Fibroblast Growth Factor-10 Prevents Asbestos-Induced Alveolar Epithelial Cell Apoptosis by a Mitogen-Activated Protein Kinase–Dependent Mechanism

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Asbestos induces alveolar epithelial cell (AEC) DNA damage and apoptosis by the mitochondria-regulated death pathway and oxidative stress. Fibroblast growth factor-10 (FGF-10), an alveolar epithelial type II cell mitogen that is required for the lung development, prevents H2O2-induced AEC DNA damage by a mitogen activated protein kinase (MAPK)/extracellular signal–regulated kinase (ERK)-dependent mechanism. In this study, we show that FGF-10 attenuates asbestos-induced AEC DNA strand break formation and apoptosis. MAPK/ERK kinase (MEK) inhibitors, U0126 or PD98059, each blocked the protective effect of FGF-10 against asbestos-induced DNA damage and apoptosis, whereas a p38-MAPK inhibitor had a negligible effect, suggesting a crucial role for MEK/ERK activation in mediating the protective effects of FGF-10. Further, we show that FGF-10 attenuates asbestos-induced change in AEC mitochondrial membrane potential and caspase 9 activation, both of which are blocked by U0126. We conclude that FGF-10 decreases asbestos-induced AEC DNA damage and apoptosis in part by mechanisms involving MEK/ERK-dependent signaling that affects the mitochondria-regulated death pathway.

Keywords: asbestos; growth factors; signal transduction; cell death; pulmonary epithelium

Oxidant-induced alveolar epithelial cell (AEC) injury is important in the pathogenesis of pulmonary toxicity from a variety of agents, including asbestos (1–8). Asbestos is directly genotoxic to relevant target cells in the lungs in part by inducing DNA strand breaks (DNA-SB) and apoptosis by mechanisms involving the mitochondria-regulated death pathway and the generation of reactive oxygen species (ROS) (3, 4, 9–15). Recently, the mitochondria were identified as the major source of oxidative stress mediating asbestos-induced cellular toxicity, and it was established that enhanced mitochondrial oxidative DNA repair by 8-oxoguanine DNA glycosylase (hOGG) was protective (14, 15). Thus, preventing free radical–induced DNA damage and promoting prompt DNA repair are crucial for restoring normal AEC barrier function and reducing DNA damage–induced apoptosis, which if extensive can result in pulmonary fibrosis (3, 13).

Growth factors, including fibroblast growth factor-10 (FGF-10), have been implicated as having an important role in pre-terminating apoptosis. FGF-10, a 13.9-kD heparin-binding protein, is a potent alveolar type II cell (AT2 cell) mitogen that is predominantly expressed by lung mesenchymal cells and is required for lung development (16–20). FGF-10 promotes epithelial cell motility, differentiation, migration, and wound healing. Among the 23 FGF family members, FGF-10, similar to keratinocyte growth factor (KGF or FGF-7), binds with high affinity to a spliced variant of fibroblast growth factor receptor 2-IIIb (FGFR2III-b) expressed exclusively on epithelial cells but, unlike KGF, also binds to FGFR1III-b (2, 16–20). We recently demonstrated that FGF-10 attenuates both cyclic-stretch and H2O2-induced DNA damage and apoptosis by mechanisms involving mitogen activated protein kinase (MAPK)/extracellular signal–regulated kinase (ERK) kinase (MEK) activation via the Grb2-SOS/Ras/RAF-1/ERK1/2 pathway as well as enhanced DNA repair (21, 22). However, there is no information whether FGF-10 is protective against asbestos-induced AEC apoptosis and, if so, whether the mitochondria are affected.

We reasoned that FGF-10 attenuates asbestos-induced AEC DNA damage and apoptosis via mechanisms involving MEK/ERK activation and inhibition of the mitochondria-regulated death pathway. In this study, we demonstrate that FGF-10 prevents amosite asbestos-induced A549 and rat AT2 cell DNA damage and apoptosis. Further, we provide evidence that the protective effects of FGF-10 against asbestos-induced AEC DNA damage and apoptosis are mediated in part by MEK/ERK activation that subsequently prevents mitochondrial membrane potential change (ΔΨm) and caspase 9 activation. These data add to the cumulative evidence implicating that MEK/ERK activation mediates the protective effects of FGF-10 against oxidant-induce AEC injury. Furthermore, our findings suggest a novel mechanism by which FGF-10–induced MEK/ERK signaling decreases the activation of the mitochondria-regulated death pathway.

