad, CA). Recombinant human TNF-α and recombinant interleukin-6 (IL-6) were obtained from R&D Systems, Inc. (Minneapolis, MN).

Cell culture. Beas-2B cells were obtained from American Type Culture Collection (Rockville, MD). They are an immortalized line of nontumorigenic human bronchial epithelium derived by infection of primary cells with adenovirus 12-SV40 virus hybrid and grown in modified LHC-9 medium supplemented with 100 units/mL each of penicillin and streptomycin. Cultures were maintained at 37°C in 5% CO_2 to 90% confluence. The nontumorigenic HBEC3KT human bronchial epithelial cell line was obtained from Drs. Jerry Shay and John Minna (Hamon Center for Therapeutic Oncology Research at University of Texas Southwestern Medical Center, Dallas, TX; ref. 12). It was immortalized by introducing cyclin-dependent kinase 4 and hTERT into normal human breast epithelial cells obtained from a 65-year-old woman without cancer. HBEC3KT was

Requests for reprints: Robert A. Casero, Jr., The John Hopkins Oncology Center, Bunting/Blaustein Cancer Research Building, Room 551, Baltimore, MD 21231. Phone: 410-955-8580; Fax: 410-614-9884; E-mail: rcasero@jhmi.edu.

doi:10.1158/0008-5472.CAN-06-3174

cultured with keratinocyte serum-free medium (Life Technologies) containing 50 μg/mL bovine pituitary extract (Life Technologies) and 5 ng/mL human epidermal growth factor (Life Technologies). Cell number and viability were determined by trypan blue exclusion.

Quantitative PCR. Total cellular RNA was extracted using Trizol reagent and RNA isolation according to the method developed by Chomczynski and Sacchi (13). To determine changes in levels of SMO/PAOh1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), quantitative PCR (qPCR) was done as reported previously (14). SMO/PAOh1 cDNA (287 bp) was amplified using the following primers: 5′-GATCCCGGCGGACCATGTGATTGTG-3′ (forward) and 5′-CTCAGGCGGGTAGAGGACATCAAA-3′ (reverse) and, as a loading control, GAPDH cDNA (188 bp) was also amplified using the following primers: 5′-GAAGGTGAAGGTCGGAGTC-3′ (forward) and 5′-GAAGATGGTGATGGGATTTC-3′ (reverse).

Quantitative Western blotting. Total cell extracts were obtained by lysing cells on ice in radioimmunoprecipitation assay buffer (PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 30 μg/mL aprotinin, 100 μmol/L sodium orthovanadate, 10 μg/mL phenylmethylsulfonyl fluoride) and insoluble material was pelleted at 12,000 × *g* for 20 minutes at 4°C. Thirty microgram total protein was loaded per lane and separated on a 10% SDS-PAGE gels transferred electrophoretically to immunoblot polyvinylidene difluoride membrane (Bio-Rad) for immunoblotting. Membranes were blocked for 1 hour in Odyssey blocking buffer as per manufacturer's instructions. The primary antibodies, an affinity-purified antisera to the human SMO/PAOh1 peptide sequence CIHWDQASARPRGPEIPR raised in rabbits by standard methods, and a mouse anti-β-actin, purchased from Sigma (St. Louis, MO), were used at dilutions of 1:1,000 and 1:1,500, respectively, with 0.1% Tween 20 in blocking buffer for 1 hour at room temperature. Following washes with PBS-T, blots were incubated with appropriate fluorescent dye-conjugated secondary antibodies (1:4,000 each, 0.1% Tween 20, in blocking buffer, protected from light, for 45 minutes). Western blot results were quantified using the LI-COR immunofluorescence system (LI-COR Biosciences, Lincoln, NE).

RNA interference and stable transfections. Stable clones expressing small interfering RNA targeting SMO/PAOh1 or spermidine/spermine N^1-acetyltransferase (SSAT) were generated as reported previously (15). Individual colonies representing Beas-2B-SMO/ctrl (nonsense vector control), Beas-2B-SMO/kd (SMO/PAOh1 stably knocked down), Beas-2B-SSAT/ctrl (nonsense vector control), and Beas-2B-SSAT/kd (SSAT stably knocked down) were chosen. Clones were selected and maintained in LHC-9 medium supplemented with either 200 μg/mL G418 (Sigma) or 100 μg/mL hygromycin (Roche Applied Science, Indianapolis, IN) as required. All data presented here are representative of multiple, independent experiments done using two clones for each cell type.

