C57BL6 mice (male, 10 weeks) were used. On Day 1, mice were anesthetized with isoflurane, xylazine, and ketamine, and a 7-day micro-osmotic pump containing 120 mg/kg of bleomycin was aseptically implanted into a subcutaneous space. On Day 8, mice were again anesthetized, and saline or $1.0x10^9$ plaque-forming units (PFU) of an adenoviral vector was instilled intratracheally. In a survival trial, one group of mice received the lower dose of $1.0x10^8$ PFU of the adenoviral vector. A second osmotic pump containing 120 mg/kg of bleomycin was implanted on Day 29 to induce pulmonary fibrosis progressively for 8 weeks.

*Survival study*

Mice were given bleomycin and the viral vector as described above. Survival was then assessed for up to 8 weeks.

*Histopathological examination*

In addition to routine hematoxylin and eosin (H&E) staining, we stained sections with Masson's trichrome to visualize collagen deposition clearly.

*Histopathological quantification of fibrosis*

The Ashcroft score was calculated for semiquantitative analysis of fibrotic change (27).

*Immunohistochemistry of lungs*

Paraffin sections were immunostained with KGF (FGF-7) antibody and rabbit polyclonal anti-surfactant protein C (SP-C) antibody, followed by processing using an EnVision System. Cells positive for SP-C were counted and are presented as a percentage of the total number of cells in the field.

*Measurement of pulmonary function*

Mice were anesthetized with xylazine and ketamine, and the trachea was cannulated by a metallic needle through a tracheostomy. Mice were ventilated with a tidal volume of 8 ml/kg at a rate of 150 breaths/min, and pulmonary function was measured using a computer-controlled animal ventilator.

*Reverse transcriptase polymerase chain reaction (PCR)*

Total RNA was extracted from the lungs and cDNA synthesis was performed; subsequent PCR reactions were performed using an RNA-LAPCR kit.

### *Real-time PCR*

Total RNA was transcribed using Superscript III reverse transcriptase (Invitrogen) and oligo-(dT). PCR amplification was performed on a Thermal Cycler Dice (Takara Bio) using SYBR Green I as a double-strand DNA-specific binding dye and continuous fluorescence monitoring according to the manufacturer's instructions.

### *Western blotting for transforming growth factor (TGF)-β1 and SP-D*

One lobe of the right lung was homogenized with an elution buffer on ice. Standardized quantities of proteins were loaded onto SDS-PAGE gel and transferred electrophoretically onto nitrocellulose membranes.

### *Statistics*

Data are reported as means and SEM for each group. Comparisons of multiple groups were made with a Tukey-Kramer *post hoc* test after analysis of variance (ANOVA). Survival rates are shown on the basis of Kaplan-Meier product limit curves, and the groups were compared by log rank test. A $p < 0.05$ was considered significant.

## Results

### *Survival rates using two different vector doses*

Mice administered bleomycin were instilled intratracheally with adenovirus vector on day 8. The survival rate of mice given the low dose ($1.0 \times 10^8$ PFU/mouse) of Ad-1w1 was similar to that of the saline-administered control (**Fig. 2A**). Mice given the low dose of Ad-KGF had a significantly higher survival rate than the saline group ($p$=0.011) and low dose Ad-1w1 group ($p$=0.032), and mice given the high dose ($1.0 \times 10^9$ PFU/mouse) of Ad-1w1 had a significantly lower survival rate than the saline group ($p$<0.001) (**Fig. 2B**). Mice given the high dose of Ad-KGF had a significantly higher survival rate compared with the saline group (p=0.002), and the percentage of surviving mice at eight weeks was the highest of all groups (68.8%). These observations suggest that (i) even the low dose of Ad-KGF

worked effectively for survival, (ii) the high dose of the adenoviral vector lacking KGF expression (Ad-1w1) had a significant negative or toxic effect on the survival of mice, but (iii) the high dose of Ad-KGF clearly overcame the negative effect of the vector on mice given bleomycin. Therefore, we selected the high dose of vector virus ($1.0 \times 10^9$ PFU/mouse) for the subsequent experiments.

*Effects of adenovirus vectors*

The effects of KGF-over expression using Ad-KGF and the adverse effects related to the adenovirus and/or KGF over-expression, including inflammation and fibrous change of lungs were evaluated histopathlogically in the lungs of mice that were given Ad-KGF. Remarkable KGF expression in the lung cells was observed one week after the administration of Ad-KGF (**Fig. 3A**). Secretion of SP-C, a product of type II pneumocytes, was remarkable one week after the administration of Ad-KGF (**Fig. 3B**). H&E staining (**Fig. 3C**) as well as Masson's trichrome staining, which stains collagen fibers blue (**Fig. 3D**), of lungs harvested from mice 3 weeks after Ad-KGF administration demonstrated minimal fibrosis in the lungs in the chronic phase. The average Ashcroft score of these lungs was $0.7 \pm 0.1$, which was comparable to that of naïve mice.

