Keratinocyte growth factor promotes alveolar epithelial cell DNA repair after H₂O₂ exposure

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Keratinocyte growth factor (KGF), a fibroblast-derived heparin-binding mitogen, prevents oxidant-induced lung damage and mortality in animals exposed to hyperoxia, bleomycin, and radiation (23, 39). Several mechanisms may be involved in protection by KGF against oxidant injury. First, KGF may exert its protective effect by stimulating proliferation of alveolar type II (ATII) cells, which could maintain or restore an intact epithelial surface (24, 33). Second, KGF may decrease lung injury in part by augmenting ATII cell expression of surfactant apoproteins (31). Third, KGF may limit oxidant-induced increases in lung epithelial cell permeability partly by protein kinase C (PKC)-dependent mechanisms (5, 34). The binding of KGF to the KGF receptor, an alternatively spliced variant of fibroblast growth factor receptor 2, stimulates tyrosine kinase and autophosphorylation pathways that can activate PKC (13, 16). Finally, KGF may also reduce oxidant-induced DNA damage by promoting DNA repair. Takeoka et al. (32) recently showed that KGF augments repair of radiation-induced DNA damage in A549 cells.

In this study, we determined whether KGF attenuates H₂O₂-induced DNA damage in cultured AECs. We show that KGF reduces H₂O₂-induced DNA-SB formation in cultured A549 and rat alveolar type II cells measured by an alkaline unwinding, ethidium bromide fluorometric technique. The protective effects of KGF were independent of alterations in catalase, glutathione (GSH), or the expression of bcl-2 and bax, two protooncogenes known to regulate oxidant-induced apoptosis. Actinomycin D and cycloheximide abrogated protective effects of KGF. Furthermore, protection by KGF was completely blocked by 1) genistein, a tyrosine kinase inhibitor; 2) staurosporine and calphostin C, protein kinase C (PKC) inhibitors; and 3) aphidicolin, butylphenyl dGTP, and 2, 3-dideoxythymidine 5′-triphosphate, inhibitors of DNA polymerase. We conclude that KGF attenuates H₂O₂-induced DNA-SB formation in cultured AECs by mechanisms that involve tyrosine kinase, PKC, and DNA polymerases. These data suggest that the ability of KGF to protect against oxidant-induced lung injury is partly due to enhanced AEC DNA repair.

METHODS

Cell culture. A549 cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA). A549 cells, which are human bronchoalveolar carcinoma-derived cells with some features characteristic of epithelial ATII cells, were maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with l-glutamine (0.3 μg/ml), nonessential amino acids, penicillin (100 U/ml), streptomycin (200 μg/ml), and 10% fetal bovine serum (FBS; Gibco, Grand Island, NY). For each experiment, cells were trypsinized and counted in a hemocytometer, and 2 ml were plated onto six-well plates at 2.5 x 10⁴ cells·ml⁻¹·well⁻¹ (Costar, Cambridge, MA).

Rat ATII cells were isolated from specific pathogen-free adult Harlan Sprague-Dawley rats (200–250 g) as previously described (17, 18). Briefly, the cells were obtained from rat lungs by elastase digestion, followed by mincing and sequential filtration. ATII cell purity was enhanced by “panning” the...
cell suspension on plates coated with rat immunoglobulin G (500 μg/ml; Sigma). The cell suspension was then washed, resuspended in DMEM plus 10% FBS with antibiotics, and plated at a density of 1 × 10^6 cells/well in a six-well plate (Costar). After isolation, cell viability was >90% by vital dye exclusion, and type II cell purity 24 h after isolation was 80–85% ATII cells as assessed by modified Papanicolaou staining (n = 2 experiments). The cells were incubated in 95% air-5% CO₂ at 37°C for 24 h before use. A previous study (17) in our laboratory using transmission electron microscopy confirmed that 70–80% of these cells contained lamellar bodies at 48 h.