MATERIALS AND METHODS

Asbestos and Chemicals

Asbestos fibers used in these experiments were Union Interna- tional Centerle Cancer Reference Standard samples supplied by Dr. V. Timbrell (23). The amosite fibers are amphiboles that are 70% respirable (length between 2 and 5 μm), whereas the remainder are > 5 μm in length. Stock solutions (5 mg/ml) of each particulate were prepared in Hanks’ balanced salt solution (HBSS) with calcium, magnesium, and 15 mM N-2-hydroxethylpiperazine-N’-2-ethanesulfonic acid (HEPES). All suspensions were autoclaved and stored at 4°C. Samples were warmed to 37°C and vigorously vortexed before usage to ensure a uniform suspension. FGF-10 was purchased from R&D Systems (Minneapolis, MN). U0126 and PD98059 were purchased from Promega (Madison, WI). SB203580 was purchased from Calbiochem (La Jolla, CA). All other chemicals were purchased from Sigma Chemicals (St. Louis, MO).

Cell Culture

A549 cells were obtained from the American Type Culture Collection (Manassas, VA) and maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with L-glutamine (0.3 μg/ml), nonessen- tial amino acids, penicillin (100 U/ml), streptomycin (200 μg/ml), and

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10% fetal bovine serum (FBS; Gibco, Grand Island, NY). For each experiment, we used a seeding density of 3.0 \times 10^5 cells/ml/well plated onto six-well plates (Costar, Cambridge, MA). The cells were grown to confluence over 24 h in a humidified 95% air–5% CO2 incubator at 37°C. Rat AT2 cells were isolated from specific pathogen–free adult Harlan Sprague-Dawley rats (200–250 g) using a technique previously described (4). Cells were plated at a density of 1 \times 10^5 cells/well in a six-well plate and were grown to confluence in a humidified 95% air–5% CO2 incubator at 37°C over 24 h.

**DNA-SB Assay**

DNA-SB formation was assessed as we previously described (4, 21). Briefly, A549 cells were treated with FGF-10 (10 ng/ml) for 1 h followed by asbestos (1.5 and 25 \mu g/cm²) for 24 h. In some experiments, cells were treated with a selective MAPK inhibitor, U0126 or PD98059, for 15 min before FGF-10 (10 ng/ml) was added for 1 h. After treatment, the cells were washed once in phosphate-buffered saline, placed on ice, and DNA-SB formation was assessed by an alkaline unwinding and ethidium bromide fluorescence. 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. Fluorescence was determined with a model 450 Sequoia Turner fluorometer (Mountain View, CA) with excitation at 520 nm and emission at 585 nm. The results were expressed as the percentage of total double-stranded DNA defined as \([F \times (Fmmin/Fmax-Fmmin)] \times 100\), where \(F\) is the fluorescence in the experimental condition, \(Fmmin\) is the background ethidium bromide fluorescence determined after converting all the DNA into single-strand form, and \(Fmax\) is the fluorescence determined from cells not exposed to alkaline unwinding conditions. The reductions in ds-DNA in this assay are due to increased DNA-SB formation.

**Apoptosis Assays**

AEC cell apoptosis was assessed by both TUNEL-stained nuclear morphology and DNA nucleosomal fragmentation ELISA (Roche Diagnostics, Indianapolis, IN) as previously described (9, 12). Briefly, A549 cells were treated with FGF-10 (10 ng/ml) for 1 h, then exposed to various doses of asbestos for 24 h, after which the cells in the supernatant and attached to the dish were collected for determination of apoptosis by both techniques. In some experiments AEC were treated with a MAPK inhibitor as described above. The DNA nucleosomal fragmentation ELISA assay detects histone-associated DNA fragments (mono- and oligonucleosomes). We previously demonstrated that these assays directly correlate with AEC apoptosis as assessed by acridine orange–stained nuclear morphology, annexin V staining, and caspase 3 activation (9).

