Gene transfer of hepatocyte growth factor by electroporation reduces bleomycin-induced lung fibrosis

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Gazdhar A, Fachinger P, van Leer C, Pierog J, Gugger M, Friis R, Schmid RA, Geiser T. Gene transfer of hepatocyte growth factor by electroporation reduces bleomycin-induced lung fibrosis. Am J Physiol Lung Cell Mol Physiol 292: L529–L536, 2007. First published October 20, 2006; doi:10.1152/ajplung.00082.2006.—Abnormal alveolar wound repair contributes to the development of pulmonary fibrosis after lung injury. Hepatocyte growth factor (HGF) is a potent mitogenic factor for alveolar epithelial cells and may therefore improve alveolar epithelial repair in vitro and in vivo. We hypothesized that HGF could increase alveolar epithelial repair in vitro and improve pulmonary fibrosis in vivo. Alveolar wound repair in vitro was determined using an epithelial wound repair model with HGF-transfected A549 alveolar epithelial cells. Electroporation-mediated, nonviral gene transfer of HGF in vivo was performed 7 days after bleomycin-induced lung injury in the rat. Alveolar epithelial repair in vitro was increased after transfection of wounded epithelial monolayers with a plasmid encoding human HGF, pCikhHGF [human HGF (hHGF) gene expressed from the cytomegalovirus (CMV) immediate-early promoter and enhancer] compared with medium control. Electroporation-mediated in vivo HGF gene transfer using pCikhHGF 7 days after intratracheal bleomycin reduced pulmonary fibrosis as assessed by histology and hydroxyproline determination 14 days after bleomycin compared with controls treated with the same vector not containing the HGF sequence (pCik). Lung epithelial cell proliferation was increased and apoptosis reduced in hHGF-treated lungs compared with controls, suggesting increased alveolar epithelial repair in vivo. In addition, profibrotic transforming growth factor-β1 (TGF-β1) was decreased in hHGF-treated lungs, indicating an involvement of TGF-β1 in HGF-induced reduction of lung fibrosis. In conclusion, electroporation-mediated gene transfer of hHGF decreases bleomycin-induced pulmonary fibrosis, possibly by increasing alveolar epithelial cell proliferation and reducing apoptosis, resulting in improved alveolar wound repair.

PULMONARY FIBROSIS is a lung disease with high morbidity and mortality. It is histologically characterized by excessive deposition of collagen in the lung, resulting in impaired gas exchange and hypoxia. There is increasing evidence that alveolar epithelial injury and aberrant alveolar wound repair are major mechanisms for the development of pulmonary fibrosis (8, 29). Previous studies indicate that efficient alveolar epithelial repair may reduce the development of pulmonary fibrosis (3). Modulation of the alveolar repair process after injury may therefore represent a novel therapeutic target in patients with lung injury and subsequent pulmonary fibrosis.

Hepatocyte growth factor (HGF) is a potent mitogenic factor for alveolar and bronchial epithelial cells (31, 32). HGF stimulates DNA synthesis in alveolar type II cells in vitro (21, 30) and in vivo (26) and has antiapoptotic properties (5). Previous in vivo studies showed reduced collagen accumulation in the bleomycin-induced lung fibrosis model after HGF administration (6), indicating an important role of HGF in the development of pulmonary fibrosis (35). However, the effect of the intratracheally administered HGF protein was short due to the short half-life of HGF (6), or HGF needed to be administered by continuous infusion in excessive amounts (39). Therefore, novel techniques to achieve sustained expression of HGF in the injured lung are needed to optimize its antifibrotic effect.

Although viral vector-mediated gene transfer is a widely used technique to express specific proteins, serious disadvantages limit its use in animal models and patients. Notably, it activates the host immune system, it brings the risk of insertional mutagenesis, and it can effect only a relatively short duration of expression by the transfected gene in the target organ. In vivo electroporation is a physical method of gene transfer by which plasmid DNA injected into the tissue is delivered into the cells by short-duration electric pulses. The pulsed electrical field creates transient pores in the cells allowing transfer of large DNA and protein molecules. Recently, electroporation has emerged as a promising gene delivery method and has been tested in a broad range of target tissues and organs such as liver (16), spinal cord (18), and skeletal muscle (22, 23).