*Histopathological evaluation*

The morphology of the lungs was evaluated with H&E and Masson's trichrome stain (insets) (**Fig. 4**). Mild fibrous changes in subpleural areas were seen in the lungs at 1 week after bleomycin administration when the vector was instilled into the lungs (**Fig. 4B**). Progressive expansion of fibrotic areas toward the lung parenchyma was seen in all groups. The fibrotic areas were larger at 4 weeks (**F, G, H**) than at 2 weeks (**C, D, E**), but the area involved was significantly smaller in the KGF group (**E, H**) than in the saline (**C, F**) and Ad-1w1 (**D, G**) groups. Lung fibrosis in the KGF group at 8 weeks (**I**) was similar to that in the KGF group at 4 weeks (**H**). Because all mice in the saline and Ad-1w1 groups died before 8 weeks, no lung tissue was available for comparison of these groups. These findings suggested that the progressive fibrosis caused by bleomycin is attenuated by Ad-KGF. Inflammatory cell infiltration associated with bleomycin administration was found in the subpleural stroma and alveolar walls (**Fig. E1**). Inflammatory cells consisted of lymphocytes and

neutrophils, and the intensity of infiltration was not considerably different among the saline, KGF, and 1w1 groups. Adenovirus-exposed lung tissue (KGF and 1w1 groups) revealed mild lymphocyte infiltration around the bronchioles as reported previously (22).

*Ashcroft score*

Lung fibrosis was quantitatively analyzed by Ashcroft score using sections stained with Masson's trichrome (**Fig. 4J**). Applying the rules described in the Methods section in the online data supplement, at least 12 grids of each specimen were evaluated. The Ashcroft score was significantly higher at 4 weeks than at 2 weeks in the 1w1 group. Less fibrosis was seen in the KGF group than the 1w1 group at 4 weeks. Although the Ashcroft score trended to increase from 4 weeks to 8 weeks in the KGF group, the change was not statistically significant.

*Lung function test*

Cst decreased in the groups that were administered bleomycin compared with that of the naïve group (**Fig. 5A**). Cst levels at 2 weeks were the same in the saline, 1w1, and KGF groups, but it had declined further at 4 weeks in the control and 1w1 groups, and Cst was then significantly greater ($p<0.01$) in the KGF group compared with that in the 1w1 group. The lung volume (TLC - FRC) was less in the saline, 1w1, and KGF groups compared with that in the naïve group (**Fig. 5B**).

*Analysis of Ad-KGF mRNA in lungs*

In the KGF group, vector-derived KGF mRNA was detectable at 2 weeks (1 week after vector administration), it had declined at 4 weeks, and then was not detectable at 8 weeks (**Fig. 6A**). Vector-derived KGF was not detectable in the other groups.

*Analysis of total KGF mRNA in lungs*

The alterations of total KGF (endogenous KGF + Ad-KGF-derived murine KGF) mRNA levels were analyzed using real-time PCR, and the values were normalized to that for the naïve group (**Fig. 6B**). The total KGF mRNA levels of saline and 1w1 groups were significantly reduced compared with the naïve group at 2 weeks. In contrast, the level of the KGF group at 2 weeks was about 12 times that of the naïve group and was also significantly

higher than that of the saline and 1w1 groups. The total KGF mRNA levels of the saline and 1w1 groups at 4 weeks were still significantly lower than that of the naïve group. The total KGF mRNA level of the KGF group at 4 weeks had declined to a level that was not significantly different from that of the naïve group, and was significantly lower than that of the KGF group at 2 weeks. At 8 weeks, the total KGF mRNA level in the KGF group had declined further and was significantly lower than that of the naïve group.

### *Immunohistochemistry of SP-C*

Surfactant proteins are secreted by type II pneumocytes. We used SP-C as a specific marker for alveolar type II cells (26). While only a few cells were SP-C-positive in the naïve, saline and 1w1 groups at 2 weeks, there were many SP-C-positive cells in the KGF group at 2 weeks (**Fig. 7 A-D**). This was confirmed by blinded observation and quantification of the samples (**Fig. 7I**). As fibrosis progressed, at 4 weeks, there were fewer SP-C-positive cells per total number of lung cells in the saline and 1w1 groups, whereas there were more SP-C-positive cells in the KGF group compared with the naïve, saline and 1w1 groups (**Fig. 7E-G, I**). SP-C-positive cells were still dominant in the lungs at 8 weeks in the KGF group (**Fig. 7 H, I**). These observations suggest that bleomycin reduced the number of type II pneumocytes, and that Ad-KGF administration increased it significantly to an even higher level than it was in the naïve group.

### *Analysis of mRNA for surfactant proteins in lungs*

mRNA expression of SP-A, -B, -C, and -D were analyzed using real-time PCR, the same method used for total KGF mRNA analysis. Basically, mRNA expression of the four isotypes of SP showed the same trend (**Fig.8**). The mRNA expression decreased in a time dependent manner in the saline and 1w1 groups. SP-A and SP-D mRNA had increased significantly at 2 weeks in the KGF group. The mRNA expression in the KGF group at 4 weeks remained at a level similar to that of the naïve group, and was higher than that in the saline and 1w1 groups.