Measurement of H_2O_2. Intracellular H_2O_2 was quantified using flow cytometry and the cell-permeable redox-sensitive dye CM-H_2DCFDA as reported previously (14). Briefly, cells were treated for the indicated times with TNF-α, harvested with trypsin, and washed with 1× PBS (Mediatech, Herndon, VA), and 1 × 10^6 cells were treated with 10 μmol/L CM-H_2DCFDA for 20 minutes at 37°C. Ten thousand cells were then analyzed by fluorescence-activated cell sorting on a BD-LSR (BD Biosciences, Rockville, MD).

Oxidative DNA damage. DNA damage was determined by binding of FITC conjugate to 8-oxo-deoxyguanosine (8-oxodG) as reported previously (16). In brief, after fixation and permeabilization, cells were washed, blocked, and incubated with OxyDNA FITC conjugate (Calbiochem, San Diego, CA) for 1 hour in the dark. Cells were resuspended in PBS and analyzed by flow cytometry for fluorescence.

Enzyme activity determination. Cells were harvested after treatment and washed in cold PBS. The enzyme activities of SMO/PAOh1 and N^1-acetylpolyamine oxidase (APAO) in cell lysates were assayed as described

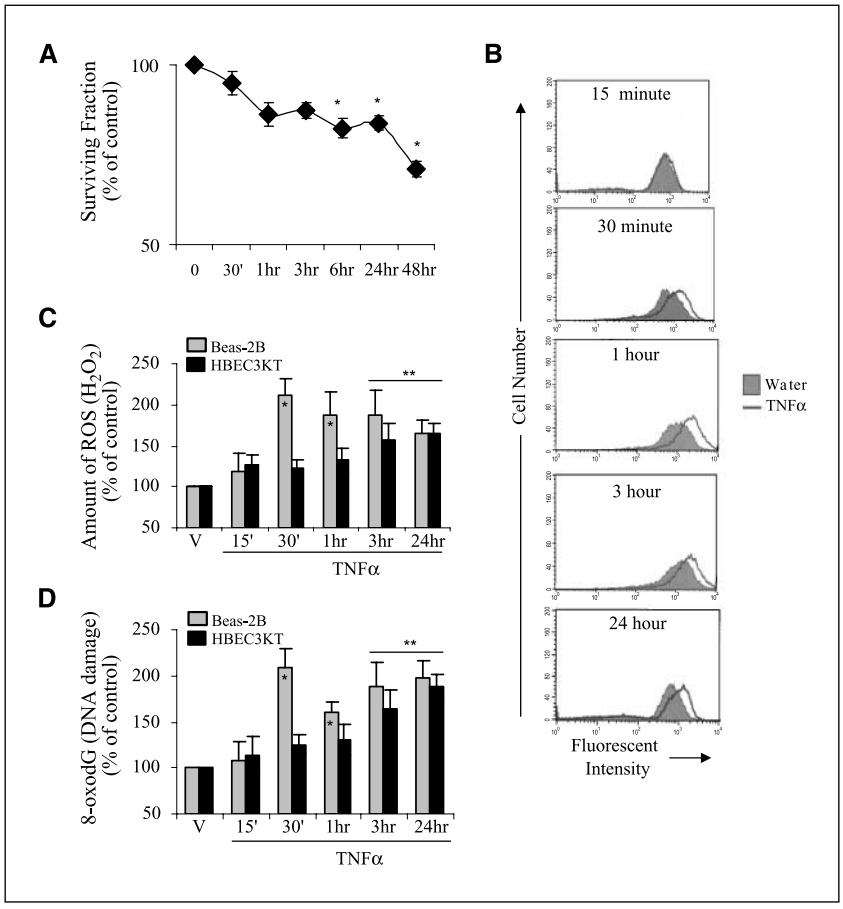
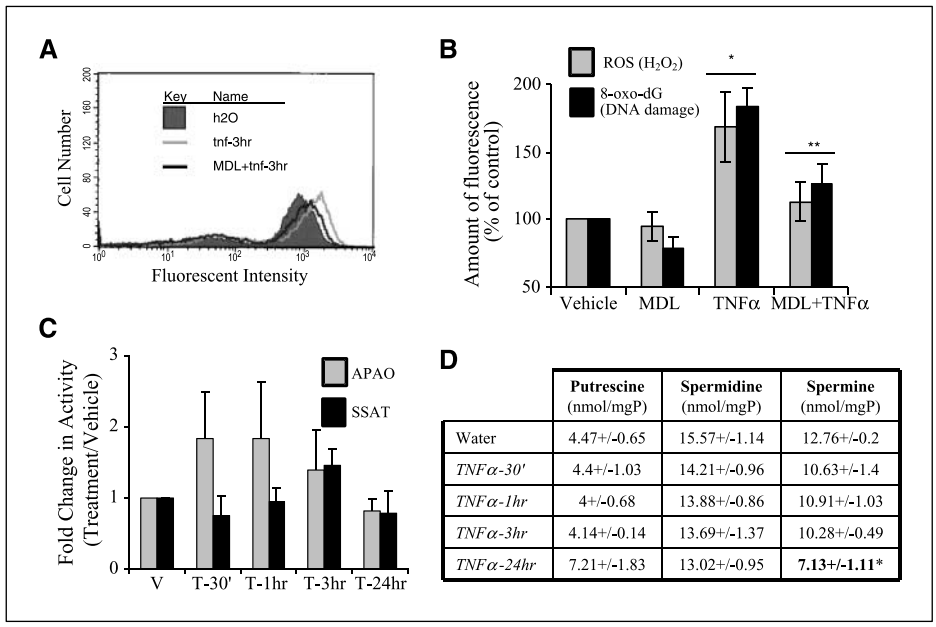