We hypothesized that HGF could be locally expressed in the bleomycin-injured lung using electroporation-mediated gene transfer to the lungs, resulting in the reduction of bleomycin-induced pulmonary fibrosis. We show that electroporation-mediated gene transfer in the lung is an efficient method for achieving HGF gene expression and that gene transfer of human HGF (hHGF) reduces pulmonary fibrosis in the bleomycin-injured rat lung, even if performed 7 days after bleomycin instillation. Because of increased proliferation and reduced apoptosis of alveolar epithelial cells and reduced TGF-β1 levels after hHGF gene transfer, we assume that local expression of HGF in the injured lung may support alveolar epithelial repair, therefore contributing to the reduction of lung fibrosis.
MATERIALS AND METHODS

Plasmids

The full-length sequence of hHGF was kindly provided by Prof. Toshikazu Nakamura, Osaka, Japan. The plasmid was constructed by inserting hHGF cDNA (2.1 kb) into the backbone of pCik, driven by the human cytomegalovirus (CMV) early promoter enhancer (pCikhHGF) (12) between the NotI and Nhel sites. Empty vector (not containing the hHGF sequence) served as control. Endotoxin-free plasmids were produced at large scale at Plasmid Factory (Bielefeld, Germany). For gene delivery, the plasmid was suspended in endotoxin-free water.

In vitro alveolar epithelial wound repair assay. Human A549 alveolar epithelial-like cells [American Type Culture Collection (ATCC), Rockville, MD] were cultured to confluence in six-well plates using RPMI 1640 containing 10% fetal bovine serum (GIBCO). The epithelial monolayer was mechanically wounded with a pipette tip (11) followed by transfection with pCikhHGF using Lipofectamine 2000 (Invitrogen) as described by the manufacturer. Control experiments were performed using pCik without containing the hHGF sequence. Images of the wound surface were captured at time 0 and after 24 h using an inverted microscope (Leitz Diavert, Wetzlar, Germany) connected to a digital camera (Nikon Coolpix). Quantification of the wound surfaces was performed using Scion image software, and wound repair was expressed as percentage of epithelial wound closure after 24 h. After the experiment, cell culture supernatants were harvested, and HGF levels were determined using ELISA (R&D Systems, Abingdon, UK). Assays were done in triplicate with at least three independent experiments.

Cell spreading and migration were determined with a modified protocol published previously (11). Briefly, wounded alveolar epithelial monolayers were fixed after the experiment and stained with Hoechst nuclear stain, and five different intercellular distances in five randomly chosen high-power fields were measured in each condition at the edge of the wound and in the intact monolayer of the same well.

5-Bromo-2'-deoxyuridine (BrdU) immunolabeling was performed to determine cell proliferation according to the manufacturer’s protocol (Roche Diagnostics, Basel, Switzerland). BrdU-positive cells were counted in five randomly chosen high-power fields at the edge of the wound.

Animals

Male Fischer F344 rats (220–240 g) were obtained from Harlan (The Netherlands). Experiments were performed in accordance to the standards of the European Convention of Animal Care. The study protocol was approved by the University of Bern Animal Study Committee.

Instillation of bleomycin. At day 1 of the protocol, F344 rats (220–240 g) were anesthetized by inhalation of 4% halothane in a glass chamber, intubated with a 14-gauge intravenous catheter (Insyte, Madrid, Spain), and instilled intratracheally with bleomycin (1.28 U/rat) to both lungs. The dosage of bleomycin was based on preliminary experiments showing induction of pulmonary fibrosis with lowest mortality.