DNA-SB assay. DNA damage was assessed by alkaline unwinding and ethidium bromide fluorescence as previously described (4, 18). Because ethidium bromide preferentially binds to double-stranded DNA (ds-DNA) in alkali, the relative amounts of nonbroken ds-DNA and broken single-stranded DNA can be assessed (4). Fluorescence was determined with a model 450 Sequoia Turner fluorometer (Mountain View, CA) with excitation at 520 nm and emission at 585 nm. Results are expressed as %ds-DNA = ([F - Fₘᵋᵣₐᵋ]/[Fₚₙᵋᵋₐᵋ - Fₘᵋᵣᵋₐᵋ]) × 100, where F is the fluorescence of the experimental condition, Fₘᵋᵣᵋₐᵋ is the background ethidium bromide fluorescence determined after conversion of all the DNA into single-strand form, and Fₚₙᵋᵋᵋ is the fluorescence determined after addition of the mercaptoethanol solution before the alkaline solution to maintain the pH at 11.0, which is well below that needed to augment unwinding of single-stranded DNA. The amount of single-stranded DNA present in alkali after sonication is proportional to the number of DNA-SB formations (either single or double) because the level of DNA-SB formation varies directly as a function of the extent of DNA unwinding (4).

Cytotoxicity/viability assays. A549 cell cytotoxicity was assessed by measuring lactate dehydrogenase (LDH) release and by trypan blue dye exclusion. A549 cells were grown to 75% confluency in 35-mm plates, washed, and then incubated with KGF (100 ng/ml) or fresh medium for 24 h. Recombinant human KGF was generously provided by Dr. W. Scott Simonet (Amgen, Thousand Oaks, CA). The cells were washed and then exposed to H₂O₂ (0.1 or 0.5 mM) for 4, 8, or 24 h. LDH was measured spectrophotometrically in the medium bathing the cells (LDHₘᵋᵋ) and in the cell lysates (LDHₜₒᵋᵋ) as previously described (17). The percentage of LDH released was calculated as [LDHₜₒᵋᵋ/(LDHₜₒᵋᵋ + LDHₘᵋᵋ)] × 100. The number of viable and nonviable cells at each time point was determined by trypan blue dye exclusion from at least 100 trypsinized cells.

Effect of KGF on H₂O₂-induced DNA damage and cell death. To determine the effect of KGF on H₂O₂-induced DNA-SB formation and cell death, we added varying concentrations of KGF to subconfluent A549 and ATII cells 1 day after plating. In some experiments, cycloheximide (0.5 μg/ml) or actinomycin D (0.01 μg/ml) was added for the last 1 h of incubation with KGF. After incubation with KGF for 24 h, the cells were washed twice with PBS and exposed to H₂O₂ (0.05, 0.1, or 0.5 mM) for 30 min. After exposure to H₂O₂, the cells were washed twice with PBS, and DNA-SB formation and cell cytotoxicity were measured.

Time course of effect of KGF on H₂O₂-induced DNA damage. To determine the time course of the protective effect of KGF, we added KGF (100 ng/ml) to subconfluent A549 cells 1 day after plating. The cells were incubated with KGF for various time periods (0, 1, 4, or 24 h), washed with PBS, exposed to 0.5 mM H₂O₂ for 30 min, and washed, and DNA-SB formation was measured.

A549 cell antioxidant levels. To determine whether KGF enhances A549 cell antioxidant defense against H₂O₂, we exposed cells to KGF, and then catalase and total GSH levels were measured. A549 cells were treated with KGF (10 and 100 ng/ml) for 24 h, washed twice in PBS, and exposed to H₂O₂ (0.1 mM) for 30 min. After treatment with H₂O₂, the cells were washed, lysed (0.1% Triton X-100), sonicated for 3 s, and centrifuged (1,400 rpm for 10 min at 0°C), and the supernatants were obtained for measurement of catalase and GSH. Catalase activity was determined spectrophotometrically at 240 nm on the basis of the reduction of H₂O₂ over time as previously described (23). Total GSH levels were determined as previously described by our laboratory (14). The levels of catalase (in units) and GSH (in μM) are expressed as means per cell number (10⁶ cells).

Effect of tyrosine kinase and PKC inhibitors on the reduction of H₂O₂-induced DNA damage by KGF. To determine whether the protective effect of KGF was due to activation of tyrosine kinase and/or PKC, we added genistein (0.1 mM), a tyrosine kinase inhibitor; calphostin C (0.1 μM), a highly selective PKC inhibitor (30); and staurosporine (0.5 μM; all from Calbiochem, La Jolla, CA), a low-specificity PKC inhibitor, to the A549 cells for 15 min. After pretreatment, KGF (100 ng/ml) was added to the A549 cells for 1 h. The cells were then washed once, exposed to H₂O₂ (0.5 mM) for 30 min at 37°C, and washed twice with PBS, and then the formation of DNA-SB was measured. Because calphostin C is only active in the light, the cells were continuously exposed to light during the pretreatment period.