Figure 1. TNF-α reduces cell survival, increases H_2O_2 production, and induces DNA damage in human lung epithelial cells. *A,* Beas-2B cells were grown overnight and then treated with 10 ng/mL TNF-α. Cells were harvested at the indicated times and counted. *Points,* average of three independent experiments. *, $P < 0.05$. *B,* Beas-2B cells were treated with 10 ng/mL TNF-α for the indicated times, harvested, and incubated with 10 μmol/L CM-H_2DCFDA for 20 minutes. *X axis,* fluorescent intensity; *Y axis,* cell number. Representative results of three experiments that gave similar results. *C* and *D,* Beas-2B (*gray columns*) and HBEC3KT (*black columns*) cells were treated with 10 ng/mL TNF-α or its vehicle (*V*) for the indicated times, harvested, and incubated with 10 μmol/L CM-H_2DCFDA to quantify H_2O_2 productions (*C*) for 20 minutes or with FITC conjugate to determine DNA damage (*D*) for 1 hour. The amount of fluorescence was quantified and plotted as a percentage of the vehicle-treated controls. *Columns,* mean of three independent experiments; *bars,* SD. *, $P < 0.05$; **, $P < 0.001$.



	Putrescine (nmol/mgP)	Spermidine (nmol/mgP)	Spermine (nmol/mgP)
Water	4.47+/-0.65	15.57+/-1.14	12.76+/-0.2
TNFα-30'	4.4+/-1.03	14.21+/-0.96	10.63+/-1.4
TNFα-1hr	4+/-0.68	13.88+/-0.86	10.91+/-1.03
TNFα-3hr	4.14+/-0.14	13.69+/-1.37	10.28+/-0.49
TNFα-24hr	7.21+/-1.83	13.02+/-0.95	**7.13+/-1.11***

Figure 2. TNF-α induces H_2O_2 production and DNA damage through polyamine catabolism. *A,* Beas-2B cells were treated with 10 ng/mL TNF-α, with or without pretreatment for 3 hours with 100 μmol/L MDL 72,527, harvested, and treated with 10 μmol/L CM-H_2DCFDA for 20 minutes. *X axis,* fluorescent intensity; *Y axis,* cell number. Representative results of three experiments that gave similar results. *B,* production of H_2O_2 (ROS; *gray columns*) and DNA damage (8-oxodG; *black columns*) *columns*) in Beas-2B cells treated with 10 ng/mL TNF-α with or without 100 μmol/L MDL 72,527 pretreatment or its vehicle for 3 hours. *Columns,* mean of three independent experiments; *bars,* SD. *, $P < 0.05$ between the vehicle- and TNF-α–treated samples; **, $P < 0.05$ between the TNF-α–treated and TNF-α+MDL 72,527–treated samples. *C,* enzyme activity for APAO (*gray columns*) and SSAT (*black columns*) protein in Beas-2B cells treated with TNF-α or its vehicle for the indicated times. Fold induction was calculated by dividing enzyme activity of the treated sample by the vehicle-treated control. *Columns,* mean of three different experiments; *bars,* SD. *D,* intracellular polyamine content in Beas-2B cells treated with either vehicle or TNF-α for 30 minutes and 1, 3, and 24 hours. The result is the mean of three different experiments done in duplicate ±SD. *, $P < 0.05$.