In vivo HGF gene transfer. Seven days after intratracheal instillation of bleomycin, the animals were randomly divided into two groups (each n = 5). One group was treated by intratracheal administration of the empty vector, pCik (control group), the second group with the plasmid containing the HGF sequence, pCikhHGF (250 μl, 1 μg/μl) followed by electroperoration of the left lung. For electroperoration-mediated gene transfer, the animals were anesthetized by inhaling 4% halothane in a glass chamber, intubated, and ventilated via a 14-gauge catheter (Insyte) with Fio2 = 1.0 and 1.5% halothane to maintain anesthesia. The breathing frequency was 100 breaths/min, and the tidal volume 10 ml/kg body wt using a rodent ventilator (model 683; Harvard Apparatus, South Natick, MA). A left thoracotomy in the fourth intercostal space was performed, and the animals were turned to the left side and placed with head up at a 45° angle to facilitate even distribution of the instillate. A volume of 250 μl of either pCik or pCikhHGF, including 5 μg of the DNase inhibitor ATA (auringicarboxylic acid; Sigma-Aldrich), was instilled to the left lung through the intubation tube using a fine 24-gauge catheter under visual control. Plate electrodes (2 × 1 cm) were placed on each side of the left lung followed by the application of four pulses of 20 ms and 200 V/cm. A small chest drain was inserted into the left hemithorax, and the thoracotomy was closed with four layers of continuous sutures (4–0 Prolene). The chest drain was removed after the animals recovered spontaneous breathing, followed by extubation. To be consistent, only the left lung was assessed in both treated and control animals.

Assessment

At day 14 (7 days after electroporation-mediated hHGF gene transfer), animals were anesthetized as described above. Thiopental (50 mg/kg body wt) was administered intraperitoneally, and the animals were ventilated via a tracheostomy with the Harvard Rodent Ventilator with FiO2 = 1.0, a frequency of 100 breaths/min, and a tidal volume of 10 ml/kg. Subsequently, the pulmonary vessels were flushed with 20 ml of 0.9% saline under pressure of 20 cmH2O. The heart-lung block was explanted, and tissue samples were collected for further analysis.

Histochemistry and immunohistochemistry. Routine hematoxylin and eosin staining was performed with formalin-fixed tissue sections. To evaluate the extent of pulmonary fibrosis, the scoring system of Ashcroft (Ref. 4) was used. Briefly, a score ranging from 0 (normal lung) to 8 (total fibrosis) was given for each of five randomly chosen microscopic fields, and the mean score of all fields was calculated. Formalin-fixed tissue sections were deparaffinized in a xylene series and rehydrated through a decreasing ethanol series. Sections were then incubated with the anti-hHGF antibody (R&D Systems, Abingdon, UK) at 1:100 dilution overnight at 4°C followed by the second antibody-horseradish peroxidase (HRP) conjugate (1:500) for 1 h. Staining was with 3,3-diaminobenzidine (DAB) using a cell and tissue staining kit (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. For Ki67 immunostaining, the sections were subjected to antigen retrieval with citrate buffer (100 mM, pH 7.0) by cooking in a pressure cooker for 10 min followed by incubation with the Ki67 antibody (NCL-Ki67P; Novacastra, Newcastle upon Tyne, UK) at a 1:100 dilution overnight. Staining was performed as described above using the HRP-DAB system (R&D Systems, Minneapolis, MN). To study apoptosis, the sections were incubated with an antibody against cleaved caspase-3 (Cell Signaling Technologies, Beverly, MA) at 1:50 dilution overnight at 4°C and stained using the HRP-DAB system (R&D Systems, Minneapolis, MN).

In situ hybridization. The hHGF cDNA fragment was subcloned into Blue Script vector (Stratagene, La Jolla, CA). The plasmid containing the cDNA insert was linearized using the restriction endonucleases NotI for in vitro transcription of the sense strand and HindIII for the antisense strand. Single-stranded sense and antisense digoxigenin (DIG)-labeled cRNA probes were transcribed in vitro using 1 μg of linearized template and 40 units of the appropriate polymerase (T7 RNA polymerase for the sense strand, T3 RNA polymerase for the antisense strand) with DIG-labeled uridine triphosphate using the DIG RNA Labeling Kit according to the manufacturer’s protocol (Roche Diagnostics).

Tissue sections were deparaffinized in a xylene series and then rehydrated through a decreasing ethanol series diluted in dimethyl pyrocarbonate-treated water. Sections were then subjected to Proteinase K treatment (10 μg/ml) at 37°C for 10 min. After sections were washed with phosphate-buffered saline (PBS), they were treated with acetic anhydride for 10 min at room temperature. The slides were rewashed with PBS and prehybridized for 2 h at 55°C in the hybridization solution containing 50% formamide (Sigma-Aldrich) and...
tRNA (10 μg/ml) before hybridization with 100 ng of either sense or antisense cRNA-DIG-labeled probes in a humid chamber at 55°C for 18 h. After hybridization, slides were washed in increasingly stringent buffers as follows: ×2 SSC and 50% formamide; ×2 SSC, 0.2% SDS for 15 min at 37°C; and two washes with ×0.1 SSC, 0.2% SDS for 15 min at 60°C. Detection was carried out by incubation with an alkaline phosphatase-conjugated anti-DIG antibody using nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indoyl phosphate according to the manufacturer’s instructions (Roche Diagnostics).