Effect of DNA polymerase inhibitors on the reduction of H₂O₂-induced DNA damage by KGF. To determine whether KGF protects AECs from H₂O₂-induced DNA-SB formation by augmenting DNA repair, we added KGF (0 or 100 ng/ml) to subconfluent A549 cells for 24 h. The cells were exposed to one of the DNA polymerase inhibitors for the last 1.5 h. Aphiidicolin (Sigma) was dissolved in dimethyl sulfoxide (DMSO; 0.1% volume), whereas butylphenyld-glTTP (BpGdTTP) (a kind gift from Dr. G. E. Wright, University of Massachusetts, Worcester, MA) and 2',3'-dideoxythymidine 5'-triphosphate (ddTTP; Boehringer Mannheim) were dissolved in DMEM. After exposure to H₂O₂ (0.5 mM) for 30 min, the formation of DNA-SB was determined.

Statistical analysis. The results of each experimental condition were determined from the means of duplicate or triplicate trials. Unless otherwise noted, at least six separate experiments were performed for each condition (n = 6). The data are expressed as means ± SE. A two-tailed Student's t-test was used to assess the significance of differences between two groups. ANOVA was used when more than two groups were compared; differences between two groups within the set were analyzed by Fisher's protected least significant difference test. P values < 0.05 were considered significant.

RESULTS

Effect of KGF on H₂O₂-induced AEC DNA damage. As expected, H₂O₂ (0.1 and 0.5 mM) caused dose-dependent reductions in A549 cell ds-DNA, consistent with DNA-SB formation (Fig. 1A). In the absence of H₂O₂, KGF (0.1–100 ng/ml for 24 h) had no significant effect on the amount of ds-DNA measured (Fig. 1A). This finding is consistent with the previous observations by Takeoka et al. (32) that KGF does not alter the cell cycle distribution in A549 cells. KGF attenuated the loss of ds-DNA caused by H₂O₂ in a KGF dose-dependent manner (Fig. 1A).
In contrast to A549 cells, KGF (10–100 ng/ml) increased the amount of ds-DNA in ATII cells after a 24-h exposure period (40% increase; P < 0.05; Fig. 1B). These findings are consistent with prior studies (24, 33) demonstrating that KGF augments ATII cell proliferation. As with A549 cells, H₂O₂ caused dose-dependent reductions in ATII cell ds-DNA, consistent with DNA-SB formation (Fig. 1B). Notably, ATII cells were more sensitive than A549 cells to H₂O₂-induced DNA damage (Fig. 1). Similar to A549 cells, KGF increased ds-DNA in H₂O₂-exposed ATII cells, again consistent with protection against H₂O₂-induced DNA-SB formation (Fig. 1B).

Time course of the effect of KGF on H₂O₂-induced A549 cell DNA damage. To determine the time course of the protective effect of KGF, we added KGF (100 ng/ml) to subconfluent A549 cells for various time periods (0, 1, 4, and 24 h), and the number of H₂O₂ (0.5 mM × 30 min)-induced occurrences of DNA-SB was determined. KGF added at the time of H₂O₂ exposure produced negligible protection (Fig. 2). As shown in Fig. 2, the protective effect of KGF against H₂O₂-induced A549 cell DNA-SB formation was noted as early as 1 h after KGF pretreatment (~20% increase in ds-DNA; P < 0.05) and was persistent at 24 h (~25% increase in ds-DNA; P < 0.05).

Effect of cycloheximide and actinomycin D on the actions of KGF. To determine whether RNA and protein synthesis are required to mediate the beneficial effect of KGF, we treated A549 cells with KGF (10 or 100 ng/ml) for 24 h, and then either cycloheximide (0.5 µg/ml) or actinomycin D (0.01 µg/ml) was added during the final 1 h. Compared with control medium, cycloheximide and actinomycin D each minimally reduced ds-DNA in A549 cells over the 1-h treatment period (10% reduction in ds-DNA; P < 0.05 vs. control medium; n = 6 experiments). As shown in Fig. 3, the protective effects of KGF were completely abolished in the presence of either cycloheximide or actinomycin D. This suggests that the protective effect of KGF against H₂O₂-induced DNA damage occurs by mechanisms that depend on both RNA and protein synthesis.