**Mitochondrial Membrane Potential Change**

The \(\Delta \psi_m\) was assessed using a fluorometric assay that we have previously described (12). Briefly, AEC were pretreated with FGF-10 (10 ng/ml) for 1 h followed by asbestos for 24 h, and were then exposed to either tetramethylrhodamine ethyl ester (TMRE; 500 nM, Molecular Probes, Eugene, OR) or Mitotracker green (1 \mu M; Molecular Probes) for 1 h at 37°C. Carbonyl cyanide trifluoromethoxyphenylhydrazide (FCCP; 20 \mu M) was added to a separate group of comparably treated cells for 1 h before adding fluorochromes to induce a maximal \(\Delta \psi_m\) by uncoupling oxidative phosphorylation and eliminating the mitochondrial proton gradient. Changes in dye fluorescence at 25°C were analyzed in a fluorometer using an excitation wavelength of 488 nm and emission wavelength of 520 or 580 nm (TMRE and Mitotracker green fluorescence, respectively). The Mitotracker green was used to label the mitochondria and is not retained in cells upon collapse of \(\Delta \psi_m\). The \(\Delta \psi_m\) was compared qualitatively based on the percentage difference in the ratio of TMRE and Mitotracker green fluorescence of untreated cells (Tc and Mg, respectively) corrected for the background fluorescence in FCCP-treated control cells (Ftc and Fmgc) and the ratio of TMRE and Mitotracker green fluorescence of treated cells (Tt and Mgt) minus the FCCP-treated cells (Ft and Fmgt, respectively) defined as follows: \(\Delta \psi_m = (Tc/Mg - Ftc/Fmg) - (Tt/Mgt - Ft/Fmgt) \times 100\).

**Caspase 9 Assays**

AEC were pretreated with FGF-10 (10 ng/ml) for 1 h followed by asbestos (0, 25, or 50 \mu g/cm²) for 24 h, washed, and then the protein from the cell lysates of the attached and floating cells were collected for use in the ELISA as previously described (12). In some experiments, AEC were pretreated with MAPK inhibitor as described above and then asbestos-induced caspase 9 activation was assessed. Asbestos-induced caspase 9 release was assessed by a commercially available ELISA assay for each caspase exactly as per the manufacturer’s protocol (Roche Diagnostics) and normalized to the total protein concentration as determined by the Bio-Rad (Hercules, CA) protein assay.

**Statistics**

All data are expressed as the mean ± SEM. An unpaired Student’s \(t\) test was used to assess the difference between two groups. ANOVA was performed when more than two groups were compared with a single control, and then differences between individual groups within the set were assessed by a multiple comparison test (Tukey) when the \(F\) statistic was < 0.05. A \(p\) value of < 0.05 was considered significant.

**RESULTS**

**FGF-10 Attenuates Asbestos-Induced A549 Cell DNA-SB Formation**

To determine whether FGF-10 prevents asbestos-induced AEC DNA damage, A549 cells were exposed to various doses of FGF-10 (1, 10, and 100 ng/ml) for 1 h, followed by amosite asbestos (25 \mu g/cm²) for 24 h, and then DNA-SB formation was assessed as previously described (4, 21). As expected, asbestos reduced A549 cell double-stranded DNA due to DNA-SB formation (Figure 1). FGF-10 attenuated asbestos-induced A549 cell DNA-SB formation by nearly 50% (Figure 1). The protective effect of FGF-10 occurred with as little as 1 ng/ml, and no clear FGF-10 dose-dependent effect was observed (Figure 1). As shown in Figure 2B, FGF-10 also nearly completely protected primary isolated rat AT2 cells from asbestos-induced DNA-SB formation. We used 10 ng/ml of FGF-10 for all subsequent experiments because this concentration of FGF-10 is maximally effective in our in vitro models (21, 22).