Hydroxyproline assay. Lungs were analyzed for collagen content as initially described by Woessner (38). The lung left was excised and snap-frozen after the wet weight was measured. The frozen lungs were homogenized, and 1 ml of the homogenate was treated with 10% trichloroacetic acid, hydrolyzed with 6 M hydrochloric acid (18 h at 110°C), and adjusted to pH 7.0. Oxidation was initiated by incubation with 1 ml of chloramine T reagent for 20 min at room temperature and stopped by addition of 1 ml of 3.15 M perchloric acid. After incubation with Ehrlich reagent (p-dimethylaminobenzaldehyde added to methyl cellosolve) for 20 min at 55–65°C, the absorbance of each sample was measured at 557 nm. A standard curve was generated using known concentrations of reagent-grade hydroxyproline (Sigma).

Determination of transforming growth factor-β1 in lung homogenates. Transforming growth factor-β1 (TGF-β1) levels were determined in lung homogenates 7 days after electroporation-mediated hHGF gene transfer. pCikhHGF and pCik-treated lungs were frozen in liquid nitrogen. The tissue was then grinded and suspended in ×1 RIPA buffer, followed by homogenization. The samples were thawed at room temperature and three “freeze and thaw” cycles were performed using liquid nitrogen and water bath at room temperature. Debris was removed by centrifugation. TGF-β1 activity was measured using the TGF-β1 ELISA kit (R&D Systems, Abingdon, UK) following the manufacturer’s protocol. Total lung protein concentration was determined using a detergent-compatible protein assay (Bio-Rad), and TGF-β1 activity was normalized for protein content.

Statistical Analysis

Data are presented as means ± SE. Statistical analysis was done by unpaired Student’s t-test or the nonparametric Mann-Whitney rank sum test, where appropriate, using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA). The results were considered significant at *P < 0.05.

RESULTS

Alveolar Epithelial Wound Repair in Vitro

Wounded monolayers of A549 alveolar epithelial cells were transfected with pCikhHGF, and alveolar epithelial wound repair was studied compared with wounded A549 cells transfected with pCik (not containing the hHGF sequence), both cultured in serum-free medium. Alveolar epithelial wound closure in vitro was increased after 24 h in pCikhHGF-transfected compared with pCik-transfected A549 epithelial cells (Fig. 1A). Consistently, physiological levels of hHGF were detected in the supernatant of pCikhHGF-transfected A549 epithelial cells (3.266 ± 156 pg/ml), whereas no HGF could be detected in the supernatant of pCik-transfected A549 epithelial cells (data not shown).

Mechanisms of HGF-induced alveolar epithelial wound repair. We determined cell spreading, migration, and cell proliferation in wounded, pCikhHGF-transfected A549 epithelial cells to determine possible mechanisms of HGF-induced alveolar epithelial wound repair. The internuclear distance of cells at the wound edge was significantly increased after transfection with pCikhHGF compared with transfection with the empty vector (pCik), indicating that cell spreading and migration contributes to alveolar epithelial wound repair induced by hHGF (Fig. 1B). Staining of the wounded and transfected monolayers with BrdU did not show a significant alteration in the number of proliferating cells at the wound edge, suggesting that proliferation does not seem to be a major mechanism of HGF-induced alveolar epithelial wound repair in our in vitro model.