Effects of KGF on A549 cell catalase activity and GSH content. To determine whether the protective effects of KGF against H₂O₂-induced DNA-SB are due to increased A549 cell antioxidant defenses, we measured A549 cell catalase and GSH levels. Compared with control medium, H₂O₂ (0.1 mM) caused no change in A549 cell catalase activity after a 30-min exposure period and induced a small but nonsignificant reduction in GSH content (57.0 ± 10.3 vs. 45.6 ± 5.3 µM/10⁶ cells; n = 4 experiments; P = 0.13; Fig. 4). Notably, KGF did not alter A549 cell catalase activity or GSH levels in the absence or presence of H₂O₂ (Fig. 4). Although a higher dose of H₂O₂ (0.5 mM) did not alter catalase activity, it reduced GSH levels by 46% com-

Fig. 1. Keratinocyte growth factor (KGF) caused a dose-dependent reduction in H₂O₂-induced DNA damage. A549 (A) and rat alveolar type II (ATII; B) cells were treated with KGF for 24 h, washed once, and exposed to H₂O₂ for 30 min, and then double-stranded DNA (ds-DNA) was quantitated as described in METHODS. Data are means ± SE; n = 6 experiments for A549 cells and n = 5 experiments for ATII cells. *P < 0.05 vs. no-KGF control. †P < 0.05 vs. H₂O₂ without KGF.

Fig. 2. Time course of KGF-mediated protection against H₂O₂-induced A549 cell DNA damage. Cells were pretreated with medium with or without KGF for 0 h (n = 4 experiments), 1 h (n = 11 experiments), 4 h (n = 6 experiments), and 24 h (n = 6 experiments). After pretreatment, cells were washed once and exposed to H₂O₂ for 30 min, and then ds-DNA was quantitated as described in METHODS. Data are means ± SE. *P < 0.05 vs. control. †P < 0.05 vs. H₂O₂.
pared with unexposed cells (n = 2 experiments). However, KGF provided negligible protection (41% reduction in GSH levels; n = 2 experiments).

Effect of KGF on H2O2-induced A549 cell death. We next determined whether KGF also decreased H2O2-induced death of A549 cells as assessed by trypan blue exclusion and LDH release. A549 cells were incubated in the presence or absence of KGF (100 ng/ml) for 24 h and then exposed to H2O2 (0.1 or 0.5 mM) for various periods (4, 8, and 24 h). Compared with control medium, H2O2 (0.1–0.5 mM) caused minimal reductions in A549 cell viability and LDH release at 4 and 8 h (Table 1). After 24 h, H2O2 caused a dose-dependent LDH release into the medium (Table 1). Although H2O2 resulted in negligible reductions in the percentage of viable attached A549 cells as measured by trypan blue exclusion (Table 1), the total number of viable cells (× 10^4) was reduced [control, 80 ± 26; H2O2 (0.1 mM), 61 ± 16; H2O2 (0.5 mM), 53 ± 22; n = 6 experiments]. KGF did not attenuate H2O2-induced LDH release or the percentage of viable attached cells (Table 1). KGF also did not prevent the reduction in the total number of viable cells (× 10^4) at 24 h [H2O2 (0.1 mM) + KGF, 55 ± 13; H2O2 (0.5 mM) + KGF, 46 ± 14; n = 6 experiments]. Collectively, these data indicate that the mechanism by which KGF attenuates H2O2-induced A549 DNA damage is not related to alterations in cell death.

Effect of tyrosine kinase and PKC inhibitors. Because Takeoka et al. (32) previously found that the protective effect of

Fig. 3. Cycloheximide (CHX) and actinomycin D (ACD) blocked the beneficial effect of KGF against H2O2-induced A549 cell DNA damage. Cells were pretreated with KGF for 23 h before addition of a protein synthesis inhibitor, CHX, or an RNA synthesis inhibitor, ACD, for the last 1 h of incubation with KGF. Cells were then washed twice and exposed to H2O2 for 30 min, and then ds-DNA was quantitated as described in METHODS. Data are means ± SE; n = 6 experiments. ○ on y-axis, %ds-DNA in control cells. *P < 0.05 vs. H2O2 alone. †P < 0.05 vs. H2O2 + KGF.