**MAPK-Dependent Pathways Mediate the Protective Effects of FGF-10 against Asbestos-Induced AEC DNA-SB Formation**

We previously reported that FGF-10 attenuates oxidant-induced DNA damage by mechanisms involving MEK/ERK activation (21, 22). To determine whether MEK/ERK activation mediates...
DNA damage. the protective effect of FGF-10 against asbestos-induced AEC used have negligible effects (4, 9, 21, 22). These data suggest and rat AT2 cells (Figures 2A and 2B, respectively). We pre- of FGF-10 against asbestos-induced DNA-SB formation in A549 15 min followed by FGF-10 (10 ng/ml) for 1 h and then asbestos blocked the protective effects of FGF-10 against asbestos-induced A549 and AT2 cell DNA-SB formation (⁎P < 0.005 control versus asbestos, †P < 0.005 asbestos versus FGF-10 + asbestos). We noted that FGF-10 near completely blocked asbestos-induced AEC apoptosis as assessed by both techniques (⁎P < 0.005 control versus asbestos, †P < 0.05 asbestos versus FGF-10 + asbestos; n = 3).

Figure 2. Asbestos-induced DNA-SB formation in A549 (A) and AT2 (B) cells is reduced by FGF-10 by a MAPK-dependent pathway. A549 and AT2 cells were pretreated with MAPK inhibitors U0126 (10 μM) or PD98059 (100 μM) before FGF-10 (10 ng/ml), then exposed to asbestos (25 μg/cm²) for 24 h. Both inhibitors blocked the protective effects of FGF-10 against asbestos-induced A549 and AT2 cell DNA-SB formation (⁎P < 0.005 control versus asbestos, †P < 0.005 asbestos versus FGF-10 + asbestos, ‡P < 0.005 FGF-10 + asbestos versus U0126 + FGF-10 + asbestos or PD98059 + FGF-10 + asbestos; n = 3).

Demonstrate that FGF-10 attenuates asbestos-induced alveolar epithelial cell apoptosis.

Asbestos-Induced AEC Apoptosis by the Mitochondria-Regulated Death Pathway Is Attenuated by FGF-10

We recently showed that asbestos causes alveolar epithelial cell apoptosis via the mitochondria-regulated death pathway (12). In this study, we determined whether FGF-10 mediates its protective effects against asbestos-induced A549 cell apoptosis by reducing activation of the mitochondria-regulated death pathway. We exposed A549 cells to FGF-10 (10 ng/ml) for 1 h, followed by asbestos (0, 25, and 50 μg/cm²) for 24 h; A549 cell ΔΨm was then assessed using a fluorometric technique as previously described (12). As shown in Figure 4A, asbestos reduced A549 cell ΔΨm in a dose-dependent manner that was similar to our previous study (12). Notably, FGF-10 completely blocked asbestos (25 μg/cm²)-induced reduction in A549 cell ΔΨm and partially blocked the effects of high-dose asbestos (50 μg/cm²), but this difference did not reach statistical significance. Because caspase 9 activation occurs via the mitochondria-regulated death pathway, we determined whether FGF-10 attenuates asbestos-induced caspase 9 activation. Similar to our prior study (12), we found that asbestos caused caspase 9 activation in an asbestos dose-dependent manner (Figure 4B). We noted that FGF-10 near completely blocked asbestos-induced A549 cell caspase 9 activation (Figure 4B). Collectively, these data demonstrate that the protective effects of FGF-10 against asbestos-induced A549 cell apoptosis are mediated via the mitochondria-regulated death pathway.