### *Quantification of SP-D protein in lung homogenate*

The SP-D protein concentration in lung homogenate was analyzed using

Western blotting (**Fig. 9A**). The intensities of SP-D signals were normalized by dividing them by those of β-actin (**Fig. 9B**). The expression of SP-D protein in the naïve group was barely detectable. SP-D proteins in the saline, 1w1, and KGF groups at 2 weeks had increased significantly compared with that in the naïve group. Among them, the increase in the KGF group was significantly greater than in the other groups. Although, SP-D protein expression had declined significantly in all groups at 4 weeks, it was still detectable in the KGF group at 8 weeks.

### *Quantitative analysis of TGF-β1*

The TGF-β1 concentrations in lung homogenates were also analyzed using Western blotting (**Fig. 9C**). The intensity of the TGF-β1 signal was normalized by dividing it by that of β-actin (**Fig. 9D**). TGF-β1 was not detected in the lungs of the naïve group and all experimental groups at 2 weeks. TGF-β1 was detectable in all groups at 4 weeks, and the level in the 1w1 group was significantly greater than in the naïve group. TGF-β1 in the KGF group was significantly lower than that in the 1w1 group at 4 weeks. These findings suggest that the TGF-β1production induced by bleomycin and/or adenovirus vector administration was reduced by KGF.

### *Analysis of collagen mRNA expression in lungs*

Collagen mRNA expression was analyzed using RT-PCR, the same method used for KGF mRNA analysis (above). (**Fig. 10**). The mRNA of collagen 1a1 in the saline group had decreased significantly at 2 weeks compared with that in the naïve group. The mRNA of collagen 1a1 in the KGF group had further decreased at 2 weeks and was lower than that in the saline group at 2 weeks. The mRNA expressions of collagen 1a1 and 3a1 in the saline group had increased significantly at 4 weeks and were significantly greater than that in the KGF group at 4 weeks.

## Discussion

In the present study, we examined the therapeutic effects of a KGF-expressing adenovirus vector on bleomycin-induced pulmonary fibrosis. We demonstrated that KGF overexpression in the lungs resulting from intratracheal administration of Ad-KGF reduced collagen deposition in the lungs, improved respiratory function, and reduced mortality. These

changes occurred concomitantly with KGF effects including alveolar epithelial cell proliferation, suppression of TGF-β1 production, and increased SPs.

We allowed a 7-day interval from the initiation of bleomycin administration to the beginning of treatment with the Ad-KGF. Because histopathological observation of the lungs taken at 7 days after the administration of bleomycin revealed early fibrotic changes, it would be safe to conclude that we administered Ad-KGF after the pathological processes of fibrosis had started within the lungs. In order to elucidate a therapy for a disease in experimental models, it is important to test the therapy when the disease is present; however, the majority of studies in the literature were concerned with the preventative use of KGF or the use of KGF within a few days after intra-tracheal instillation of bleomycin when fibrosis was not yet established. For example, Deterding *et al.* demonstrated that while administration of KGF before intra-tracheal bleomycin administration reduced lung injury, KGF treatments at 24 and 48 hours after bleomycin administration did not prevent lung injury and fibrosis (21). The difference between their results and ours may be due to different study designs including the routes for administration of bleomycin (intratracheal vs. subcutaneous), the form of KGF administered (protein vs. vector), and the timing of the administration of KGF (1 and 2 days vs. 1 week after bleomycin). Morikawa et al. demonstrated KGF overexpression in the lungs of rats after instillation of a KGF-expressing adenovirus vector (27). However, the authors used unaffected animals and no information is available about the effect of KGF gene transduction on injured lungs. As far as we know, the present study is the first to demonstrate the *therapeutic* (as opposed to *preventative*) effects of KGF.

We speculate that the mechanism by which KGF ameliorated the bleomycin-induced lung fibrosis in the present study involves multiple complex functions of KGF. The main mechanism of lung fibrosis is incomplete repair of impaired lung tissues. The proliferation of alveolar epithelial cells induced by KGF might enhance the repair of the lungs (11, 28) and thus reduce the fibrosis (**Fig. 4**) because regenerative alveolar type II cells were found in the lungs of mice that were administered Ad-KGF (**Fig. 7**). These cells might restore damaged tissue to its normal condition. Second, KGF is known to suppress the production of TGF-β1, which is the most

potent regulator of connective tissue synthesis in several different organs (14). The reduction in the concentration of TGF-β1 in the lung homogenate in the group administered Ad-KGF (**Fig. 9C, D**) might be responsible for the reduction in the fibrous change in the lungs. Third, KGF increases the secretion of surfactant proteins (10). The predominant function of pulmonary surfactants is to reduce the surface tension at the alveolar air/liquid interface and thereby prevent lungs from collapsing at the end of expiration. In addition, the pulmonary surfactant system exhibits host-defense properties (29). Secreted surfactant proteins in mice in the KGF group may be involved in preventing lung injuries induced by bleomycin (**Figs. 8 and 9A, B**).