previously by using 250 μmol/L spermine (Sigma) or N^1-acetylspermine (Sigma), respectively, as the substrate (11). SSAT activity was determined by measuring the amount of labeled N^1-acetylspermidine synthesized from [^{14}C]acetyl-CoA and unlabeled spermidine, as described previously (17). Protein was determined by Bradford assay.

Statistical analysis. All experiments were done in triplicates and repeated at least thrice. qPCR assays were done at least thrice. Representative experiments or mean values ± SD are shown. Statistical differences were determined by Student's *t* test. A *P* value of <0.05 was considered significant.

Results and Discussion

TNF-α induces ROS production and increased DNA damage in lung epithelial cells. TNF-α, a common mediator of inflammation, has the ability to activate many intracellular

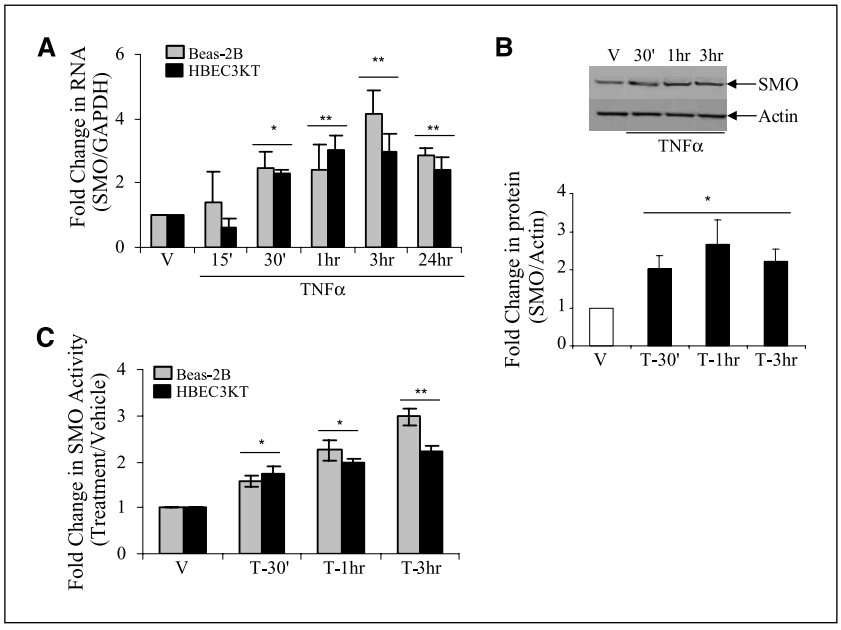


Figure 3. TNF-α induces SMO/PAOh1 in Beas-2B cells. *A,* quantitative reverse transcription-PCR (qPCR) for SMO/PAOh1 expression from Beas-2B (*gray columns*) and HBEC3KT (*black columns*) cells treated with TNF-α or its vehicle for the indicated times. *Columns,* mean of three different experiments; *bars,* SD. *, $P < 0.05$; **, $P < 0.001$. *B,* representative Western blot and quantified results for SMO/PAOh1 protein in Beas-2B cells treated with TNF-α for 30 minutes, 1 hour, and 3 hours. *Columns,* mean of five independent experiments; *bars,* SD. *, $P < 0.05$. *C,* enzyme activity for SMO/PAOh1 protein in Beas-2B (*gray columns*) and HBEC3KT (*black columns*) cells treated with TNF-α or its vehicle for the indicated times. Relative luciferase units were calculated after normalizing to the protein in the cell lysates. Representative of three experiments. *Columns,* fold induction relative to the activity in the presence of vehicle alone; *bars,* SD. *, $P < 0.05$; **, $P < 0.001$.