MEK/ERK Signaling Is Crucial for Mediating the Protective Effect of FGF-10 against Asbestos-Induced AEC Apoptosis via the Mitochondria-Regulated Death Pathway

Given the importance of MEK/ERK signaling pathways in mediating the protective effects of FGF-10 noted in our earlier studies (21, 22) as well as against asbestos-induced DNA-SB formation described above, we reasoned that FGF-10–induced MEK/ERK

FGF-10 Attenuates Asbestos-Induced A549 Cell Apoptosis

Because FGF-10 reduces AEC apoptosis caused by oxidative stress from H₂O₂ or cyclic stretch (21, 22), we explored whether FGF-10 also prevents asbestos-induced AEC apoptosis. A549 cells were treated with FGF-10 (10 ng/ml) for 1 h and then exposed to asbestos (0, 25, and 50 μg/cm²) for 24 h. As expected, asbestos induced A549 cell apoptosis in a dose-dependent manner as assessed by both TUNEL staining (Figure 3a) and a highly sensitive DNA nucleosomal fragmentation ELISA (Figure 3b) (9). The novel finding in this study is that FGF-10 completely blocked asbestos-induced TUNEL staining and partially prevented DNA fragmentation by ~40% (Figure 3). These findings

the protective effect of FGF-10 against asbestos-induced DNA-SB formation, A549 and rat AT2 cells were treated with a specific MEK/ERK inhibitor (U0126: 10 μM or PD98059: 100 μM) for 15 min followed by FGF-10 (10 ng/ml) for 1 h and then asbestos for 24 h. Both MEK/ERK inhibitors blocked the protective effect of FGF-10 against asbestos-induced DNA-SB formation in A549 and rat AT2 cells (Figures 2A and 2B, respectively). We previously showed that each MEK/ERK inhibitor alone cause negligible DNA-SB formation and that the levels of DMSO (0.05%) used have negligible effects (4, 9, 21, 22). These data suggest that MEK/ERK-dependent pathways are important in mediating the protective effect of FGF-10 against asbestos-induced AEC DNA damage.

FGF-10 reduces AEC apoptosis caused by oxidative stress from H₂O₂ or cyclic stretch (21, 22), we explored whether FGF-10 also prevents asbestos-induced AEC apoptosis. A549 cells were treated with FGF-10 (10 ng/ml) for 1 h and then exposed to asbestos (0, 25, and 50 μg/cm²) for 24 h. As expected, asbestos induced A549 cell apoptosis in a dose-dependent manner as assessed by both TUNEL staining (Figure 3a) and a highly sensitive DNA nucleosomal fragmentation ELISA (Figure 3b) (9). The novel finding in this study is that FGF-10 completely blocked asbestos-induced TUNEL staining and partially prevented DNA fragmentation by ~40% (Figure 3). These findings
FGF-10 attenuates asbestos-induced A549 cell mitochondrial membrane potential change (A) and caspase 9 activation (B): A549 cells were exposed to FGF-10 (10 ng/ml) for 1 h followed by asbestos (25 or 50 μg/cm²) for 24 h. As expected, asbestos reduced A549 cell ΔΨm (A) as assessed by a fluorometric technique with TMRE and Mitotracker green and activated caspase 9 (B) as assessed by an ELISA as described in MATERIALS AND METHODS. FGF-10 attenuated both asbestos-induced ΔΨm and caspase 9 activation (*p < 0.005 control versus asbestos, †p < 0.05 asbestos versus FGF-10 + asbestos; n = 3).

**Figure 4.** FGF-10 attenuates asbestos-induced A549 cell mitochondrial membrane potential change (A) and caspase 9 activation (B): A549 cells were exposed to FGF-10 (10 ng/ml) for 1 h followed by asbestos (25 or 50 μg/cm²) for 24 h. As expected, asbestos reduced A549 cell ΔΨm (A) as assessed by a fluorometric technique with TMRE and Mitotracker green and activated caspase 9 (B) as assessed by an ELISA as described in MATERIALS AND METHODS. FGF-10 attenuated both asbestos-induced ΔΨm and caspase 9 activation (*p < 0.005 control versus asbestos, †p < 0.05 asbestos versus FGF-10 + asbestos; n = 3).