Although the high dose of the adenoviral vector ($1.0 \times 10^9$ PFU) lacking KGF expression (Ad-1w1) had significant negative effects on the survival of mice, we employed this dose of adenovirus vector because the survival rate for mice given the higher dose of Ad-KGF ($1.0 \times 10^9$ PFU) was greater than that for mice given the lower dose of Ad-KGF ($1.0 \times 10^8$ PFU). Thus the KGF expression obtained by the higher dose of the vector was expected to overcome the negative effects of the vector on mice.

There are some limitations in the current study. First, bleomycin was administered twice (Days 1 to 7 and then Days 29 to 35) to prevent spontaneous improvements of pulmonary fibrosis. Therefore, one may argue that our results represent the prophylactic effects of KGF for the second administration of bleomycin, instead of the post-disease effects of the first administration. However, Ad-KGF instillation had improved the Ashcroft scores and pulmonary functions at 4 weeks (before the second bleomycin administration was started), suggesting that Ad-KGF reduced the impact of the first bleomycin administration. Second, we tested the effects of Ad-KGF at only one time point, which was 1 week after the first administration of bleomycin. Therefore, it is not clear whether Ad-KGF administered at later time points, when fibrosis is more advanced, would similarly exert beneficial effects. Third, it is not clear whether Ad-KGF reverses the fibrosis towards the physiological status. However, pulmonary fibrosis is a progressive disease, and treatments that reduce the speed of the pathological progression would be useful. In this respect, the beneficial effects of KGF due to instillation of Ad-KGF after the onset of fibrosis shown in the present study have

therapeutic utility. Lastly, there is no proof of a mechanism by which KGF protects against fibrosis. KGF mRNA expression in the lungs decreased after bleomycin administration. In contrast, collagen mRNA expression in the lungs increased after bleomycin administration, and the increase was significantly attenuated by the administration of Ad-KGF, which resulted in less severe fibrosis in the lungs. These results strongly suggest direct and/or indirect effects of KGF in preventing the pulmonary fibrosis caused by bleomycin.

In conclusion, administration of a KGF-expressing adenoviral vector to mice with preexisting bleomycin-induced pulmonary fibrosis reduced fibrosis of the lungs, improved respiratory function, and reduced mortality. These phenotypes were associated with KGF effects including alveolar epithelial cell proliferation, suppression of TGF-β1 production, and increased surfactant protein secretion. These observations suggest the therapeutic utility of the KGF-expressing adenoviral vector in pulmonary fibrosis.

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## Current address

The current address for H. Sato is Department of Anatomy, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki, Kanagawa 216-8511, Japan

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# Figure legends

**Fig. 1.** Structure of Ad-KGF (AxCAmKGF) (i) and Ad-1w1 (Ax1w1) (ii).

The arrow indicates the direction of transcription. Solid triangles under the Ad genome represent deleted Ad sequences.

CAG, cytomegalovirus enhancer and chicken β-actin promoter; GpA, rabbit β-globin poly (A) signal; mKGF, mouse KGF cDNA (from Ref. #22, with permission).

**Fig. 2.** Survival rates after first bleomycin administration. Seven days after the administration of bleomycin, mice (n = 15 to 16 per group) were given (A) $1.0 \times 10^8$ PFU of Ad (Ad-KGF or Ad-1w1) (low dose) or (B) $1.0 \times 10^9$ PFU of Ad (high dose) intratracheally. Data are expressed as Kaplan-Meier product limit curves and were compared by log rank test. *: $p < 0.05$ vs. Saline group, #: $p < 0.05$ vs. 1w1 group. (A) When the low-dose vector was administered, the survival rate of the 1w1 group was the same as that of the Saline group, whereas the survival of the KGF group was significantly increased compared with that of the Saline and 1w1 groups. (B) When the high-dose vector was administered, the survival rate of the 1w1 group was significantly reduced compared with that of the Saline group; however, the survival rate of the KGF group was significantly increased compared with both the Saline

and 1w1 groups.

**Fig. 3.** Representative micrographs of lung tissue of mice given Ad-KGF alone. Shown are immunohistochemistry images for KGF (A) and SP-C (B) 1 week after Ad-KGF administration. High magnification images are shown in insets. Diffuse hyperplasia of cuboidal cells positive for KGF is seen in alveolar region. The cells are also positive for SP-C, suggesting that they have features of type II pneumocytes. Inflammation was minimal. Lung sections harvested 3 weeks after Ad-KGF administration are stained with H & E (C) or Masson's trichrome (collagen stained blue) (D). Hyperplasia of cuboidal cells still exists at 3 weeks, but there was no sign of fibrosis. Black bars: 50 μm.