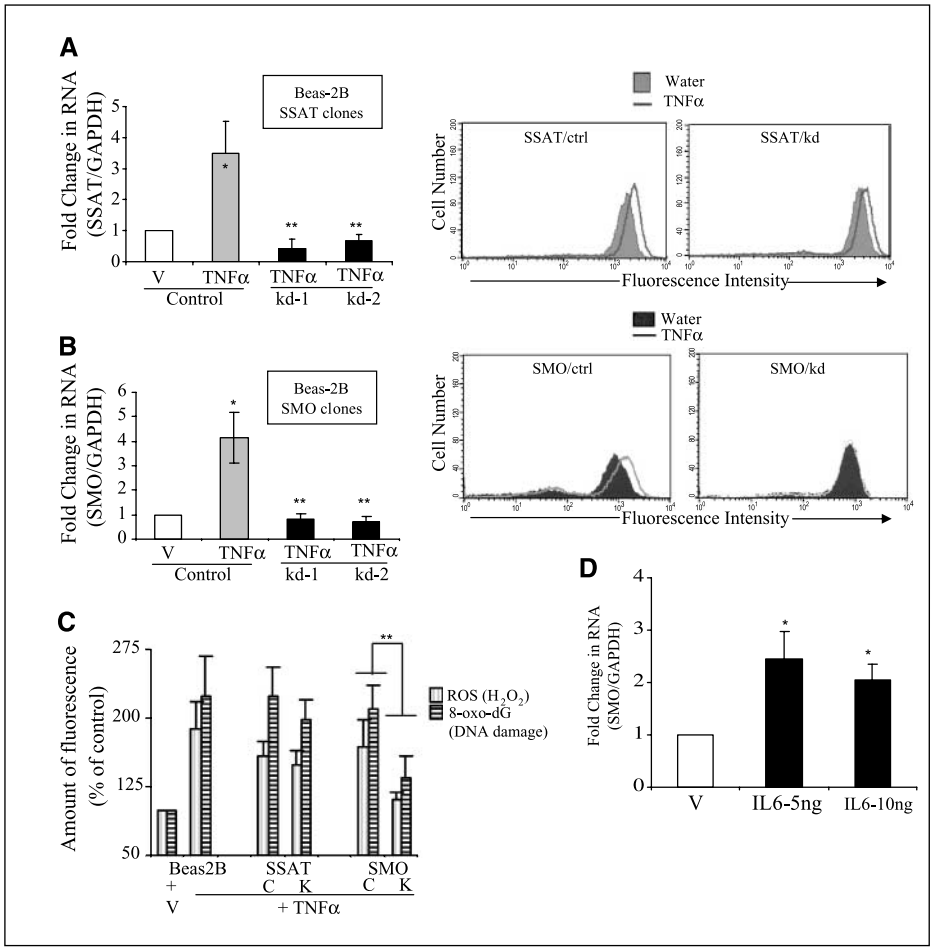


Figure 4. Knockdown of SMO/PAOh1, but not SSAT, expression abolished H_2O_2 production and reduces DNA damage in Beas-2B cells. *A,* left SSAT mRNA measured in Beas-2B-SSAT/ctrl and Beas-2B-SSAT/kd cells treated with either vehicle or 10 ng/mL TNF-α for 3 hours. *Columns,* mean of three different experiments; *bars,* SD. *, $P < 0.05$ between the vehicle- and TNF-α–treated SSAT/ctrl samples. **, $P < 0.05$ between the TNF-α–treated SSAT/ctrl and SSAT/kd samples. Right Beas-2B-SSAT/ctrl or Beas-2B-SSAT/kd cells were treated with 10 ng/mL TNF-α or its vehicle for 3 hours, harvested, and treated with 10 μmol/L CM-H_2DCFDA for 20 minutes. *X axis,* fluorescent intensity; *Y axis,* cell number. Representative results of one of three experiments that gave similar results. *B,* upper SMO/PAOh1 mRNA measured in Beas-2B-SMO/ctrl and Beas-2B-SMO/kd cells treated with either vehicle or 10 ng/mL TNF-α for 3 hours. *Columns,* mean of three different experiments; *bars,* SD. *, $P < 0.05$ between the vehicle- and TNF-α–treated SMO/ctrl samples. **, $P < 0.05$ between the TNF-α–treated SMO/ctrl and SMO/kd samples. Lower Beas-2B-SMO/ctrl or Beas-2B-SMO/kd cells were treated with 10 ng/mL TNF-α or its vehicle for 3 hours, harvested, and treated with 10 μmol/L CM-H_2DCFDA for 20 minutes. Data are representative results of one of three experiments that gave similar results. *C,* H_2O_2 and DNA damage as assessed by production of ROS (*vertical lines columns*) and 8-oxodG (*horizontal lines bar*). All cells were treated with 10 ng/mL TNF-α or vehicle for 3 hours, harvested, and incubated with either CM-H_2DCFDA or FITC conjugate. *Columns,* mean of three different experiments; *bars,* SD. *, $P < 0.05$ between the vehicle- and TNF-α–treated Beas-2B samples. **, $P < 0.05$ between the TNF-α–treated Beas-2B-SMO/ctrl and Beas-2B-SMO/kd samples. *D,* qPCR for SMO/PAOh1 expression from Beas-2B cells treated with 5 or 10 ng IL-6 (*black columns*) or vehicle (*white columns*) for 1 hour. *Columns,* mean of three different experiments; *bars,* SD. *, a significance with $P < 0.05$.