**DISCUSSION**

Accumulating evidence shows that growth factors play an important role in preventing oxidant-induced lung injury (2, 5–8, 16). In particular, we recently showed that FGF-10, which is critical for lung development and is a potent AT2 cell mitogen, prevents oxidant-induced DNA damage in part by MEK/ERK signaling pathways and by augmenting DNA repair (17–19, 21, 22). However, it is unknown whether FGF-10 prevents mineral dust–induced AEC DNA damage and apoptosis, two events that are mediated by an oxidative stress. The major finding of this study is that FGF-10 prevents asbestos-induced AEC DNA damage. This was shown by a reduction in DNA-SB formation, as assessed by an alkaline elution technique, as well as apoptosis, as assessed by TUNEL staining and DNA fragmentation. The protective effects of FGF-10 were evident after a treatment period of as little as a 1 h, suggesting an important role for signaling mechanisms in mediating the protective effects of FGF-10 rather than other biologic actions, such as cell proliferation (2, 18). In this study, we provide evidence that MEK/ERK signaling mechanisms mediate the protective effects of FGF-10 against asbestos-induced DNA damage and apoptosis by the mitochondria-regulated death pathway. Collectively, these data suggest an important role for FGF-10 in protecting the alveolar epithelium against asbestos-induced DNA damage and apoptosis.

DNA damage surveillance mechanisms are crucial for maintaining genome integrity and cell survival (13). DNA-SB formation is among the earliest abnormalities that occur in cells exposed to oxidative stress such as H₂O₂, asbestos, radiation, and mechanical stretch (4, 7, 8, 21). The alkaline unwinding, ethidium bromide fluorescent assay for measuring DNA-SB is one of the most sensitive assays for detecting DNA damage, with a detection threshold of one break per chromosome (24). Using this assay, we showed that FGF-10 reduces asbestos-induced DNA-SB formation in both A549 and rat AT2 cells (Figures 1 and 2). DNA damage, if extensive, is a potent trigger of apoptosis. Previous studies, including ones from our group, have established that asbestos causes AEC apoptosis (9–12, 14, 15). In this study, we extend these observations by demonstrating that an important pulmonary growth factor, FGF-10, attenuates asbestos-induced apoptosis as assessed by both TUNEL staining and the highly sensitive DNA fragmentation assay (Figure 3). Apoptosis occurs by two principal pathways, the mitochondria-regulated apoptotic death (“intrinsic”) pathway and the death receptor (“extrinsic”) pathway. Mitochondria are the central regulators of apoptosis in mammalian cells exposed to a wide array of noxious stimuli including DNA damage, ROS, growth factor deprivation, calcium overload, and microtubule damaging agents (25). Work by others as well as our group has established that asbestos fibers, unlike inert particulates (e.g., glass beads or titanium dioxide), cause apoptosis by a mitochondria-regulated death pathway (12, 14, 15). In particular, the integrity of the mitochondrial DNA appears critically important in regulating the survival signals that determine whether the cells live or die in response to oxidative stress, such as with asbestos exposure (13, 14). One of the major findings of this study is that FGF-10 reduces asbestos-induced apoptosis resulting from mitochondrial dysfunction (Figure 3 and Table 1). FGF-10 completely prevented asbestos (25 μg/cm²)-induced reduction in A549 cell ΔΨm and caspase 9 activation, a caspase activated by the mitochondria-regulated death pathway (Figure 3). FGF-10 provided partial protection against A549 cell ΔΨm and caspase 9 activation by high-dose asbestos (50 μg/cm²), that can cause cell death by generating nonmitochondrial sources of ROS production (15).

Although the molecular mechanisms underlying the protec-
The protective effects of FGF-10 noted in our model are not fully established, several possibilities were considered. First, FGF-10 may augment antioxidant defenses. There is some evidence that KGF recently demonstrated that FGF-10 reduces H2O2-induced AEC apoptosis via the mitochondria-regulated death pathway.