Effect of DNA polymerase inhibitors. Because Takeoka et al. (32) previously found that the protective effect of
KGF against radiation-induced AEC DNA damage was blocked by DNA polymerase inhibitors, it was determined whether the beneficial effect of KGF observed in the present study was due to enhanced DNA repair. After incubation with KGF for 24 h, A549 cells were exposed for 1.5 h to either aphidicolin (1 µM), an inhibitor of DNA polymerase-α, -δ, and -ε (21); BuPDGTP (10 µM), which strongly blocks DNA polymerase-α and weakly inhibits DNA polymerase-δ and -ε (38); or ddTTP (10 µM), a specific inhibitor of DNA polymerase-β; blocked the reduction of KGF of H2O2-induced A549 cell DNA damage. Cells were pretreated for 24 h with control medium or KGF, as well as either aphidicolin, BuPDGTP, or ddTTP for final 1.5 h. After incubation, cells were washed, and H2O2-induced DNA damage was quantified as described in METHODS. Data are means ± SE. *P < 0.05 vs. corresponding result without H2O2 of KGF. †P < 0.05 vs. H2O2 alone. §P < 0.05 vs. H2O2 + KGF.

**DISCUSSION**

KGF ameliorates various forms of oxidant-mediated lung injury but the protective mechanism(s) have not yet been elucidated. The purpose of this study was to determine whether KGF reduces DNA damage in cultured AECs exposed to H2O2. We showed that KGF attenuates H2O2-induced DNA-SB formation in both A549 cells, a human AEC line, and primary isolated rat ATII cells. The protective effects of KGF were dose dependent and occurred within the concentration range for other biological actions of KGF, such as increasing ATII cell proliferation and the expression of surfactant apoproteins and Na⁺-K⁺-ATPase (5, 24, 31).

Several experiments were performed to determine the mechanism by which KGF reduces H2O2-induced AEC DNA damage. First, we found that KGF does not increase the activity of two antioxidants involved in H2O2 clearance from AEC, catalase and GSH. The lack of effect of KGF on antioxidant defenses of cultured AECs demonstrated in this study is similar to the previous finding (23) that KGF did not augment catalase or superoxide dismutase activity in rats exposed to hyperoxia. On the other hand, utilizing differential-display RT-PCR, Frank et al. (7) recently demonstrated that KGF preferentially augments keratinocyte expression of a gene for non-selenium GSH peroxidase (GPX), an enzyme that utilizes GSH to decrease the toxic effects of H2O2 and organic peroxides. Although KGF did not alter A549 cell GSH levels with or without H2O2, the protective effects of KGF after a 24-h pretreatment

### Table 1. H2O2-induced A549 cell death: effect of KGF

<table>
<thead>
<tr>
<th>Condition</th>
<th>Trypan Blue Exclusion, %</th>
<th>LDH Release, %total</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>97.7 ± 0.5 (10)</td>
<td>6.0 ± 3.5 (9)</td>
</tr>
<tr>
<td>H2O2 (0.1 mM)</td>
<td>89.5 ± 1.5*</td>
<td>5.4 ± 3.1</td>
</tr>
<tr>
<td>H2O2 (0.1 mM) + KGF</td>
<td>95.0 ± 1.4†</td>
<td>6.3 ± 3.2</td>
</tr>
<tr>
<td>H2O2 (0.5 mM)</td>
<td>94.3 ± 1.9*</td>
<td>2.6 ± 0.8</td>
</tr>
<tr>
<td>H2O2 (0.5 mM) + KGF</td>
<td>94.3 ± 1.0*</td>
<td>3.2 ± 1.3</td>
</tr>
<tr>
<td>8 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>97.4 ± 0.9 (8)</td>
<td>4.2 ± 2.0 (8)</td>
</tr>
<tr>
<td>H2O2 (0.1 mM)</td>
<td>94.9 ± 1.4</td>
<td>4.4 ± 1.9</td>
</tr>
<tr>
<td>H2O2 (0.1 mM) + KGF</td>
<td>96.4 ± 1.0</td>
<td>5.2 ± 1.8</td>
</tr>
<tr>
<td>H2O2 (0.5 mM)</td>
<td>94.1 ± 1.8</td>
<td>5.8 ± 2.5</td>
</tr>
<tr>
<td>H2O2 (0.5 mM) + KGF</td>
<td>97.1 ± 0.9</td>
<td>4.4 ± 1.0</td>
</tr>
<tr>
<td>24 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>96.9 ± 0.8 (10)</td>
<td>8.8 ± 1.8 (6)</td>
</tr>
<tr>
<td>H2O2 (0.1 mM)</td>
<td>98.0 ± 0.6</td>
<td>15.7 ± 1.8*</td>
</tr>
<tr>
<td>H2O2 (0.1 mM) + KGF</td>
<td>97.9 ± 0.8</td>
<td>21.5 ± 14.4*</td>
</tr>
<tr>
<td>H2O2 (0.5 mM)</td>
<td>97.2 ± 0.7</td>
<td>22.1 ± 5.2*</td>
</tr>
<tr>
<td>H2O2 (0.5 mM) + KGF</td>
<td>98.2 ± 0.9</td>
<td>33.1 ± 31.3*†</td>
</tr>
</tbody>
</table>