Electroporation-mediated gene transfer is an efficient method in bleomycin-induced lung injury. Electroporation-mediated HGF gene transfer was performed 7 days after intratracheal bleomycin instillation using pCikhHGF. In situ hybridization and immunohistochemistry performed 7 days after electroporation-mediated HGF gene transfer showed expression of hHGF on both the RNA and protein level in HGF-treated lungs (Fig. 2), whereas no hHGF could be detected in lungs from control animals treated with pCik only. Both mRNA and protein expression was mainly localized in bronchial and alveolar epithelial cells and, to some extent, in alveolar macrophages.
We measured hHGF levels in the plasma of pCikhHGF and pCik-treated rats to evaluate if hHGF expressed in the lung can be detected in the systemic circulation. In the plasma of pCikhHGF-treated rats, the mean hHGF level was 1.6 ± 0.7 ng/ml, whereas no hHGF could be detected in rats treated with the empty vector (pCik) 7 days after electroporation-mediated hHGF gene transfer. In addition, no hHGF could be detected in the plasma obtained from pCikhHGF-treated rats 14 days after hHGF gene transfer (data not shown).

Electroporation-mediated gene transfer of hHGF reduces lung fibrosis. Electroporation-mediated gene transfer of hHGF markedly reduced lung injury and fibrosis compared with the control group as assessed 14 days after bleomycin instillation and 7 days after electroporation (Fig. 4). Whereas severe inflammation and fibrotic changes were detected in lugs of control animals, considerable improvement could be seen in hHGF-treated lungs (Fig. 4, A and B). The fibrotic changes of the lung were further assessed and quantified by the Ashcroft score (Ref. 4). Electroporation-mediated gene transfer of hHGF reduced the score from 3.4 ± 0.47 (control) to 1.7 ± 0.3 (P < 0.01) (Fig. 4C). In accordance, collagen content, as assessed by the hydroxyproline assay, was significantly reduced in the animals treated with hHGF compared with the control group (2,036 ± 410 vs. 5,428 ± 418 μg/mg; P < 0.01) (Fig. 4D).

Electroporation-mediated gene transfer of hHGF increases alveolar epithelial cell proliferation. Since HGF is a potent mitogen for alveolar epithelial cells, we studied cell proliferation 7 days after hHGF treatment by performing immunohistochemistry with the proliferation-specific Ki67 antibody. Cell proliferation increased in lungs treated with pCikhHGF compared with the control group (286 ± 77.2 positive cells/high-power field for pCikhHGF vs. 78 ± 17.5 positive cells/high-power field in the control group; P < 0.05) (Fig. 5, A–C). Based on their localization, the majority of Ki67-positive cells were alveolar and, to a lesser extent, bronchial epithelial cells (Fig. 5B, inset).

Electroporation-mediated gene transfer of hHGF reduces apoptosis. Since both cell proliferation and cell death can modulate alveolar repair, we also studied the number of apoptotic cells in bleomycin-injured lungs followed by electroporation-mediated hHGF gene transfer. Activated (cleaved) caspase-3 positive cells were markedly fewer in hHGF-treated lungs compared with controls. There were 29 ± 8 caspase-3-positive cells/high-power field for the pCikhHGF-treated animals vs. 65 ± 11 positive cells/high-power field for controls (P < 0.05) (Fig. 6).

TGF-β1 is decreased in pCikhHGF-treated lungs. Since TGF-β1 was repeatedly shown to be a profibrotic mediator in the bleomycin-induced lung injury model, we studied TGF-β1 expression after electroporation-mediated hHGF gene transfer. TGF-β1 was decreased after treatment with pCikhHGF compared with treatment with the empty vector pCik (Fig. 7), suggesting that TGF-β1 is involved in the hHGF-induced reduction of bleomycin-induced lung fibrosis.

DISCUSSION

This study shows that electroporation-mediated gene transfer of hHGF reduces bleomycin-induced pulmonary fibrosis in the rat lung. Increased proliferation and decreased apoptosis of alveolar epithelial cells suggests that improved alveolar repair may contribute to the observed reduction of pulmonary fibrosis in hHGF-treated lungs. Our in vitro data using an alveolar epithelial wound repair model indicates that hHGF-transfected
cells have an increased repair capacity, further supporting the concept of improved alveolar epithelial repair after electroporation-mediated hHGF gene transfer in the injured lung.