Table 1: Mitogen-Activated Protein Kinase Signaling Pathways Mediate the Protective Effects of Fibroblast Growth Factor-10 Against Asbestos-Induced Alveolar Epithelial Cell Apoptosis via the Mitochondria-Regulated Death Pathway

<table>
<thead>
<tr>
<th></th>
<th>TUNEL (% positive cells at 24 h)</th>
<th>Mitochondrial Potential (% change at 24 h)</th>
<th>Caspase 9 Relative Units (% control at 24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 3)</td>
<td>(n = 3)</td>
<td>(n = 3)</td>
</tr>
<tr>
<td>Control</td>
<td>5.5 ± 0.9</td>
<td>0 ± 0</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>FGF-10</td>
<td>5.7 ± 0.2</td>
<td>7.7 ± 9.4</td>
<td>106.7 ± 3</td>
</tr>
<tr>
<td>U0126</td>
<td>6.6 ± 0.5</td>
<td>−3.9 ± 5.3</td>
<td>102.8 ± 1.8</td>
</tr>
<tr>
<td>Asbestos (25 μg/cm²)</td>
<td>38.2 ± 0.1*</td>
<td>−27.2 ± 2.9*</td>
<td>132.3 ± 4.6*</td>
</tr>
<tr>
<td>FGF-10 + Asbestos</td>
<td>10.2 ± 0.8*</td>
<td>−0.6 ± 7.3*</td>
<td>114 ± 5.8*</td>
</tr>
<tr>
<td>U0126 + Asbestos</td>
<td>33.0 ± 3.3*</td>
<td>−30.1 ± 6.5*</td>
<td>171.6 ± 18.6*</td>
</tr>
<tr>
<td>U0126 + FGF-10 + Asbestos</td>
<td>38.0 ± 4.5*</td>
<td>−40.5 ± 3.2*</td>
<td>177.4 ± 7.5*</td>
</tr>
</tbody>
</table>

Definition of abbreviation: FGF-10, fibroblast growth factor-10.

* P < 0.05 versus control.
† P < 0.05 versus asbestos.
‡ P < 0.05 versus FGF-10 + asbestos.

asbestos-induced AEC DNA damage and apoptosis through mechanisms that are independent of cell proliferation.

Third, FGF-10 may augment mitochondrial DNA repair. We recently demonstrated that FGF-10 reduces H2O2-induced AEC DNA damage and apoptosis in part by enhancing DNA repair mechanisms as evidenced by the lack of protection in the presence of ice-cold conditions or a DNA polymerase inhibitor (22). There is also evidence that enhanced mitochondrial oxidative DNA repair by overexpressing hOGG in HeLa cells can prevent asbestos-induced apoptosis (14). Further studies are warranted to determine whether FGF-10 affects AEC mitochondrial DNA repair and, if so, to elucidate the mechanisms involved.

Finally, we explored the signaling mechanisms activated by FGF-10 that may account for the protective effects in our model. Because FGF family members bind specific tyrosine kinase receptors (FGFR) that are coupled to multiple signaling pathways, including MAPK (2). Several lines of evidence presented in this study implicate a crucial role for MEK/ERK activation in mediating the protective effects of FGF-10 against asbestos-induced AEC DNA-SB formation, apoptosis, and mitochondrial dysfunction. First, MEK/ERK inhibitors (U0126 and PD98059) blocked the protective effects of FGF-10 against asbestos-induced A549 and AT2 cell DNA-SB formation (Figure 2). Second, MEK/ERK inhibitors prevented the protective effects of FGF-10 against asbestos-induced A549 apoptosis and mitochondrial dysfunction (Table 1), whereas inhibitors of p38-MAPK