Data are means ± SE; nos. in parenthesis, no. of separate experiments. A549 cells were incubated in presence or absence of keratinocyte growth factor (KGF; 100 ng/ml) for 24 h and then exposed to H2O2 for times indicated. Significant difference (P < 0.05) from: *control; †0.1 mM H2O2; ‡0.5 mM H2O2.
period in our study could be partially due to increased GPX expression. We found that the actions of KGF were blocked by either an RNA or a protein synthesis inhibitor, indicating that expression of new protein(s) is important. Further studies will be necessary to determine the role of this newly described non-selenium GPX protein in the protective effect of KGF in the lung.

Because KGF is a potent ATII cell mitogen (24, 33), we questioned whether the protective effects of KGF were due to increased AEC proliferation. Several lines of evidence suggest that this was not the mechanism of protection. First, using flow cytometry, Takeoka et al. (32) previously showed that a high percentage of A549 cells are in the proliferative stages of the cell cycle (S phase, 34% and G2/M phase, 10%) and that KGF (100 ng/ml) did not alter these percentages. Second, the protective effects observed in this study occurred after incubation with KGF for as briefly as 1 h (Fig. 2), which is substantially less than the average A549 cell doubling period of 16 h under our conditions (Pollack and Kamp, unpublished observations). Finally, KGF did not increase A549 cell DNA synthesis as assessed by bromodeoxyuridine labeling over 24 h (M. Nadeem and R. Panos, unpublished observations). Collectively, these data suggest that KGF attenuates H2O2-induced AEC DNA damage through mechanisms independent of the proliferative state of the cell.

Growth factors may decrease oxidant-induced cellular damage by inhibiting apoptosis, a central pathway regulating cellular homeostasis (36). Basic fibroblast growth factor attenuates radiation-induced bovine aortic endothelial cell apoptosis and maintains reproductive integrity (9, 10). In this study, H2O2 caused relatively minimal A549 cell death after a 4- to 8-h exposure period but increased LDH release in a dose-dependent manner after 24 h (Table 1). Furthermore, KGF did not attenuate H2O2-induced LDH release at 24 h. Our data suggest that the protective effect of KGF against H2O2-induced AEC DNA damage is unlikely to be due to reduced cell death.

There is evidence to suggest that specific signal transduction pathways mediate the protective effects of growth factors against oxidant-induced cellular toxicity. For example, basic fibroblast growth factor activation of PKC inhibits radiation-induced apoptosis in bovine aortic endothelial cells (9, 10). Binding of KGF to the KGF receptor results in receptor dimerization, which, in turn, activates diverse signaling events, including tyrosine kinase, as well as phosphorylation of phospholipase C-γ and a 90-kDa protein (13, 16, 27). Activation of the PKC pathway can also result from phosphorylation of phospholipase C-γ, which subsequently augments intracellular diacylglyceride levels. KGF also induces the phosphorylation of mitogen-activated protein kinases, a common downstream effect of activated tyrosine kinase and PKC (27). We showed that KGF reduces H2O2-induced DNA damage after as short as a 1-h preincubation period, which suggests a role for signal transduction pathways. Further evidence implicating specific signal transduction mechanisms was suggested by our observations that genistein, a tyrosine kinase inhibitor, as well as two PKC inhibitors, staurosporine and calphostin C, each totally blocked the protective effect of KGF. Although inhibitors such as genistein may scavenge H2O2 (35), this was unlikely to account for the effects observed in this study because the inhibitors were removed from the cells before H2O2 was added. Our data are consistent with those of Waters et al. (34), who demonstrated that KGF attenuated H2O2-induced albumin flux across cultured airway epithelial cell monolayers by a PKC-dependent mechanism. These investigators showed that either inhibition of PKC by calphostin C and staurosporine or depletion of PKC by pretreatment with phorbol 12-myristate 13-acetate completely blocked protection by KGF. Inhibitor studies are limited by their cross-reactivity with other kinases. However, our findings that inhibitors of tyrosine kinase or PKC each blocked the protective effects of KGF are consistent with the biological effects of KGF on cellular signaling pathways mentioned above. Although the detailed signaling mechanisms that account for the beneficial effects of KGF against H2O2-induced AEC DNA damage require further study, our data suggest that tyrosine kinase and PKC signal transduction pathways are important.