pathways leading to the regulation of downstream genes, which are involved in cell-survival and cell-death pathways (5). One of the downstream signaling events of TNF-α is the generation of ROS. ROS can be DNA-damaging agents that increase mutation rates and promote oncogenic transformation (18). To study the effects of TNF-α inflammatory stress, we treated Beas-2B cells, a nontumorigenic human lung epithelial cell line with 10 ng/mL of TNF-α and estimated the changes in H_2O_2. TNF-α exposure led to a decrease in cell viability (Fig. 1*A*) concurrent with a rapid and significant increase in the ROS production as indicated by the shift to the right of the fluorescence peaks in the presence of CM-H_2DCFDA, an oxidation-sensitive fluorescent probe (Fig. 1*B* and *C*). DNA damage produced by ROS can result in base modifications of which 8-oxodG is the most common (19). We quantified DNA modifications induced by TNF-α exposure, using an 8-oxodG-targeted FITC conjugate, and found that TNF-α significantly increased 8-oxodG levels (Fig. 1*D*). The time frame for the generation of H_2O_2 is similar to the production of 8-oxo-dG, which suggests a casual relationship between the two. Further, this time frame is consistent with that observed for other complex signaling networks activated by TNF-α (20). The induction of H_2O_2 production and DNA damage by TNF-α was not limited to Beas-2B cells as a similar trend was observed in the HBEC3KT human bronchial epithelial cells (Fig. 1*C* and *D*). It is important to note that measurable DNA damage persists throughout the entire 24 hours of treatment consistent with the hypothesis that chronic inflammation has the potential to lead to the accumulation of DNA damage in epithelial cells.

TNF-α induces H_2O_2 production by inducing spermine oxidation in lung epithelial cells. H_2O_2 can be produced by many different enzymes (predominantly oxidases) in the cells. The possibility that polyamine oxidation could be the source of inflammation-induced ROS is suggested by the results with

H. pylori showing that infection leads to the production of ROS by inducing the SMO/PAOh1 (10). Spermine is a polyamine that can be oxidized intracellularly by two pathways, both of which can produce ROS (21). Spermine can act as a direct substrate for SMO/PAOh1, which converts spermine into spermidine, H_2O_2, and 3-aminopropanal or spermine can be first acetylated by SSAT and then oxidized by APAO producing H_2O_2 as a byproduct. To determine if polyamine catabolism is the source of TNF-α–induced H_2O_2 production, MDL 72,527, an inhibitor of both APAO and SMO/PAOh1 (22), was used in combination with TNF-α treatment. Pretreatment of cells with 100 μmol/L MDL 72,527 attenuated the TNF-α–induced H_2O_2 production, indicating that oxidation of polyamines by SMO/PAOh1 and/or APAO plays a central role in the production of H_2O_2 in response to TNF-α (Fig. 2*A*). Consistent with the effects of H_2O_2 on the production of 8-oxodG, MDL 72,527 pretreatment also led to a significant reduction in the formation of 8-oxodG bases compared with the TNF-α alone (Fig. 2*B*).