**Fig. 4.** Representative micrographs of lung tissue stained with H&E. Masson's trichrome-stained images are shown in insets. Lungs from the naïve group are shown (A). Lungs taken 1 week after the administration of bleomycin show subpleural fibrosis (B). Two weeks after bleomycin treatment, lungs in the Saline (C), 1w1 (D), and KGF (E) groups had developed subpleural fibrosis. Four weeks after bleomycin treatment, expansion of fibrous areas towards the lung parenchyma was seen in the Saline (F), 1w1 (G), and KGF (H) groups; however, the area involved was significantly smaller in the KGF group than in the other two groups. Fibrosis did not progress much in the KGF group from 4 weeks (H) to 8 weeks (I). No mice in the Saline and 1w1 groups survived to 8 weeks. Black bar: 100 μm. (J) Quantitative analysis of lung fibrosis by Ashcroft score.

Each lung section was placed over 2 mm square grids, the lung morphology of each grid was scored, and the scores of all the grids were averaged. 1W is the score for the lungs taken 1 week after the administration of bleomycin and before any intratracheal (includes adenovirus vector) administration. 2W, 4W, and 8W are the scores for the lungs taken 2, 4, and 8 weeks after the administration of bleomycin and 1, 3, and 7 weeks after the vector administration. Values are mean ± SEM; n = 3-6 except at 2 weeks in the saline (n = 2) and KGF (n = 2) groups. *: $p<0.05$. N/A: no data were available because all mice had died by this time.

**Fig. 5.** Lung function tests.

(A) Quasi-static compliance (Cst) was measured under mechanical ventilation. (B) The lung volume (TLC - FRC) was calculated from a pressure-volume curve. Values are mean ± SEM; n = 3-5, except at 2 weeks (n = 2), and in the Saline group at 4 weeks (n = 2). *: $p<0.05$.

**Fig. 6.** Semi-quantitative analysis of KGF mRNA.

(A) Ad-KGF-derived KGF mRNA levels at 2, 4, and 8 weeks after bleomycin administration (1, 3, and 7 weeks after Ad-KGF administration, respectively) were quantitatively analyzed by RT-PCR and were normalized to that for β-actin. Values are mean ± SEM; n = 3 in each group.

(B) Total KGF (endogenous KGF + Ad-KGF derived KGF) mRNA levels were analyzed using real-time PCR and the values were normalized to that of the naïve group. Values are mean ± SEM; n = 3 in each group.

*: $p<0.05$. N/A: no data were available because all mice had died by this time.

**Fig. 7.** Immunohistochemistry for SP-C.

Lungs from the naïve group are shown (A). Two weeks after bleomycin treatment, lungs in the Saline (B) and 1w1 (C) groups had very few SP-C-positive cells, but there were many SP-C-positive cells in KGF group (D). Fewer SP-C positive cells per total number of lung cells were seen in the saline (E) and 1w1 (F) groups at 4 weeks. SP-C-positive cells were more prominent in the KGF group at 4 weeks (G). SP-C-positive cells were still dominant in the lungs at 8 weeks in the KGF group (H). No mice in the Saline and 1w1 groups survived to 8 weeks. Black bar: 50 μm. (I) SP-C-positive cells were counted in a blinded fashion and described as the percentage of SP-C-positive cells per total number of cells in the lungs. Values are mean ± SEM; n = 3-6, except at 2 weeks in the Saline (n = 2) and KGF (n = 2) groups. *: $p<0.05$. N/A: no data were available because all mice had died by this time.

**Fig. 8.** Levels of surfactant protein mRNA.

mRNA levels of SP-A, -B, -C, and -D after bleomycin administration were quantitatively analyzed by real-time PCR and the values were normalized to that for the naïve group. Values are mean ± SEM; n = 3 in each group.

**Fig. 9.** Protein concentrations of SP-D and TGF-β1.

Representative results of Western blotting for SP-D (A) and TGF-β1 (C) are shown. 2w, 4w, and 8w represent samples taken 2, 4, and 8 weeks, respectively, after bleomycin administration. Semi-quantitative analyses of SP-D proteins (B) and TGF-β1 (D) in lung homogenate are presented. The density and area of each signal obtained by Western blotting were analyzed using NIH Image. The results for quantitative SP-D or TGF-β1 protein were normalized to β-actin. Values are mean ± SEM; n = 3-4 except at 2 weeks in the saline (n = 2) and KGF (n = 2) groups.

*: $p < 0.05$. N/A: no data were available because all mice had died by this time.

**Fig. 10.** Expression of collagen mRNA in lungs.

mRNA expression of collagen 1a1 and collagen 3a1 after bleomycin administration were quantitatively analyzed by real-time PCR and the values were normalized to that for the naïve group. Values are mean ± SEM; n = 3 in each group.