Although H2O2 attacks various molecules within cells, DNA represents one of the important biological targets (3, 11). The mechanism by which H2O2 causes DNA damage and is subsequently repaired in eukaryotic cells is complex and not well established. As reviewed elsewhere (11), H2O2-induced DNA damage is primarily caused by ROS derived from iron-mediated Fenton reactions that occur at specific sites on DNA where iron is bound and, to a lesser extent, is also due to diffusible hydroxyl radicals (·OH) or -OH-like species generated by H2O2. Repair of H2O2-induced DNA damage mainly occurs by base excision but may also occur by at least three other pathways, including direct restitution by hydrogen donation from a sulfhydryl, nucleotide excision, and recombination (11). DNA polymerase-β, -δ, and -ε have each been implicated in various forms of DNA repair, whereas DNA polymerase-α functions primarily for semiconservative DNA replication (37). Hu et al. (12) recently demonstrated that H2O2 can inhibit DNA repair mechanisms in human peripheral mononuclear leukocytes. Our results suggest that KGF may partially reverse the suppressive effects of H2O2 on AEC DNA repair. We showed that aphidicolin, an inhibitor of DNA polymerase-α, -δ, and -ε (21); BuPdGTP, which strongly blocks DNA polymerase-α and weakly inhibits DNA polymerase-δ and -ε (38); and ddTTP, a specific inhibitor of DNA polymerase-β (6, 25), each completely blocked the protective effect of KGF against H2O2-induced AEC DNA damage (Fig. 6). Thus, similar to radiation, the mechanism by which KGF augments AEC DNA repair capability after H2O2 exposure is due partly to DNA polymerase-α, -δ, and -ε but, in contrast to radiation, is also mediated in part by DNA polymerase-β. However, the beneficial effects of KGF noted in this study are not a universal feature of growth factors because insulin and insulin-like growth factor reduce DNA repair in irradi-
ated A549 cells by impairing the rejoicing of DNA double-strand breaks (15).

The mechanism accounting for the difference between KGF enhancement of AEC DNA repair after H2O2 or radiation exposure was not addressed in this study. However, there are at least three possible explanations. First, this difference may be due to distinct mechanisms of -OH production; H2O2 utilizes an iron-catalyzed Fenton reaction to produce -OH at the site of DNA-bound iron, whereas radiation induces -OH formation independently of iron (11). H2O2 induces nearly 50 DNA base alterations that are similar but not identical to those caused by radiation (11). Second, DNA polymerase-β, which is involved in one nucleotide patch repair during base excision repair (37), may be a more important AEC DNA repair pathway after exposure to H2O2 than radiation. Our data implicating DNA polymerase-β in facilitating repair of H2O2-induced AEC DNA damage concur with evidence in fibroblasts demonstrating that DNA polymerase-β is involved in the repair of bleomycin-induced DNA damage (20). In contrast, Takeoka et al. (32) and others (21) have found that DNA polymerase-β does not participate in the repair of radiation-induced DNA damage. Thus the role of different DNA polymerases in DNA repair synthesis appears to be directly associated with the specific agent used to cause DNA damage. Finally, differences in the activation of signal transduction pathways and the expression of transcriptional factors after exposure to H2O2 or radiation may also account for some of the differences observed because ROS regulate DNA repair by affecting these pathways (1, 29). Together, these findings underscore the complexity of effects that ROS and growth factors have in regulating DNA repair.

In summary, we showed that KGF attenuates H2O2-induced DNA damage in cultured A549 and rat ATII epithelial cells and in alveolar epithelial cells in primary culture. Although the precise mechanism by which KGF reduces AEC DNA damage was not established in this study, our data suggest that the effects of KGF are partly due to activation of signal transduction pathways involving tyrosine kinase and PKC as well as the activity of DNA polymerase-α, -β, -δ, and -ε. Further investigation will be necessary to determine the specific pathways mediating the protective effects of KGF as well as the in vivo relevance of our findings. We propose that one mechanism by which KGF attenuates oxidant-induced lung damage is by augmenting DNA repair in AECs.

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