Pulmonary fibrosis is a progressive disorder with limited therapeutic options (13). Increasing evidence suggests that the changes present in pulmonary fibrosis result from alveolar epithelial injury and abnormal wound repair (29). Alveolar epithelial cell injury induces the proliferation of fibroblasts and their differentiation to myofibroblasts. The increased production of extracellular matrix by activated fibroblasts/myofibroblasts results in the excessive deposition of extracellular matrix and the development of pulmonary fibrosis. Efficient alveolar epithelial repair can inhibit the development of pulmonary fibrosis since the presence of an intact alveolar epithelial layer was shown to suppress fibroblast proliferation and matrix deposition (2). This property of alveolar epithelium has been confirmed in several animal models including the bleomycin lung injury model, demonstrating that delaying alveolar epithelialization after lung injury leads to an enhanced fibrotic response (3). We therefore hypothesized that long-term administration of a growth factor for alveolar epithelial cells may reduce pulmonary fibrosis in the bleomycin-induced lung injury model due to improved proliferation and repair.

HGF is a pleiotropic growth factor that has recently been shown to have mitogenic, morphogenic, motogenic, and antiapoptotic properties (24, 25, 36, 37). In particular, it is a potent mitogen for alveolar type II epithelial cells in vitro (21) and in vivo (26). The therapeutic potential of HGF in lung injury and

![Image](image1.png)

**Fig. 4. Electroporation-mediated gene transfer of hHGF reduces bleomycin-induced pulmonary fibrosis.**

A: bleomycin-induced lung injury followed by electroporation-mediated transfer of pCik (empty vector not containing the HGF sequence) 7 days after bleomycin (control). B: bleomycin-induced lung injury followed by electroporation-mediated hHGF gene transfer (pCikhHGF) 7 days after bleomycin (A and B hematoxylin and eosin stainings, objective magnification ×20). C: assessment of lung fibrosis using the Ashcroft score for grading pulmonary fibrosis 14 days after bleomycin instillation. n = 5 in each group; *P < 0.05. D: reduction of hydroxyproline content after electroporation-mediated gene transfer of hHGF (pCikhHGF) in bleomycin-injured lungs. Values are means ± SE; n = 5 in each group; **P < 0.01.

![Image](image2.png)

**Fig. 5. Cell proliferation after electroporation-mediated hHGF gene transfer (pCikhHGF) in bleomycin-injured lungs.** Immunochemical Ki67 staining for cell proliferation in bleomycin-injured lungs transduced with pCik (vector not containing the HGF sequence) (A) and after electroporation-mediated hHGF gene transfer (pCikhHGF) (B); objective magnification ×40 in A and B, ×100 in inset of B. C: number of Ki67-positive cells per high-power field were increased after electroporation-mediated gene transfer of pCikhHGF. Values are means ± SE; n = 5 in each group; *P < 0.05.
fibrosis has been reported previously. Yaekashiwa and coworkers (40) administered continuous infusion of recombinant HGF protein, whereas Dohi and coworkers (6) injected recombinant hHGF intratracheally after bleomycin injury, resulting in reduced pulmonary fibrosis. Since enhanced cell proliferation already decreased 2–3 days after HGF administration, other approaches are needed to achieve an optimal antifibrotic effect of HGF (6). We therefore established an electroporation-mediated gene transfer technique that allows expressing hHGF for a longer period of time in the injured lung. Using this technique, we show that electroporation-mediated hHGF transfer efficiently reduces pulmonary fibrosis in bleomycin-treated lungs.

Electroporation, a technique routinely used to transfer DNA to bacteria, yeast, and mammalian cells in vitro, is now used in various in vivo applications as well (15, 16, 18, 19, 22, 23, 33). When applied at appropriate field strength, electroporation is safe, efficient, and a reproducible technique requiring low doses of plasmid DNA. In previous experiments, we (7) showed that application of controlled electric pulses on normal lung tissue has no adverse effect on the structure and function of the lung. In particular, we could not detect increased inflammation after electroporation-mediated gene transfer. Thus the use of in vivo electroporation for plasmid DNA transfer appears to be a reproducible and safe method for efficient gene transfer.

hHGF was detected on the RNA level by in situ hybridization and on the protein level by immunohistochemistry in the lung of treated animals. Positive signals for hHGF were mainly seen in the bronchial and alveolar epithelium and in alveolar macrophages, whereas increased cell proliferation was mainly localized in the alveolar epithelium. These data indicate that the main target cells of the transferred hHGF, the alveolar epithelium, may be stimulated by autocrine (and paracrine) mechanisms in our model. Since the levels of hHGF in the plasma were very low and transient after hHGF gene transfer, we do not expect significant systemic effects of hHGF in our bleomycin lung injury model.