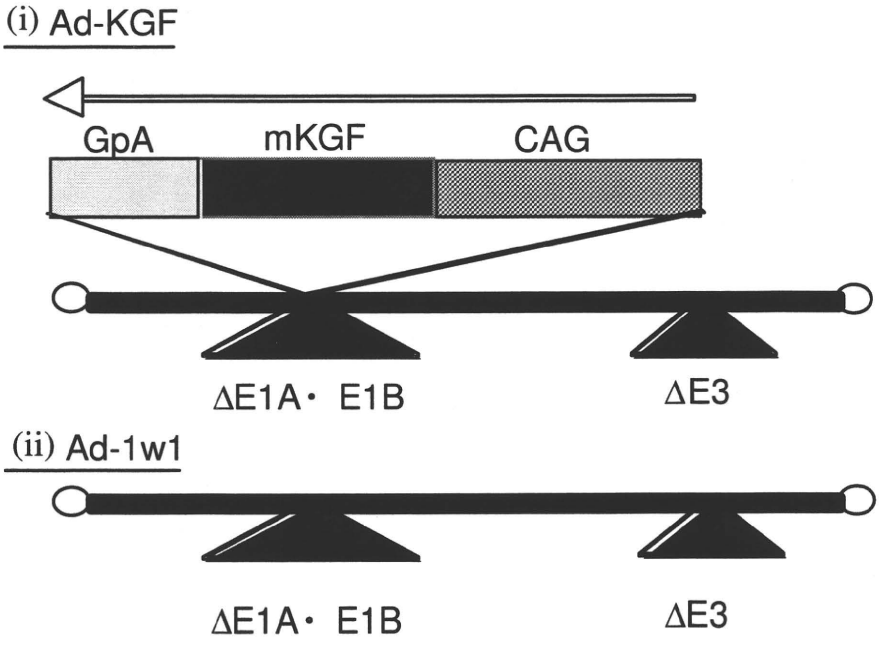


Fig. 1

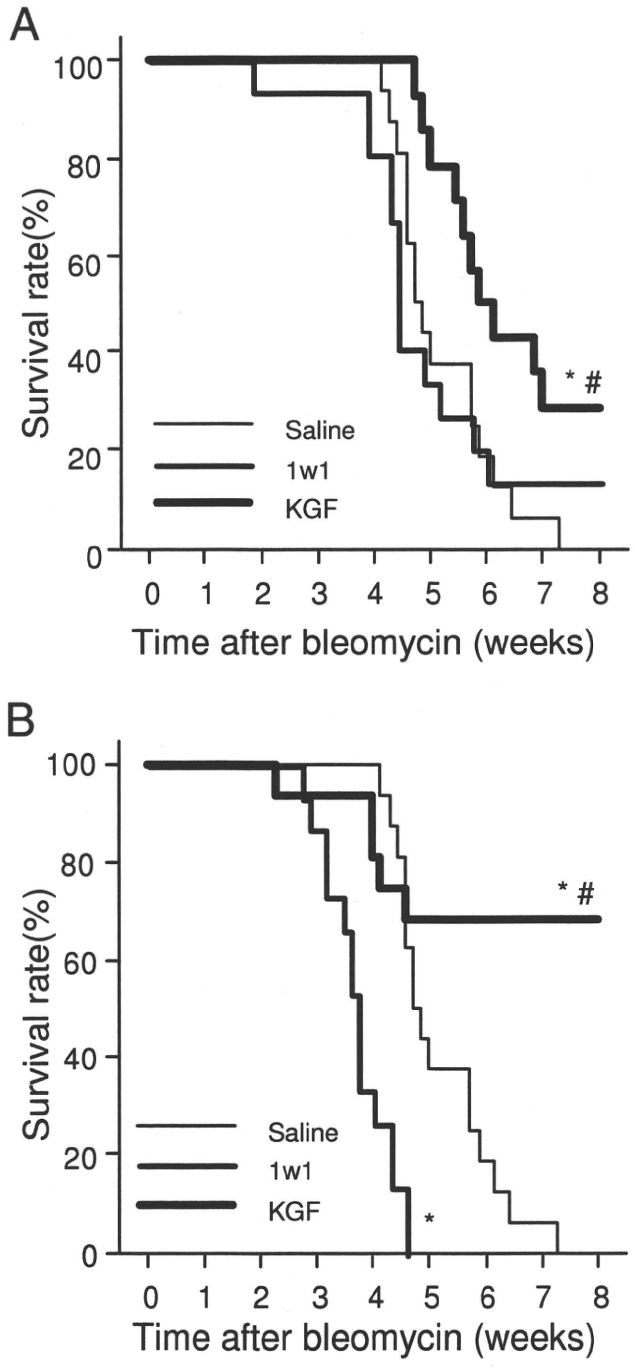
A
100
80
60
40
20
0
Survival rate(%)
Saline
1w1
KGF
* #
0 1 2 3 4 5 6 7 8
Time after bleomycin (weeks)
B
100
80
60
40
20
0
Survival rate(%)
Saline
1w1
KGF
* #
*
0 1 2 3 4 5 6 7 8
Time after bleomycin (weeks)