Figure 5. Inhibition of MEK/ERK signaling pathways block the protective effects of FGF-10 against asbestos-induced reductions in A549 cell ΔΨm. A549 cells were pretreated with either an MEK/ERK inhibitor (U0126 [0.1 or 10 μM] or PD98059 [25 or 100 μM]), a p38-MAPK inhibitor (SB203580; 20 μM), or a p38K inhibitor (wortmannin; 100 nM) before FGF-10 (10 ng/mL), then exposed to asbestos (25 μg/cm²) for 24 h. MEK/ERK inhibitors blocked the protective effects of FGF-10 against asbestos-induced A549 ΔΨm in a dose-dependent manner, whereas inhibitors of p38-MAPK or P38K had negligible effects (* P < 0.05 versus control, † P < 0.05 versus asbestos, ‡ P < 0.05 versus low-dose MEK/ERK inhibitor; n = 6).
or PI3K had negligible effects (Figure 5). Third, we showed that a U0126 does not augment asbestos-induced A549 cell apoptosis or mitochondrial dysfunction, suggesting that MAPK activation is critical in mediating the protective effects of FGF-10 rather than inducing apoptosis or mitochondrial dysfunction (Table 1). We previously established that FGF-10 activates A549 cell MAPK via the Grb2-SOS/Ras/Raf-1 pathway as assessed by inhibitor studies. Western analysis of the activated form of extracellular signal–regulated kinases (ERK), and the use of a dominant/negative Ras construct (22). It is known that oxidative stress, such as from asbestos or H2O2, can activate MAPK, but whether apoptosis or proliferation occurs is cell type– and stimulus-specific (27–31). MAPK signaling pathways are critically important in fetal rat lung branching morphogenesis, a key role shared by FGF-10 (32). Although activation of the PI3K/protein kinase B pathways is a well-established survival signal from growth factors (2, 16), we show that these pathways unlikely account for our findings because the PI3K inhibitor, wortmannin, had negligible effects on the protective effects of FGF10 against asbestos-induced Δαm (Figure 5). In this study we noted that MEK/ERK inhibitors block the protective effects of FGF-10, which is unlike studies implicating the PI3K pathway, where MAPK inhibitors are ineffective. Also, previous studies have shown that MEK/ERK inhibitors block the protective effects of FGF-10, prevent DNA damage and apoptosis from other noxious agents (21, 22, 33, 34). Collectively, these data firmly implicate the MEK/ERK pathway in mediating the protective effects of FGF-10 in our model.

There are at least three possible mechanisms by which MEK/ERK activation prevents mitochondrial-regulated apoptosis: (1) phosphorylating pro-apoptotic Bcl-2 family members (e.g., Bax) that renders them inactive, (2) transcriptionally increasing anti-apoptotic Bcl-2 family members (e.g., Bcl-2 or Bcl-XL), and (3) translationally up-regulating Bcl-2 and Bcl-XL (33–35). In this study, the latter two possibilities seem unlikely to account for the protective effects of FGF-10 because we noted protection after only a 1-h treatment period. Growth factors may affect the cell cycle via MEK/ERK-dependent regulation of G1 cyclins and cyclin-dependent kinases, which results in G1 cell cycle arrest and modulation of apoptosis (35, 36). However, this mechanism seems unlikely, because we previously showed that the protective effects of KGF and FGF-10 against AEC oxidative DNA damage occur independently of cell cycling (7, 8, 21, 22).

Our data showing that FGF-10 prevents asbestos-induced mitochondrial dysfunction after a 1-h treatment periods suggest that the first mechanism in part accounts for our findings. A study in small cell lung cancer shows that FGF-2 prevents mitochondria-regulated apoptosis by MEK/ERK signaling and transcriptional regulation of proteins (37). Further studies are necessary to determine the downstream molecular mechanisms by which FGF-10–induced MEK/ERK activation mediates survival signals that prevent asbestos-induced DNA damage and apoptosis.

In summary, we have shown that FGF-10 attenuates asbestos-induced AEC DNA damage and apoptosis. Furthermore, our findings implicate an important role for MEK/ERK activation in mediating these effects, in part by preventing mitochondria-regulated cell death caused by altered mitochondrial membrane potential and caspase 9 activation. These findings add to the accumulating body of evidence that FGF-10–induced MEK/ERK signaling mechanisms are important in AEC survival. A hypothetical model summarizing some of the key branch points that can result in AEC survival or apoptosis is shown in Figure 6. Future studies are necessary to determine the downstream molecular mechanisms mediating the protective effects of FGF-10 as well as the in vivo relevance of our findings. We reason that FGF-10 has an important role in preventing oxidant-induced lung injury, including that resulting from asbestos exposure.

Conflict of Interest Statement: D.U. has no declared conflicts of interest; V.P. has no declared conflicts of interest; and D.W.K. has no declared conflicts of interest.

References