Although the MDL 72, 527 inhibitor experiments are consistent with the hypothesis that polyamine catabolism is involved in the TNF-α–induced H_2O_2, they do not indicate precisely which enzyme or enzymes are involved. Therefore, the effects of TNF-α exposure on the expression of these three enzymes involved in the spermine catabolism were determined at the same times when ROS production is induced. TNF-α exposure led to an induction in SSAT mRNA (data not shown), but no effect on the SSAT and APAO enzyme activities were observed (Fig. 2*C*). Most importantly, TNF-α led to an induction in the SMO/PAOh1 expression at the levels of mRNA (Fig. 3*A*), protein (Fig. 3*B*), and enzyme activity (Fig. 3*C*). The intracellular spermine concentrations of Beas-2B cells seemed to decrease with increased TNF-α exposure time. However, this decrease only became significant after 24 hours (Fig. 2*D*). The maintenance of relative polyamine homeostasis, even in the presence of increased SMO/PAOh1, is consistent with observations that TNF-α also up-regulates polyamine biosynthesis (23). Thus, the flux through the polyamine metabolic pathway may play a role in chronic inflammation by continually providing substrate for oxidation.

SMO/PAOh1, but not SSAT, is required for the TNF-α–induced oxidative DNA damage. Although there was no significant change in APAO and SSAT activity in response to TNF-α, the SSAT/APAO pathway could not be formally excluded as the source of H_2O_2. Therefore, to determine which catabolic pathway was responsible for the TNF-α–induced H_2O_2 production, RNA interference (RNAi) was used to knock down the expression of each enzyme in Beas-2B cells. These RNAi sequences have been used recently to efficiently reduce the related mRNAs in breast cancer cells (15). Beas-2B clones exhibiting the greatest decrease in SSAT and SMO/PAOh1 expression were chosen for further study. It is important to note that, identical to our results in the breast cancer lines (15), the knockdown of one gene did not effect the expression of the untargeted gene. Two clones for each cell line were analyzed for both SSAT and SMO/PAOh1 induction and H_2O_2 production after TNF-α treatment for 3 hours. Beas-2B clones with SSAT knockdown failed to induce SSAT expression and had no significant effect on the production of H_2O_2 by TNF-α (Fig. 4*A*). TNF-α–treated Beas-2B clones with SMO/PAOh1 knockdown did not exhibit induced SMO/PAOh1 expression and totally blocked the production of ROS (Fig. 4*B*). Further, suppression of SMO expression, but not of SSAT, blocked the production of 8-oxodG by TNF-α in the Beas-2B cells (Fig. 4*C*), which shows an integral role for SMO/PAOh1 activity leading to oxidative DNA damage by TNF-α. To determine if the induction of SMO/PAOh1 was unique to TNF-α or if another mediator of inflammation could also induce SMO/PAOh1 the effects of IL-6, another cytokine produced in response to inflammation was examined (24, 25). Exposure to IL-6 at 5 and 10 ng/mL led to a significant induction in SMO/PAOh1 mRNA (Fig. 4*D*) and enzyme activity (data not shown) after 1 hour of exposure. Further, IL-6 treatment also led to an induction in the production of ROS in these cells (data not shown), which suggests a role of SMO/PAOh1 in the induction of ROS by IL-6.

The above results are consistent with a model that directly links inflammation and DNA damage through the production of the ROS, H_2O_2 by the oxidation of spermine by SMO/PAOh1 in lung epithelial cells. Further, these data suggest a common pathway by which inflammation from multiple sources can lead to the mutagenic changes necessary for the development and progression of multiple epithelial cancers. Although additional studies will be necessary to determine what specific inflammatory signaling pathways are involved the in TNF-α–dependent induction of SMO/PAOh1, these data have considerable significance to both the understanding of the etiology of inflammation-associated cancers and to the development of chemopreventive strategies.

Acknowledgments

Received 8/28/2006; revised 10/9/2006; accepted 10/20/2006.

Grant support: NIH grants CA 51085 and CA 98454.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Leslie Mesler for technical assistance with flow cytometry and Drs. Jerry W. Shay and John D. Minna for providing the HBEC3KT cell line.