Fig. 2

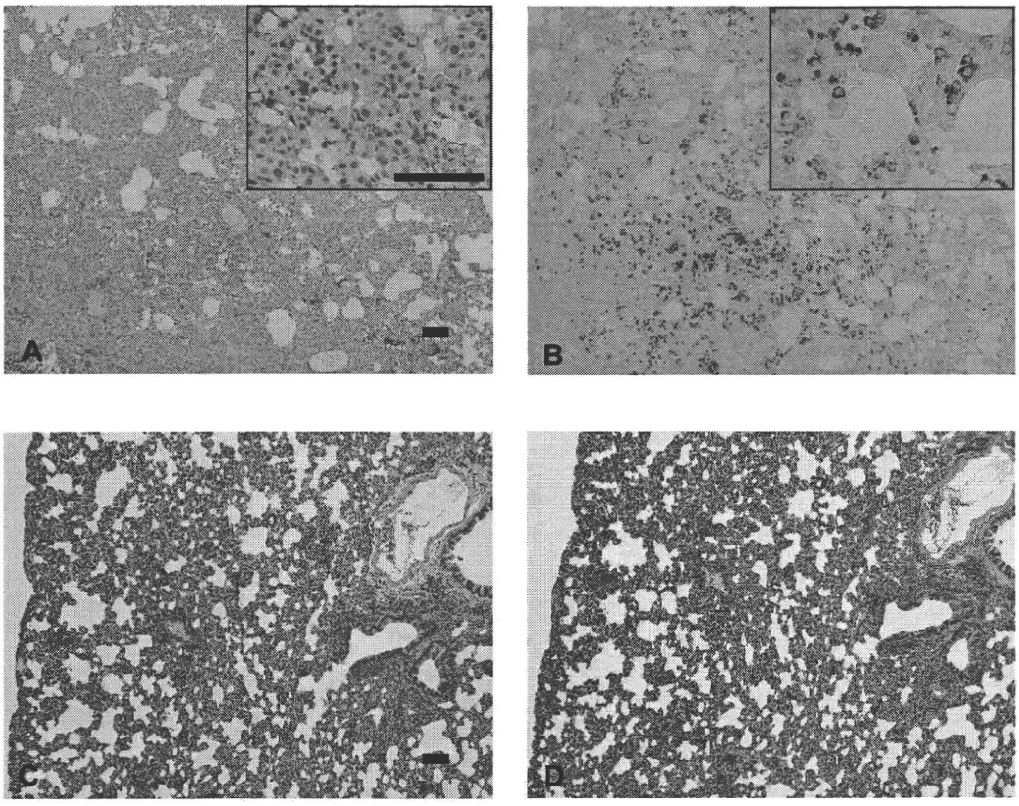


Fig. 3

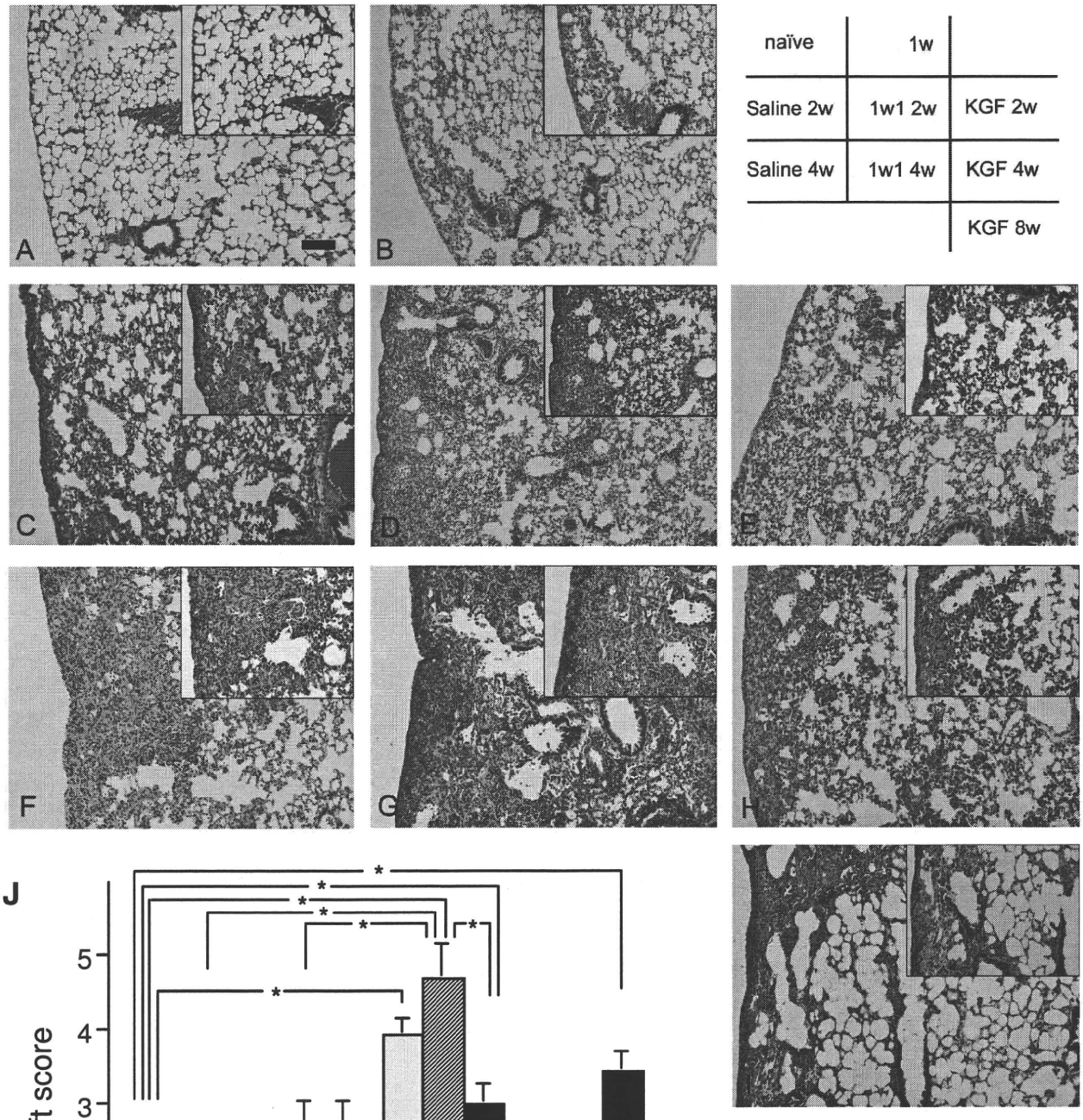
naïve
1w
Saline 2w
1w1 2w
KGF 2w
Saline 4w
1w1 4w
KGF 4w
KGF 8w
A
B
C
D
E
F
G
H
J
Ashcroft score
0
1
2
3
4
5
N/A N/A
naive
Saline
1w1
KGF
0 w
1 w
2 w
4 w
8 w