Since HGF was shown to increase migration (17) and proliferation (21) of alveolar type II epithelial cells in vitro, we hypothesized that HGF may stimulate alveolar epithelial repair in vitro and studied alveolar epithelial repair using our in vitro epithelial wound repair model with human A549 alveolar epithelial cells (9). Wounded A549 epithelial cells were transfected with the same plasmid pCikhHGF that was also used in the in vivo experiments. Transfected cells produced high levels of HGF compared with medium control, resulting in improved alveolar epithelial repair in vitro. These data support the hypothesis that improvement of pulmonary fibrosis in hHGF-treated animals may at least partially be due to improvement of alveolar repair in vivo. Previous studies showing a timely correlation between HGF concentrations in bronchoalveolar lavage fluid and proliferating alveolar epithelial cells in the bleomycin-induced lung injury model further support this hypothesis (1). Moreover, our results show that hHGF reduces

![Graph](http://ajplung.physiology.org/)
apoptosis in the bleomycin-injured lung. Reducing apoptosis is a known mechanism for inhibiting the development of pulmonary fibrosis in the bleomycin lung injury model (14). We therefore speculate that suppression of cell apoptosis by hHGF may have preserved the alveolar epithelium, resulting in improved alveolar epithelial wound repair (10).

TGF-β1 plays a central role in the development of pulmonary fibrosis. TGF-β1 induces myofibroblast differentiation and synthesis of extracellular matrices and inhibits matrix degradation. HGF was shown to reduce fibrogenesis by down-regulation of TGF-β1 in several experimental models of fibrosis such as chronic renal fibrosis (41) and liver fibrosis (39). We demonstrate that the levels of TGF-β1 decreased after hHGF gene transfer, indicating that the reduction of TGF-β1 may contribute to the antifibrotic activity of HGF observed in the bleomycin-induced lung fibrosis model.

There is increasing evidence that HGF may play an important role in patients with pulmonary fibrosis. Levels of HGF in bronchoalveolar lavage fluid are increased in patients with idiopathic pulmonary fibrosis (IPF), lung fibrosis associated with rheumatoid arthritis and sarcoidosis (28). In IPF, HGF was mainly detected in hyperplastic alveolar type II epithelial cells (28), indicating a role of HGF in alveolar epithelial repair. However, isolated fibroblasts from lung tissue obtained from patients with IPF produced less HGF in culture than fibroblasts isolated from control lungs, indicating a defect in HGF secretion by fibroblasts in IPF (20). These data support the concept of HGF administration for the treatment of patients with pulmonary fibrosis.

Recently, other techniques of electroporation-mediated gene transfer have been reported. Machado-Aranda et al. (19) performed efficient transfer of the Na⁺-K⁺-ATPase β1 to the lung by extracorporeal electroporation. This approach has the advantage that no thoracotomy is needed; however, the electric pulses cannot be targeted specifically to the injured organ. We preferred targeted electroporation to the lung to be able to induce HGF gene transfer specifically to the injured left lung. Moreover, electroporation-mediated HGF gene transfer to the skeletal muscle was reported for the treatment of pulmonary fibrosis in mice (34). This study by Umeda et al. (34) supports our findings that HGF may be beneficial in the bleomycin-injured lung. However, a direct comparison of these data with our results is difficult due to differences in the experimental protocol and the animal species that was used. We chose electroporation-mediated gene transfer directly to the lung to avoid as much as possible the potential systemic effects of HGF, as previously reported, in case of electroporation-mediated gene transfer of hHGF to skeletal muscle (27). Hence, the concept of a peripheral route of gene transfer in combination with a tissue or even cell-specific gene targeting has to be further evaluated.

In summary, we show an effective, nonviral technique of gene transfer of hHGF by electroporation to the bleomycin-injured lung, resulting in marked improvement of pulmonary fibrosis. Electroporation-mediated transfer of hHGF-induced proliferation and reduced apoptosis mainly of the alveolar epithelium, suggesting improvement of alveolar wound repair in vivo. The technique of in vivo electroporation-mediated gene transfer of HGF may therefore represent a novel therapeutic strategy in patients with pulmonary fibrosis.

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