References

1. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–7.
2. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-β, PGE_2, and PAF. J Clin Invest 1998;101:890–8.
3. Hodge-Dufour J, Marino MW, Horton MR, et al. Inhibition of interferon γ induced interleukin 12 production: a potential mechanism for the anti-inflammatory activities of tumor necrosis factor. Proc Natl Acad Sci U S A 1998;95:13806–11.
4. Beutler BA. The role of tumor necrosis factor in health and disease. J Rheumatol Suppl 1999;57:16–21.
5. Balkwill F. Tumor necrosis factor or tumor promoting factor? Cytokine Growth Factor Rev 2002;13:135–41.
6. Szlosarek PW, Grimshaw MJ, Kulbe H, et al. Expression and regulation of tumor necrosis factor α in normal and malignant ovarian epithelium. Mol Cancer Ther 2006;5:382–90.
7. Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. Cancer Res 2000;60:184–90.
8. Schulze-Osthoff K, Beyaert R, Vandevoorde V, Haegeman G, Fiers W. Depletion of the mitochondrial electron transport abrogates the cytotoxic and gene-inductive effects of TNF. EMBO J 1993;12:3095–104.
9. Chandel NS, Schumacker PT, Arch RH. Reactive oxygen species are downstream products of TRAF-mediated signal transduction. J Biol Chem 2001;276:42728–36.
10. Xu H, Chaturvedi R, Cheng Y, et al. Spermine oxidation induced by *Helicobacter pylori* results in apoptosis and DNA damage: implications for gastric carcinogenesis. Cancer Res 2004;64:8521–5.
11. Wang Y, Devereux W, Woster PM, Stewart TM, Hacker A, Casero RA, Jr. Cloning and characterization of a human polyamine oxidase that is inducible by polyamine analogue exposure. Cancer Res 2001;61:5370–3.
12. Ramirez RD, Sheridan S, Girard L, et al. Immortalization of human bronchial epithelial cells in the absence of viral oncoproteins. Cancer Res 2004;64:9027–34.
13. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987;162:156–9.

14. Babbar N, Hacker A, Huang Y, Casero RA. Tumor necrosis factor α induces spermidine/spermine N^1-acetyltransferase (SSAT) through nuclear factor κB in non small cell lung cancer cells. J Biol Chem 2006;281:24182–92.
15. Pledgie A, Huang Y, Hacker A, et al. Spermine oxidase SMO(PAOh1), not N^1-acetylpolyamine oxidase PAO, is the primary source of cytotoxic H_2O_2 in polyamine analogue-treated human breast cancer cell lines. J Biol Chem 2005;280:39843–51.
16. Tsurudome Y, Hirano T, Yamato H, et al. Changes in levels of 8-hydroxyguanine in DNA, its repair and OGG1 mRNA in rat lungs after intratracheal administration of diesel exhaust particles. Carcinogenesis 1999;20:1573–6.
17. Casero RA, Jr., Celano P, Ervin SJ, Porter CW, Bergeron RJ, Libby PR. Differential induction of spermidine/spermine N^1-acetyltransferase in human lung cancer cells by the bis(ethyl)polyamine analogues. Cancer Res 1989;49:3829–33.
18. Jackson AL, Loeb LA. The contribution of endogenous sources of DNA damage to the multiple mutations in cancer. Mutat Res 2001;477:7–21.
19. Helbock HJ, Beckman KB, Ames BN. 8-Hydroxydeoxyguanosine and 8-hydroxyguanine as biomarkers of oxidative DNA damage. Methods Enzymol 1999;300:156–66.
20. Janes KA, Gaudet S, Albeck JG, Nielsen UB, Lauffenburger DA, Sorger PK. The response of human epithelial cells to TNF involves an inducible autocrine cascade. Cell 2006;124:1225–39.
21. Wang Y, Casero RA, Jr. Mammalian polyamine catabolism: a therapeutic target, a pathological problem, or both? J Biochem (Tokyo) 2006;139:17–25.
22. Wang Y, Murray-Stewart T, Devereux W, et al. Properties of purified recombinant human polyamine oxidase, PAOh1/SMO. Biochem Biophys Res Commun 2003;304:605–11.
23. Tantini B, Pignatti C, Fattori M, et al. NF-κB and ERK cooperate to stimulate DNA synthesis by inducing ornithine decarboxylase and nitric oxide synthase in cardiomyocytes treated with TNF and LPS. FEBS Lett 2002;512:75–9.
24. Scheller J, Rose-John S. Interleukin-6 and its receptor: from bench to bedside. Med Microbiol Immunol (Berl) 2006;195:173–83.
25. Scheller J, Ohnesorge N, Rose-John S. Interleukin-6 *trans*-signalling in chronic inflammation and cancer. Scand J Immunol 2006;63:321–9.