Fig. 4

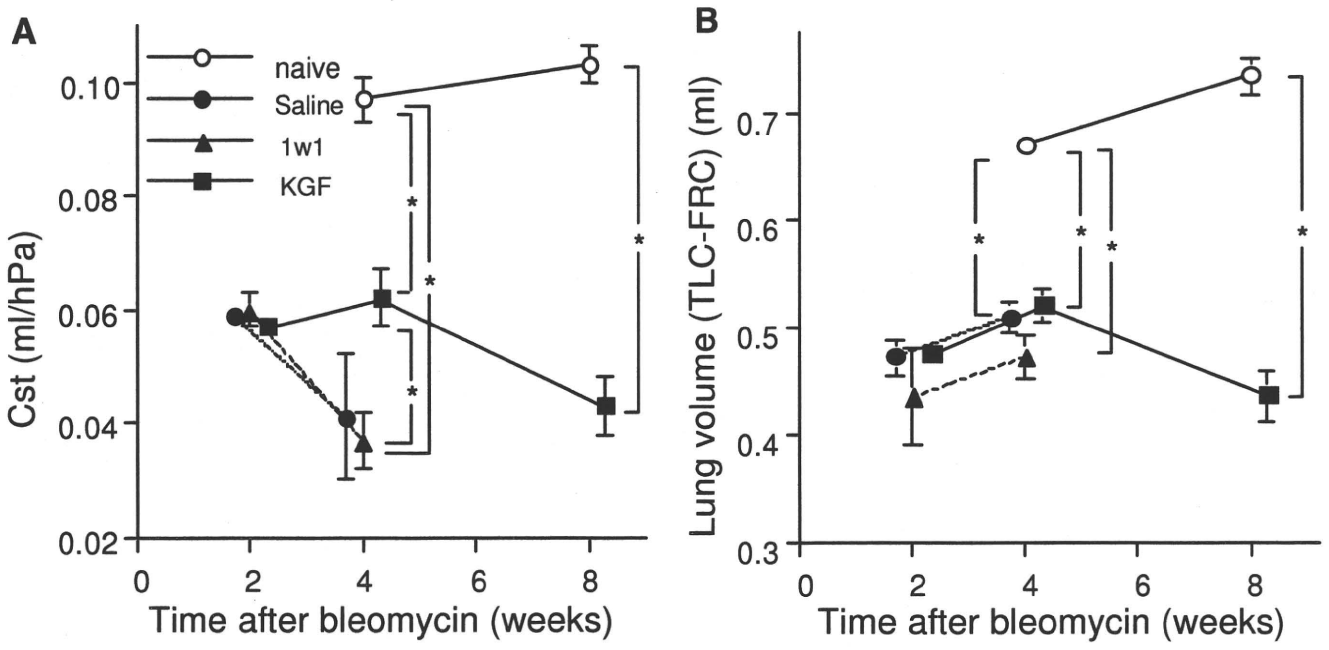
A
naive
Saline
1w1
KGF
Cst (ml/hPa)
0.10
0.08
0.06
0.04
0.02
0
2
4
6
8
Time after bleomycin (weeks)
B
Lung volume (TLC-FRC) (ml)
0.7
0.6
0.5
0.4
0.3
0
2
4
6
8
Time after bleomycin (weeks)

Fig. 5